The human *myc* gene family: structure and activity of L-*myc* and an L-*myc* pseudogene

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We have determined the nucleotide sequence and transforming activity of the human L-myc gene and a processed L-myc pseudogene (L-myc ψ). We demonstrate by cotransformation assays that a 10.6-kb EcoRI fragment derived from a human placental library contains a complete and functional L-myc gene including transcriptional regulatory sequences sufficient for expression in rat embryo fibroblasts. Organization of the Lmyc gene was determined by comparing its sequence to those of the L-myc ψ gene and an L-myc cDNA clone derived from a human small cell lung carcinoma. Our results show that L-myc has a three-exon organization similar to that of the c-myc and N-myc genes. The putative L-myc gene product consists of 364 amino acids and contains five of the seven homology regions highly conserved between c-myc and N-myc. These conserved regions are located along the entire length of the putative L-myc protein and are interspersed among nonconserved regions. While the putative L-myc gene product is of a smaller size when compared to the c- and N-myc proteins, the relative positions of certain conserved residues occur in corresponding locations along the peptide backbone of the three proteins. In addition, comparison of the human and murine L-myc gene sequences indicate that the relatively large 5' and 3' untranslated regions are evolutionarily conserved, but that these sequences are totally divergent between the L-, c-, and N-myc genes. Finally, we demonstrate that, like the N- and c-myc genes, the L-myc gene can cooperate with a mutant Ha-ras gene to cause malignant transformation of rat embryo fibroblasts in culture. Our analyses clearly prove that L-myc represents a functional member of the myc oncogene family and further delineate structural features that may be important for the common and divergent functions of the members of this gene family.

[Key Words: Nucleotide sequences; cDNA; cotransformation assay; processed pseudogene; myc gene family]

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The myc oncogene family consists of three known members: c-, N-, and L-myc (for review, see Alt et al. 1986; DePinho et al. 1987). The c-myc gene was first identified as the cellular homolog of an avian retroviral transforming gene, v-myc (Sheiness and Bishop 1979). Amplified N-mvc genes were identified in human neuroblastomas on the basis of homology to the c-myc gene (Kohl et al. 1983; Schwab et al. 1983). The c-myc and N-myc genes have a three-exon organization with a major coding domain located within exons 2 and 3 (Battey et al. 1982; DePinho et al. 1986; Kohl et al. 1986; Stanton et al. 1986). They encode similarly sized nuclear phosphoproteins (Abrams et al. 1982; Donner et al. 1982; Alitalo et al. 1983; Hann et al. 1983; Ikegaki et al. 1986; Persson et al. 1986; Ramsay et al. 1986; Sullivan et al. 1986; Slamon et al. 1986) which contain highly conserved clusters of amino acids (DePinho et al. 1986; Kohl et al. 1986; Stanton et al. 1986) that are likely to be important for nuclear targeting (Stone et al. 1986), nucleic acid binding (Donner et al. 1982; Persson and Leder 1984; Watt et al. 1985; Spector et al. 1987), and in vitro transforming activities (Land et al. 1983; Schwab et al. 1985; Yancopoulos et al. 1985; Stone et al. 1987). L-myc was identified first as a myc-related sequence that was amplified in a subset of human small cell lung carcinomas (SCLC) (Nau et al. 1985) and independently isolated from normal murine and human genomes on the basis of homology to N-myc (Zimmerman et al. 1986; see below). Limited nucleotide sequence analysis of a portion of the human L-myc gene revealed two short stretches of nucleotide sequence that were highly homologous to sequences conserved between the N- and c-myc genes (Nau et al. 1985), suggesting that L-myc might be an additional member of the myc gene family.

The physiological role of *myc*-family genes is not known; however, it is generally believed that these genes play a fundamental role in cellular proliferation and perhaps differentiation (for review, see Alt et al. 1986; Kelly and Siebenlist 1986). That they serve essential but distinct physiological function(s) is strongly supported by conservation of c-, N-, and L-*myc* as independent sequences over a large phylogenetic distance (King et al. 1986; Van Beneden et al. 1986; Collum and Alt, unpubl.). A potential role for c-*myc* expression in

development was suggested by the observation that deregulated expression of this gene affects B-cell differentiation in transgenic mice (Adams et al. 1985) as well as the differentiation of myeloid erythroleukemias in vitro (Coppola and Cole 1986; Dmitrovsky et al. 1986; Lachman et al. 1986). Analysis of myc-family gene expression during murine (Jakobovits et al. 1985; Zimmerman et al. 1986) and human (DePinho and Alt, in prep.) development demonstrated that the c-myc gene is expressed at substantial levels in most developing tissues; in contrast, high-level N- and L-myc expression is very restricted with respect to tissue and developmental stage. A striking example of differential mycfamily gene expression occurs in the B-cell differentiation pathway. Precursor B-lymphoid cells express both N- and c-myc, but only c-myc is expressed in later stages of B-lymphoid cell development (Zimmerman et al. 1986). These differential myc-family gene expression patterns led to the suggestion that differential or perhaps, combinatorial expression of these genes could be related to the progression of certain cell lineages through differentiation (Zimmerman et al. 1986). Recent evidence suggests that there may be additional members of the myc-family (Alt et al. 1986).

To help elucidate the potential content and function of the myc-gene family, we have isolated and determined the structure of the complete human L-myc gene and a related human genomic sequence. In addition, we have demonstrated for the first time that the human L-myc gene has oncogenic potential; the cloned gene can cooperate with an activated Ha-ras gene to cause malignant transformation of rat embryo fibroblasts in vitro. In parallel, we have also characterized the nucleotide sequence of the murine L-myc gene (Legouy et al. 1987). Our analyses define structural elements that may be important for overlapping and distinct myc protein properties and point to sequences that could potentially serve a regulatory role in myc gene expression. Together, our findings demonstrate the L-myc represents a third functional member of the myc-family of cellular oncogenes.

Results

Isolation and structural characterization of the L-myc gene

To determine if *myc*-related sequences, in addition to Nand c-*myc*, existed in the human genome, we assayed *Eco*RI-digested human genomic DNA by Southern blotting procedures for hybridization with second and third exon probes derived from N-, c-, and v-*myc* genes. Under low stringency conditions, these probes hybridized to numerous fragments that did not correspond to either the N- or c-*myc* genes (Alt et al. 1986). The novel *myc*homologous fragments that hybridized to an N-*myc* exon 2 probe were isolated from a human genomic library that was prepared by cloning human placental DNA digested to completion with *Eco*RI into the λ vector Charon 30A. Two recombinant phage clones, R1.3 and R11.1, that hybridized strongly to probes derived from exons 2 and 3 of both the c-, and N-*myc* genes

were characterized in detail. Restriction mapping and hybridization experiments demonstrated that regions cross-hybridizing with exons 2 and 3 were closely positioned in the 8.8-kb R1.3 insert and widely separated in the 10.6-kb R11.1 insert. Preliminary sequence analyses revealed that phage clone R11.1 contained the short stretch of sequences previously identified within a partial human L-myc clone (Nau et al. 1985) and that phage clone R1.3 harbored stretches of sequence highly related to those of R11.1. To examine the structure and organization of these L-myc-related human sequences, we determined the nucleotide sequence of the appropriate regions of both clones; clone R11.1 (referred to hereafter as L-myc) was found to contain the entire L-myc genomic sequence and the clone R1.3 (referred to hereafter as L-myc ψ) was found to contain a processed L-myc pseudogene (Figs. 1 and 2; and see below). Comparison of the L-myc genomic nucleotide sequence with the processed L-myc ψ sequence indicates that L-myc contains three regions of significant homology with L-myc ψ separated by large stretches of unrelated sequence (Fig. 1). At each of the boundaries between conserved (exons) and divergent sequence (introns), there exists a consensus donor and acceptor splice recognition sequence (Mount 1982) (Fig. 1C).

To confirm L-myc intron/exon structure suggested by the L-myc ψ gene, we prepared a λ phage Charon 16A cDNA library from cytoplasmic poly(A)+ mRNA of H510 SCLC cell line and from that of an L-myc-transformed rat embryo fibroblast (REF) cell line. A 5'-L-mycexon 3 synthetic oligonucleotide primer (Fig. 1B) or oligo(dT) primer was hybridized to cytoplasmic RNA and extended as described previously (Kohl et al. 1986; Legouy et al. 1987). Recombinant phage were screened for hybridization to various L-myc probes (Fig. 2, L-myc probes 1-4). Several cDNA clones were identified that hybridized to probes containing putative L-myc exon 1 and exon 2 sequences. Characterization of multiple cDNA clones by restriction mapping, nucleotide sequence determination, and S1 nuclease protection experiments indicates that several distinct L-myc transcripts result from the differential processing of the first intron (with some species retaining the first intron) and from the alternative utilization of polyadenylation signals located in the second intron (DePinho and Alt, in prep.). Direct nucleotide sequence analysis of one such primer-extended cDNA clone, pPEIII.5, confirmed the intron/exon organization suggested by analysis of the L-myc ψ gene (see above). Primer extension analyses (not shown) indicate that the pPEIII.5 cDNA clone extends to within several basepairs of a major transcription initiation site (Fig. 1A, position 138); just upstream from this major cap site is a potential TATAA element (Fig. 1A, position 88) and a region homologous to sequences occurring near the N-myc promoter (DePinho et al. 1986). On the basis of the data presented here, however, the existence of additional 5' exons cannot be excluded.

The location of the polyadenylation signal downstream to the third exon of the L-myc gene (Fig. 1, position 5452) was determined by comparing the genomic

10 STCGACTGCCCG	20 Tagtasgcasge	30 5 4646666666 6	40 Getteccci	50 TAGGGCCCGC	60 CCCCCAGTC:	70 CCTGGGTCCCG	80 48383888	90 Acgagatataa	100 Gecaetcaeg	110 IAAACAATSCG	120 Ectecaecte	130 Secteece	PE III.5
160 CC666CC6CC6T6	170	180	190	200	l 210	220	230	240	250	260	270	290	1 290
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460 CTTT6AACTC6C	470	480	490	500	510	520	530	540	550	560	570	580	590
610	620	630	640	650	660	670	680	690	700	710	720	730	740
6T6CC6A666AC	CCGGGGGGGGCACCT					8A3833377333	GEAGCESAC	ATSGACTACE	ACTOBTACCA	GCACTATITC		CTECEESEAS	GATTTCTAC
760 C66C6CCCA6C6 hrAlaProSer6	770 AGGACATCTGG/ LuAspileTrpi	780 NAGAAATTCGA .ysLysPheG1	790 GCT66T6CC4 uLeuValPro	800 ATC6CCCCCCA SerProProT	810 ICGTCGCCGC IhrSerProPi	820 CCT6666CTT6 roTrp61yLeu	830 661000660 61yPro61y	840 CGCAGGGGGGCC (A1aG1yAspP	850 CGGCCCCCGG roAlaProGl	860 Gattsgtccc ylleSlyPro	870 CC66A6CC6T6 Pro61uProTi	880 Gecccebagee rpPro61y61y	890 TECACCEEA CysThr61y/
910 CGGAATCCCGGG LaGLuSerArgG	920 GCCACTCBAAAA LyHisSerLys	930 GGCTGGGGGCAG GlyTrpGlyAr	940 IGAACTACSCI IGAShTyrAla	950 CTCCATCATAC Ser IleIle4	960 SECCETEACT IrgArgAspC	970 GCATGTGGAGC ysHetTrpSer	980 GGCTTCTCE GlyPheSer	990 GCCC666AAC AlaAro6luA	1000 GGCTGGAGAG rgLeuGluAr	1010 AGCTGTGAGC pAlaValSer	1020 GACCGGCTCG AspArgLeuA	1030 CTCCT66C6C6 LaPro61vAla	1040 CCCC966666 ProArg61vi
1060 CCAAGSCGTECG	1070 ECGCCCCGGACT	1080 IGCACTCCCAG	1090 ICCTCSAAGCO	1100 CEECAACCCEE		1120 CCCCCTGTCCG	1130 CT 666C6A A	1140 NECCAAGACEE	1150 AGGCCTGCTC	1160 C666TCC6A6	1170 ABCCCAABCB		1190
roLysAlaSerA 1210	1220	1230	1240	1250	1260	1270	1280	1290	1300	1310	1320	1330	1340
CCAA6A66666C	1370	1380	1390	1400	1410	1420	1430	1440	1450	1460	1470	1480	1490
GTTGGTAAACAG 1510	11166AAAA611 1520	66C6T666A60 1530	1540	TTTGATGATTI 1550	1560	CA66666ACAA6 1570	1580	1590	6C6CTTA6A6	1610	1620 1620	1630 1630	1640
AGAGACACTECA	NACCTEAA6TT1 1670	1680	16001011100	CTAGGAAACT 1700	CACACTCCC	CTA66 6666 66 1720	1730	6AGCCTTTT6 1740	16CAAA6CCA 1750	AAACCTTCGT 1760	CETTTTAAAAA	ACCTAGETETE 1780	CAGTTGGCT 1790
AAAATGCCAATA			CTCCCCACCA	CCACTTACCA	TCGTSCATC	CCTEAGACAGE	GAGEGAAG	ATGAACACTO	CCCATTAACI	GATEGAAAAA	CTGASGCTTA	BAGATAGACA	TCACTACAA
CCASCITICISC	CATCTAGECAGE	0001111000				1870 CCTTGATGTTT	1880 171778888	1890 IGNNTSGTAGC					
1960 TTCTTGCTCCCC	1970 CTCAATAGATC	1980 TCCAGCGTCA	1990 1990 1990	2000 BGCATTCAAC	2010 Raatattcac	2020 TGGCCCCTACT	2030 TTGT GG CA	2040 Atct61656Ct	2050 Acatectee	2060 Setcaasscae	2070 Stegasctcta	2080 66CCCTCCTC	2090 ICCCATECTT
2110 GTGCAACCTCGC	2120 Teagescasac	2130 T SEGECA TCC	2140 Teteccacta	2150 AACTACATTS	2160 TTCTTATTC1	2170 IGSCATCTTAG	2180 ACCTCCACA	2190 CCC GAGAGAA I	2200 Atcct 66A6A	2210 Gestatttti	2220 Stagaststae	2230 Actgt56cta	2240 GTGACAAATA
2260 Accaagaaagct	2270 Cactetagett	2280 TTAGSAATAA(2290 CTTTTACAES	2300 Accattigati	2310 Agggaactge	2320 666aat6666ta	2330 166aa611	2340 TTCCTACACTI	2350 G AGAGA AAA	2360 Nataggataa(2370 Caraanttaar	2380 Astettttt	2390 Itcct66tcc
2410 TAAGGTCATTTT	2420 TAACCASCTTSC	2430 CTTTCTACACO	2440 Caagagtttai	2450 ISTITSTITA	2460 11660166AA	2470 Agagaatetts	2480 Agatgaaa	2490 MAACCAATAAA	2500 Gatgtatct(2510 CTACAACGGCT	2520 166168A6166	2530 Tagagtebaat	2540 Gagcattgc
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2710 ATTEGAGAATAT	2720 TITICCITCITG	2730 CTAATSTCCCC	2740 CICCTITCCT	2750 Icactotocci	2760 CTTACCACAT	2770 Tacaaatgaat	2780 Casctttc1	2790 Igeteaceteg	2800 Attigtatat	2810 Iatctaaatte	2820 Maaaaatgtc	2830 Tectacette	2940 Ceaageacea
2860 Cagetaaagete	2870 Tagggtctatg	2880 TTT6T6TTTC	2890 TCAT666AT6	2900 T6TTTCTTCT	2910 CTTGATCTC1	2920 ITTTCTC66AC4					2970 GCABICICIGE gGlnSerLeuf		
3010 TGCGAGCAGACC alArgAlaAspF	3020 CCCT66ATCCC TraleuAspPro	3030 TGCATGAAGC CysMetLysH	3040 ATTTCCACAT isPheHisIl	3050 CTCCATECAT eSer IleHis	3060 CAGCAACAG(61 n61 n61 n1	3070 CACAACTATGC tisAsnTyrAla	3080 IGCCCGTTT All aArgPh	3090 TCCTCCAGAA/ eProProGlui	3100 NGCTGCTCCC SerCysSerG	3110 AAGAAGAGGC Ingluglual	3120 TTCABABABBB aSer BluArge	3130 IGTCCCCAAGA Il yProGlaGl	3140 AGAGGTTCTG uGluValLet
3160 ATECTECASEE SpALaALaELyE	3170 AAAAGGAAGAT	3180 Gaggaggatg	3190 AABAGATTGT	3200 Gasteccea	3210 CCTGTAGAAA	3220 AGTGASSETSCI	3230 CCASTCCTS	3240 CCACCCCAAAI	3250 CCTGTCAGTT	3260 Ctgatactga	3270 66at6t6acc4	3280 Nagaggaagaa	3290 TEACAACTTE
3310 GCAAGAGGC65	3320	3330	3340	3350	3360	3370	3380	3390	3400	3410	3420	3430	3440

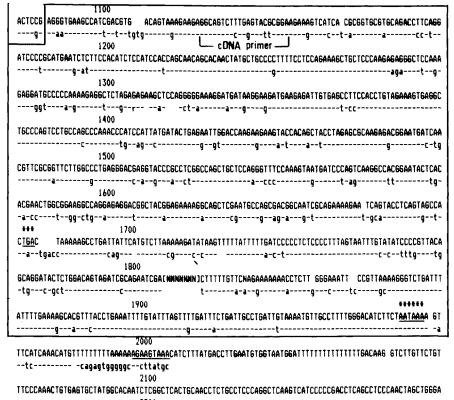
Figure 1. (See page 1315 for legend.)

3460 CTACAGAGAAAAGA laThrGluLysArg	3470 CAGCTCCGATC GloleuAroCy	3480 CCGGCAGCA	3490 GCASTISCAS aStateuSta	3500 AAAAGAATTG	3510 CATACETCA	3520 GTGGCTACTAA Ar Riv Tyr End	3530 Ctgaccaaa	3540 Aageetgaea	3550 Bitcibiciti	3560 Acgaagacaca	3570 AAGTTTATTT	3580 TTTAACCTCC	3590 CTCTCCCCTT	. 3600 Tagtaat
3610 TTECACATTITEGT	3620	3430	3640	3650	3660	3670	3680	3690 Cattetteea	3700 AACETTGAAA	3710 CCCAGCTCTC	3720 CCTCTTCCCT	3730 6actcat666	3740 Astectstati	3750 BITCTCI
3760	3770	3780	3790	3800	3810	3820	3830	3840	3850	3860	3870	3880	3890	3 90 0
GGCGCCTTTGGCCC	NGCABGCASCI	Igactgagga	GCCTTGGGGT	CTGCCTAGCT	Cactagete	Tsaagaaaasg	CTEACAEAT	Sctatscaaci	A66T66T66A	Tettgtcagb	Gectccagcc	Tecateaaat	CTCACACTCTI	6cat sa 6
3910	3920	3930	3940	3950	3960	3970	3980	3990	4000	4010	4020	4030	4040	4050
CTTTAGGCTAGGAA	Aggatgetcei	Caactgetet	CTCT666616	Atecaaegac	AGCT666CC	Tegatectete	CCTGAGGCT	CCTTTTTCCAI	Saasacacaci	Gagetgtetti	Gegtgaagaci	AASCTIGCAS	Actigaticaa	Catt6ac
4060	4070	4080	4090	4100	4110	4120	4130	4140	4150	4160	4170	4180	4190	4200
Cattacctcactst	CAGACACTIT	Acagtabcca	A66A6TTE64	AACCTTTAT	Atattatgat	GTTAGCTGACC	CCCCTTCCTC	CCACTCCCAA	TSCTGCGACC	CTEEGGAACAC	TTAAAAAGCT	TGGCCTCTAG	ATTCTTTGTC	TCAGAGC
4210	4220	4230	4240	4250	4260	4270	4280	4290	4300	4310	4320	4330	4340	4350
CCTCT666CCTCCT	CT6A666A66	BACCTTTCTI	ITCCTCACAA	IGGACTITIT	Tettccatte	ATECCTTETTA	TSCAAT666	CTCTACABCAC	CCTTTCCCAC	AGGTCAGAAA	MATTTCCCCA	IAGACACA666	MAAATCGGTCC	TAGCCTG
4360	4370	4380	4390	4400	4410	4420	4430	4440	4450	4460	4470	4480	4490	4500
666601566664146	CTT66AGTCC	Teeccate	AACTTEATCCI	TSCCCASST	GTTTTCCSAG	Gegeacttea	Gecccastc	Itttctcaagg	Cagetetaag	Acaceteaba	1666AGAACTS	Itactgctgcc	TCTTTCCCAC	CTECCTC
4510	4520	4530	4540	4550	456 0	4570	4580	4590	4600	4610	4620	4630	4640	4650
Atetcaateettga	Sceceaett	TGAAGTTCTT	ICTEGAACCA1	GCAAATCTG	Tectectest	GCAATTCCAAE	SGAGCTTGCT	66010160A6	CCACECT666	CCCCTTCCAG	CCT6CCATC6	Caatcagata	TCTTTCCCAG	AATCT66
4660	4670	4680	4690	4700	4710	4720	4730	4740	4750	4760	4770	4780	4790	4800
BCGTTTCTGAAGTT	TT666666666	Ctetteegaa	ETCATCCATCI	AGTECTCCA	Saagetgaac	STIECTICIEG	Tegettttai	AGGAACCTCC	Aggagatatg	Icttagccaac	Catsatsgat	TTTACCCCAE	CT66ACTC66	Casctcc
4810	4820	4830	4840	4950	48 60	4870	4880	4890	4900	4910	4920	4930	4940	4950
AASTEGAATCCACE		Agtctggga4	AASTCACCCAI	NCCTAGCAGT	Tetcatetge	66 TAACCTCA6	Beaceteta	AGECTGTECTG	Igaagaaggac	CAGCAGECCO	CTECAGAACTE	CTECCTAGEAG	CABCA66T6CC	Tecteec
49 60	4970	4980	499 0	5000	5010	5020	5030	5040	5050	5060	5070	5080	5090	5100
TCT666TTT66AA6	1166661666	TAGGGGGGTGG	Staastaasti	ACTATATATA	Getetsbaar	ACCASCISCI	Acticcaaa	ICTATISTECA		CTTTCT64661	ITGCTTCTTG8	SCCTCA6A666	ACCCTA6666A	Tettt e g
5110 AAATAGCCTCTCT	5120	5130	5140	5150	5160	5170	5180	5190	5200	5210	5220	5230	5240	5250
5260	5270	5280	5290	5300	5310	5320	5330	5340	5350	5360	5370	5380	5390	5400
STCTTTTTTTTT	Atatigtati	ITTGTAT6CC	Stittgcaaag	TEGTETTAA	Ctettttet	Acaagbaaaaa	MAACTOTT66	IGGCAATTTCC	Tettecaage	Stctbattta	Ittt6AAASG	CAAGTTCACC	TGAAATTTTG	Tatitagi
5410 Teteattacteat	5420 TGCCT6ATTT	5430 Taaaatgtte	5440 SCCTTCT6666	5450 CATCTTCTA		5470 ITCTCAAACATI	5480 Stcagagtei	5490 Geecaectta	5500	5510 ISTECTECTEA	5520 ACCACGGAAA	5530 ACTATTICAE	5540 Gegtagecaca	5550 AGTGATCC
	. <u> </u>													

5560 5570 5580 5590 5600 5610 5620 5630 5640 5650 5660 AGAGGGGCTGCACTTETCTAACCATGTTGCTAACETCAGTCATCTACGACATGTTGAAACAATGTAGGCTCAGTACTCAGTGAACACGGAATTC

> 100 Β. 200 300 400 TTCAAABAGATGTCAAGACCATGCAATTGGAAAAGAATAATCTCTTCAACAAATGGTGCTGGAATACTTGGATACTCACATGCAAAAGAATGAAGCTCCG 500 CCCTTECCTCACACCATTTACAAAAAATAACTCAAAATEAACCAAAAACAAAAATATAAAAAATTETAAGCCTCTTAGAAATAAATEAAAATAAATEAAAATAAATEAAAA gggggg-gcgcgcg- ---t----GTCGCGCGCTCGGTGGCGGCGTGCG GCGTGTGGAGTGCCCTGCTGCCCCCAGCTGG ASGGAACTAGTCTGCTCCAAGTGGCAAGCTGCGTGAGCAAG +++ 600 CAAGECAACAT66ACC6C6ACTC6TACCATCACTATTTCTAC6ACTAT6AC66C6666A66ATTTCTACC6CTCCAC66C6C6C6A66ACATCT66A 700 AGAAATTTGAGTTGATAC COCCOCCCT GGACTIGGGT CCGCAGCCGGGAACCCAGCCCTCAGCTITGGTCTCCTGGA CCG G 800 SCC66TA666T6C6CT66A6AC6A6AC66AATCCCA66ACTACT6T6AAA6CT1666AC6C6AACTAC6CCTCCCTCATCC6CC6T6ACT6CAT6T66A6 900 1000 TACACTCCCGAGCTCGAAGCCGGCAACCTAGCGCCCATCTTCCCCTCTTTGTTGGGCGAGCCCAAGATCCAGGCCTGCTCCAGGTCTGAGAGCCCAAGCG

> > Figure 1. (See facing page for legend.)



2200 CCACAGECTCEASCCACCECATGECTAETTTTTETACTTTTGETASAGACAGEGTTTCACCATGTTSCCCAGECT66TCTCAAACTCCT6A6CTCAA6 2300

TGATCTGCCCACCTTGSCCTTCCAAAGTGCTGGGATTAGAGGCGTGAGCCACCCACACCTAGCCNAGGGGGGGNAAGAGGATCC

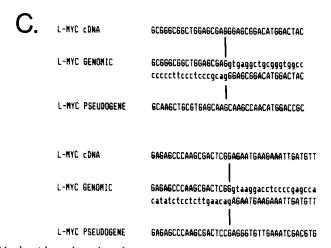


Figure 1. (A) Nucleotide and predicted amino acid sequence of the human L-myc gene. The nucleotide sequence displayed was derived from the L-myc genomic clone R11.1 and was determined by the method of Maxam and Gilbert. Sequencing strategy is outlined in Fig. 2. The 5' border of homology between the L-myc and L-myc ψ and the 5' end of the L-myc cDNA clone pPEIII.5 is indicated. The polyadenylation signal is underlined. Boxed areas represent exons. (B) Nucleotide sequence comparison between L-myc (cDNA) and L-myc ψ genes. The pseudogene sequence is shown in uppercase letters, L-myc cDNA sequence is shown in lowercase letters. The L-myc cDNA sequence was determined in the pPEIII.5 clone, which was derived from the H510 SCLC cell line. The lowercase sequence 3' to the synthetic oligonucleotide cDNA primer is derived from the R11.1 L-myc genomic sequence. (-) Symbolizes nucleotide homology. At positions 1805–1815, the series of Ns represents a 2.4-kb region that was not sequenced in the L-myc ψ gene. (C) Nucleotide sequence comparison of the exon-intron boundaries of the pPEIII.5 L-myc cDNA clone, R11.1 L-myc genomic clone, and R1.3 L-myc pseudogene clone sequences (exons 1/2 = upper panel; exons 2/3 = lower panel). Uppercase letters represent exon sequences; lowercase letters represent intron sequences. A vertical line indicates exon boundaries.

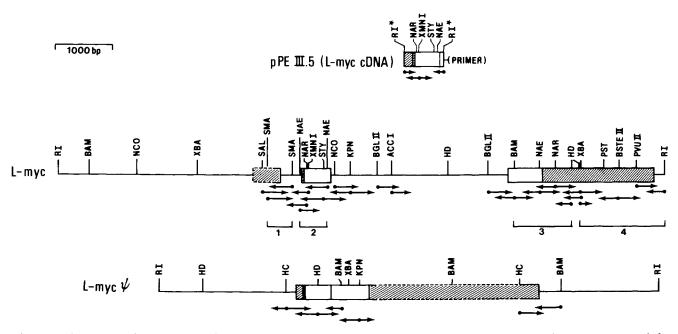


Figure 2. Structure and organization of the L-myc cDNA fragment pPEIII.5; the 10.6-kb L-myc genomic fragment R11.1 and the 8.8-kb L-myc pseudogene genomic fragment R1.3. A partial restriction map indicates restriction endonuclease sites used in the sequence analysis. An asterisk (*) indicates a restriction site created as a result of linker addition during cloning of the cDNA. Horizontal arrows represent sequencing strategy. The boxed areas indicate the position of exons; shaded areas represent untranslated regions, whereas open areas represent putative translated regions. The dashed border of exon 3 in the L-myc pseudogene represents a 2.4-kb region not subjected to nucleotide sequence analysis. Below the restriction map of the L-myc genomic fragment are shown four DNA probes used in screening the L-myc cDNA library. Probe 1 is a 400-bp SmaI fragment encompassing exon 1 and a portion of intron 1. Probe 2 is a 450-bp NaeI fragment that contains exon 2. Probe 3 is a 1200-bp BamHI-HindIII fragment containing exon 3 translated sequences.

L-myc sequence with the 3' termini of the mouse L-myc cDNA (Legouy et al. 1987) and the L-myc ψ gene. A consensus polyadenylation sequence motif AATAAA (Proudfoot and Brownlee 1976) occurs at corresponding locations in all the L-myc genes and is followed by a poly(A) stretch in the mouse L-myc cDNA and the L-myc ψ (Fig. 1A,B). Thus, at least one form of the human L-myc gene consists of a three-exon organization similar to that of the c- and N-myc genes (Fig. 2): exon 1 is about 170 bp in length; exon 2 is 505 bp; and exon 3 is 2529 bp. The combined size of these sequences, including a poly(A) stretch of variable length, is approximately 3.5 kb; this length could account for the size of the largest noted human L-myc mRNA sequence (-3.5-3.8 kb), which appears to represent a major form of the L-myc mRNA (Nau et al. 1986; Fig. 5).

A long open reading frame (ORF) in the L-myc gene begins with an ATG codon at position 684 (9 bp downstream from the 5' boundary of exon 2) and extends 1092 nucleotides to an in-phase terminator at position 3522 in exon 3. Multiple termination codons are present in the other two reading frames. If we assign the first ATG of this ORF as coding for the amino terminal residue of the protein, L-myc would consist of 364 amino acids with a predicted molecular weight of approximately 38 kD. Recent evidence has indicated the possibility that translation initiation of the c-myc mRNA can occur at nonmethionine residues within exon 1 (King et al.

1316 GENES & DEVELOPMENT

1987); such upstream initiation could theoretically extend the amino terminus of the L-myc coding domain an additional 38 or 160 amino acids (in the presence or absence of first intron splicing, respectively) before an inphase termination codon would be reached at position 202 in exon 1.

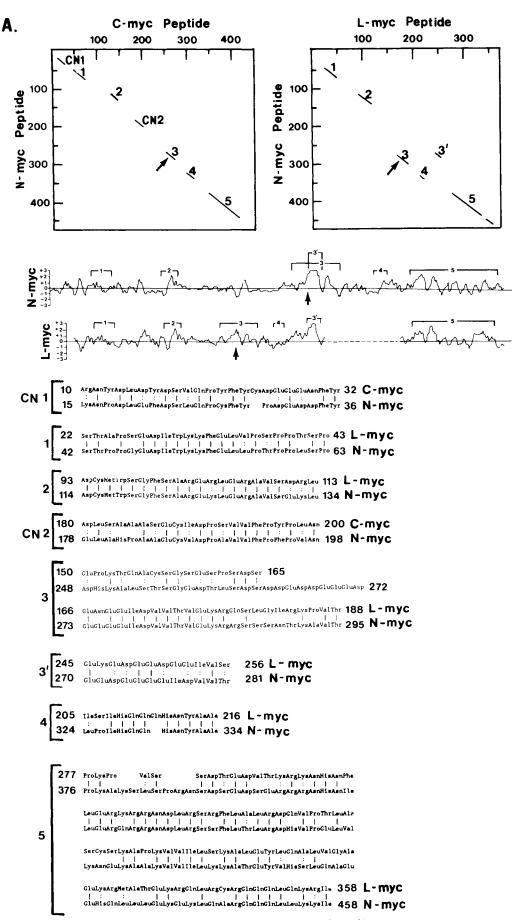
The increased size of the L-myc mRNA relative to Nand c-myc appears to result primarily from its larger 3' untranslated region-1900 bp compared with 900 bp for N-myc (Kohl et al. 1986; DePinho et al. 1986; Stanton et al. 1986) and 350 bp for c-myc (Battey et al. 1982; Bernard et al. 1983). Similar to c-myc (Bernard et al. 1983) and N-myc (DePinho et al. 1986), the 5' and 3' untranslated regions of the L-myc mRNA exhibit about 80% nucleotide sequence conservation between the mouse and human genes (Fig. 3). This degree of homology is comparable to that seen in the translated regions (data not shown). While the untranslated regions of the individual c-, N-, and L-myc genes are conserved across species, the untranslated regions of each type of myc gene diverge extensively from one another (data not shown).

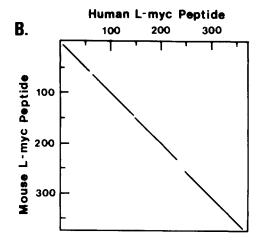
myc family gene products are highly related

The putative human and mouse L-myc proteins contain 364 and 368 amino acids, respectively (Fig. 4B). Allowing for the four-amino-acid insertion near the 5' border of

A.	B.
1 GTC6ACTECCC51A51A65CA656A6A6565C6565T116TCCC41A6565CC5CCCCC 598C-A56A6A66C6AC1-CAAAC	352 TAACTGACCAAAAAGCCTGACAAAGCTTGTGTGTGACGACAAAAGTTATTTTAHULULULULULULULULULULUHAAMHITTAGACAAAAAGCCTGACAAAAGCCTGACAAAAGCCTGACAAAAGCCTGACAAAGCCTGACAAAGCCTGACAAAGCCTGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAAGCCGACAGCCGACAGCACAGCGACAGCGACAGCGACAGCGACAGCGACAGCGACAGCGACAGCGACAGCAG
60 ABTCCCTBGBTCCCG56CGCGCGCGAGAAAAAAGGCAGTCAGGAAACAATGCGCC 658 TCCT6CAA-CA	3638 AGAICCCA6AAIGCAIIGCA6CCG GIGCACAAIAAAGGCIIGCAIICIIGBAAACCIIGAAACCCAGCICCACICICIC CIGACCCAGGG6668676C161A1611C1C16 5396CC68-C
116 TECAECTCSCECTCCCECEGATCCCEABAECETCCSESECCECCETECSCEAECBAE	3752 BCBCCTITEB CCCABCABCCABCTBACTBABCSCTTEB65CTCTA6CTCABCTCACTAAGAAAABBCTGACABATGCTATBCAACABGIGEGEGATGTTATGCAB 5515CTT-T
174 66A666C5C5C56666666666C5C1 616A616C 666C5C5C1C1C 66C65WC 7786CACC-CC6	3849 CAGCCTGCATSAAATCTCACACTCTGCATSAGCTTTA66CTA65AAAG6GATGCT C CCAACT6GTETCTCTG6565 T6AT5CAA66ACAGCT666ATECTCTCCCTGA65CTCC 5632A-6
229 A1616C616161678C168C166C6666C16CCCC6A6CC6666666A6CC661CC66C1CCA6 838	- 3986 TITTTCCABAAGACACAGAGACTGEGTTEGABGCAAGCTTGCAGAGCTTGATCAACATTGACCATTACCTCACTGTGCAGAGCATTACCTTACGATAGCCAAGGAGCATGTT A 5752 g66846CCCC
289 6156C666C666C666C6466T6466C16C666T656CC46666C4C656 C6C 656TC 898	4103 IAIATIAEATEAECTEGCCCTTCCTCCCCATECTECEGAAGCCTEGEAACACTTAAAAAECTEGCECCTAEAATTAETCTTEICTCAGAECCCTCTEGEGCCTCTEAEBEA 587161666CCACT66-TA4666CTT6C- CTA-661717
346 CCGC66T6CG66CT66CT6CCT6CCT1CT6686CAC66C5CCCC56CC58CCCC 958 -1-6C-61-CAAA	423 866ACCTITCTITCCTCACAA666ACTITTT6TTCCATAT6CCATA6CCAT666CTCTACA6CACCCTTTCCCAA66CACA66CACA666CACA666AATC66CA 5966
406 GCCGGGCCCT8GBAGCTGCGCTCCG6SGCGCGCT8GCABAGTT1GCTTTGAACTCGCTGC 10151-1CCA	4342 CCTA66CCT66666CT66G6ATA6CT166A6TCCT66ACCA16AAC TT6ATCCC16CCCA66I6ITITCC5A86666CACT16A66CCCA6 T CTT11C1CA486CA66T 61 6166 - ACCAAA6CCACCA-TC-AAC-6T
466 CCACAGICG661CC6C6C6C16C6A1T66CTTCCCCTACCACTCT6ACCC6866CCC68C 1075fc-f8C166-6-64	4451 AAGCACCTCAGAGGGAGAA CTGTACTGCTCCTTTCCCACCTGCCTC AICCAATCCTTGABC56CGAGTTTGAAGTTCTTCTG5AAACCATSCAAATCTGTCCTCGTG5CAA 6225 C6161AC6-cCTCTTC-6TC-66AT T- TCCAA646
52& TTCCC6666AC6C6666ACT666C6C6666CT6CAA6CT66156666T76666A68AC6A6A 1132A6CAAC-A66	4568 FICCAR66AGCTISCIGGCCTCTGCAGCCCCTGGGCCCTCCAGCCTGCGCATCAGATATCTTICCC AGAATCFGGGCTTTCIGA AG TITISG68AGAGCTGTT5656AC 6341 C
	4685 TCATCCATCCAGTGCTCAGAGGTGAACTTGCTT CTGGTGGGTTTTAAGGGAACCTCCAGGGATATGCTTAGCCAACCATCATGGAGGAGCTCGGGACCTCGGCGCCGAG 6448 B-TGC-CAACG-C-BT-TG-C-BT-TACGC-ACA-BTGTTCT
645 66TCA6CACBTC CCCCTTCCCTCCC6CA666666666C ATG 1241 4TT	4804 TESAATCCACETECAS CTTCTASTCTESEAAASTCCCCAACCTASCASTTSTCATETESETAAC CTCASSCACCTCTAASCCTGTASCASAAASAASAACSACCASCASCCCCTCCASAAA 6563AAC-CTC
	4922 TCT6CCTA66ACA6CA6 616CCT6CT66CTCT666TT166AA6TT6666T 666F466F66TA46FAA6TAA6TAAFTATG6CTCT66AAAACCA6CT6CTACTTCCAAAT 6679ACATCTTCC
	5034 CTATTBTCCATAATGBTTTCTTTCTGAGBTTBCTTCTGBCCCTCABABGACCCTAG6668AT8 TTT56AAATAGCCTCTCTACCCTTCT66A6ATTAGCATGACAAAGCCAGCT6ACTTC 6799 -6-CT-CCCTGATGECCCCAAAC
	5153 T66AATTGTCTAT66A66ACAGTTT66E51AAGETTACT6ATGTCTCAACT6AATA6CTTGT8TTTATAAGCT6CT8TTAT6CT66666AGT CT 6912C-AT6A6-6-T-AACA-AT6C66-T6CCTTTTT6TTTT6TTTT6
	525 11111111 AIAITGIAITTIGIAIGC CITTIGCAAAGIGGIGTTAACTGITTITGIACAAGAAAAAAACTGITGGGGGAATTICCTGITGCAAGGGICIGATTIATTITGAAAGG 7029
	5373 CAAGTICACCTGAAATTTTGTTTTGTTTGTTGTGTGTGCTGATTTTAAAATGTTGCGTGCTGTGGGGGCATCTTCTAAAA 7148 TGCAT
Figure 3. Nucleotide sequence comparison between human numbered positions in the human sequence correspond to t	Nucleotide sequence comparison between human (upper line) and mouse (lower line) L-myc 5' (A) and 3' (B) untranslated regions. A dash indicates a match. The positions in the human sequence are derived from Legouy et al. (1987).

GENES & DEVELOPMENT 1317





 ${\tt MetAspTyrAspSerTyrGlnHisTyrPheTyrAspTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrPheTyrAspCysGlyGluAspPheTyrPheTyrPheTyrAspCysGlyGluAspPheTyrP$ 1 MetAspPheAspSerTyrGlnHisTyrPheTyrAspTyrAspCysGlyGluAspPheTyr ArgSerThrAlaProSerGluAspIleTrpLysLysPheGluLeuValProSerProPro ArgSerThrAlaProSerGluAspIleTrpLysLysPheGluLeuValProSerProPro ThrSerProProTrpGlyLeuGlyProGlyAlaGlyAspProAlaProGlyIleGlyPro ThrSerProProTrpGlySerGlyProGlyAlaValAspProAlaSerGlyIleAsnPro ProGluProTrpProGlyGlyCysThrGlyAspGluAlaGluSerArgGlyHisSerLys GlyGluProTrpProGlyGlyGlyGlyAlaGlyAspGluAlaGluSerArgGlyHisSerLys ${\tt GlyTrpGlyArgAsnTyrAlaSerIleIleArgArgAspCysMetTrpSerGlyPheSerContent and the set of the set of$ AlaTrpGlyArgAsnTyrAlaSerIleIleArgArgAspCysMetTrpSerGlyPheSer AlaArgGluArgLeuGluArgAlaValSerAspArgLeuAlaProGlyAlaProArgGly
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 AlaArgGluArgLeuGluArgValValSerAspArgLeuAlaProGlyAlaProArgCly
AsnProProLysAlaSerAlaAlaProAspCysThrProSerLeuGluAlaGlyAsnPro AsnProProLysAlaProAlaThrProAspGlyThrProSerLeuGluAlaSerAsnPro ${\tt AlaProAlaAlaProCysProLeuGlyGluProLysThrGlnAlaCysSerGlySerGluSerGluB$ AlaProAlaThrGlnCysGlnLeuGlyGluProLysThrGlnAlaCysSerGlySerGlu SerProSerAspSerGluAsnGluGluIleAspValValThrValGluLysArgGlnSer SerProSerAspSerGluGlyGluGluIleAspValValThrValGluLysArgArgSer LeuGlyIleArgLysProValThrIleThrValArgAlaAspProLeuAspProCysMet LeuAsplleArgLysProValThrIleThrValArgAlaAspProLeuAspProCysMet LysHisPheHisIleSerIleHisGlnGlnGlnHisAsnTyrAlaAlaArgPheProPro GluSerCysSerGlnGluGluAlaSerGluArgGlyProGlnGluGluVal Leu GluSerCysSerGlnGluGlyAspProGluProGlyProGlnGluGluAlaProGluIle Glu ArgAspAlaAlaGlyGluLysGluAspGluGluAspGluGluIleValSer GluAlaProLysGluLysGluGluGluGluGluGluGluGluGluGluGluGluGluIleValSer ProProProValGluSerGluAlaAlaGlnSerCysHisProLysProValSerSerAsp ProProValGlySerGluAlaProGlnSerCysHisProLysProValSerSerAsp $\label{eq:leuarg} LeuArgSerArgPheLeuAlaLeuArgAspGlnValProThrLeuAlaSerCysSerLys$ LeuArgSerArgPheLeuAlaLeuArgAspGlnValProThrLeuAlaSerCysSerLys $\label{eq:label} A la ProLysValValII e LeuSerLysAlaLeuGluTyrLeuGlnAlaLeuValGlyAlabel{eq:labeleuSerLysAlabeleuSer$ GluLysArgMetAlaThrGluLysArgGlnLeuArgCysArgGlnGlnGlnLeuGlnLys ArgIleAlaTyrLeuSerGlyTyrEnd 364 Human L-myc ArgIleAlaTyrLeuSerGlyTyrEnd 368 Mouse L-mvc

exon 3 in the mouse sequence, the overall homology between mouse and human L-myc proteins is 90% with half of the changes representing conservative amino acid substitutions (Fig. 4B). The predicted size of the L-myc protein is significantly smaller than that of the observed size of human c-myc (Battey et al. 1983; Bernard et al. 1983) or N-myc (Kohl et al. 1986; Stanton et al. 1986) proteins which measure 439 and 464 amino acids, respectively.

Several observations indicate that the proposed L-myc coding region is correct. Detailed comparisons reveal clusters of amino acids that are highly conserved among the three myc proteins. Dot matrix comparison of the human N-myc and c-myc proteins identifies seven clusters of highly conserved amino acids (Fig. 4A, top, first panel). The L-myc protein possesses five of these homology regions which are interspersed between divergent regions and which span the entire length of the proteins (Fig. 4A, top, second panel). One of these five common homology regions (region 3) is encoded by sequences that span the exon 2-exon 3 splice junctions of all three proteins (Fig. 4); the shorter size of the L-myc protein appears to result from the absence of approximately 80 (mostly nonconserved) amino acid residues found within exon 2 between homology regions 2 and 3 of c- and N-myc (Fig. 4). The latter alteration occurs in a region previously noted to be subject to structural variations between myc-related proteins (Ralston and Bishop 1983; Kohl et al. 1986). Hydropathy plots also demonstrate considerable relatedness among c-myc, N-myc, and L-myc. N-myc and c-myc plots are virtually superimposable (data not shown); and N-myc and L-myc have a highly related pattern (Fig. 4A, middle). However, the truncated length of L-myc exon results in closer positioning of homology regions 3 and 4 relative to regions 1 and 2. In this regard, a portion of L-myc homology region 3 appears to be missing a major internal stretch of acidic polyglutamic/aspartic acid residues (termed homology region 3'; Fig. 4A); of potential significance, such an acidic stretch is encoded by sequence further downstream within exon 3 of the L-myc gene. This potential transposition of coding sequences results in the conservation of the spatial relationship within the L-myc protein of this highly acidic amino acid cluster and L-myc

Figure 4. (A) (Top) Dot matrix computer analysis of protein sequence homology between human N-myc and human c-myc (left panel) and human N-myc and L-myc (right panel) was performed with a window of 8 and a stringency of 60%. Regions of homology (CN1, CN2, 1-5) are indicated. An arrow denotes the position of the exon 2-3 exon splice junction. (Middle) Hydrophobic (-) and hydrophilic (+) residues are plotted across the length of the peptide and assigned a hydropathy value, ranging from -3 to +3, as determined by the method of Hopp and Woods (1981). (Bottom) The amino acid sequence of homology regions between c-, N-, and L-myc proteins is shown. A solid vertical line indicates identity between residues; a double dot indicates a conservative amino acid substitution. (B) Dot matrix analysis of protein sequence homology between the mouse and human L-myc protein was performed using a window of 20 and stringency of 90%. Solid vertical lines indicate identity; a double dot indicates a conservative substitution.

homology regions 1 and 2, thereby maintaining the position of these residues along the L-*myc* peptide backbone relative to their position in the N- and c-*myc* proteins (Fig. 4A, top and middle).

In recent studies, in vitro mutagenesis analyses of the human c-myc gene uncovered a number of regions that appear essential for nuclear targeting, nuclear localization, and transformation activity (Stone et al. 1987). Comparison of these regions among the c-, N-, and L-myc proteins demonstrates areas of strong conservation (for summary, see Alt et al. 1987). Gene fusion studies have demonstrated that c-myc residues 351-381 are capable of directing the movement of cytoplasmic pyruvate kinase to the nucleus (Stone et al. 1987). There is 70% conservation within this region among all myc proteins. Other studies demonstrate that c-myc residues 106-143 and 371-412 appear necessary for retention within the nucleus, as opposed to targeting (Stone et al. 1987). Both of these domains are also highly conserved among all myc proteins. It is notable that within these coordinates there are residues that are identical in the three proteins (e.g., c-myc residues 132-140 are the same as L-myc residues 93-101 and N-myc residues 113 - 120].

L-myc can cooperate with ras to transform normal cells in culture

L-myc gene amplification occurs in a subset of human SCLC (Nau et al. 1985). By analogy to N-myc gene amplification in human neuroblastomas (Kohl et al. 1983; Schwab et al. 1983), the increased expression of L-myc as a result of gene amplification may confer an increased growth potential to malignant cells. The c-myc (Land et al. 1983) and N-myc (Schwab et al. 1985; Yancopoulos et al. 1985) genes possess similar transforming activities in the rat embryo fibroblast (REF) cotransformation assay. Therefore, to assay the oncogenic potential of the L-myc gene and the L-myc ψ gene, we tested their ability to cooperate with an activated Ha-ras gene to transform early-passage REFs in culture. The 10.6-kb EcoRI fragment containing the L-myc gene and the 8.8-kb EcoRI fragment containing the L-myc ψ gene were subcloned into a retroviral expression construct, pVcos7 (Table 1, bottom): the L-mvc fragment was inserted in both orientations relative to the transcriptional orientation of two Moloney murine leukemia virus long terminal repeats (LTRs). The L-myc ψ gene was subcloned only in the same orientation as the Moloney promoter. These constructs were cotransfected with and without the mutant Ha-ras gene into early-passage rat embryo fibroblasts.

L-myc constructs that contained the L-myc gene in the same transcriptional orientation as the flanking LTRs (pL-myc-S) were not able to transform REFs alone but did cooperate with a mutant Ha-ras gene. In the c-, N-, and L-myc/ras cotransfections, dense foci became noticeable 7–15 days after the transfection. Foci consisted of individual cells with clearly transformed morphology, rapidly overgrowing and displacing the normal monolayer. The frequency of focus formation in the

various L-myc/ras cotransformations was significantly greater than the focus formation observed in the pVcos7/ras or ras alone controls but was significantly less than the positive cotransfection controls, c-myc/ras and N-myc/ras (Table 1). The L-myc gene in the same transcriptional orientation as the LTRs cotransfected with ras exhibited the greatest efficiency of transformation (Table 1; pVL-myc/ras; p < 0.001). The L-myc gene in the opposite transcriptional orientation (pVL-myc-AS) and the L-myc gene without neighboring LTRs (pL-myc) also cooperate with ras to induce transformation at a significant efficiency above background controls (Table 1; pVL-myc-AS, $p \leq 0.01$; pL-myc, $p \leq 0.05$), but the efficiency was somewhat less than the pVL-myc-S/ras combination. L-myc also conferred upon the transformants a high frequency of anchorage-independent growth and tumorigenic potential in young syngeneic hosts (Table 1).

Analysis of DNA prepared from the transformed REF lines demonstrated the presence of multiple copies of the introduced ras and myc genes (data not shown). Total RNAs prepared from REF transformants and controls were screened for expression of the introduced oncogenes. All of the REF transformants expressed high levels of the introduced ras gene (Fig. 5, middle panel). L-myc expression could be detected only in permanent REF lines transformed with the various L-myc/ras combinations (Fig. 5, bottom panel). The range of L-myc expression varied in each of the L-myc/ras transformants but greatly exceeded the level of L-myc expression seen in normal developing tissues and approached the levels seen in L-myc-expressing SCLC. pL-myc/ras transformants expressed an approximately 10- to 20-fold lower amount of normal L-myc message than the pVL-myc-S/ ras cell lines (Fig. 5, bottom panel). L-myc expression levels in tumors derived by injecting transformed REF lines into syngeneic hosts were similar to those observed in the corresponding cultured cell lines (data not shown). Total cellular RNA derived from pVL-myc-AS/ ras transformants was also assayed with SP65-generated L-myc antisense and sense probes and found to express normal-sized L-myc message (data not shown).

Total RNAs derived from the L-myc/ras transformants contain L-myc-hybridizing sequences of 3.8, 2.2, and 1.8 kb; sequences of the same size are found in the total RNA of the H510 SCLC (Fig. 5, lower panel) and in human L-myc-expressing embryonic tissues (R. De-Pinho and F. Alt, unpubl.). L-myc/ras transformants also exhibited a 5.0-kb transcript that initiates from the viral promoter (Fig. 5, lower panel). The presence of normalsized L-myc message in each of the L-myc/ras transformants, including the antisense construct, indicates that L-myc promoter elements sufficient for expression in REF lines are contained in the 10.6-kb L-myc clone. ras alone transformants did not express L-myc (Fig. 5, panel C).

L-myc ψ is a processed L-myc pseudogene

The genomic organization of the L-myc ψ gene corre-

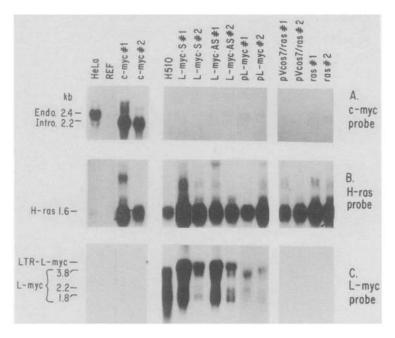
Table 1.	Transformation of	REFs with myc-far	mily and ras gene	combinations

		es with transform foci/total plates	ned	Growth in soft agar, lines positive/	Tumorigenicity, rats with tumors/ injected	
Transfected DNA	Exp. 1	Exp. 2	Exp. 3	lines tested		
pkomyc + T24-ras	9/12	9/9	9/9	2/2	2/2	
pV-Nmyc + T24-ras		12/16	8/12		_	
pV-Lmyc-S + T24-ras	6/11	16/36	17/36	3/4	9/9	
pV-Lmyc-AS + T24-ras	6/11	_	7/18	4/4	2/5	
pL-myc + T24-ras	3/11		4/15	2/2	4/4	
pL- <i>myc</i> ψ-S + T24- <i>ras</i>	0/12		1/12		_	
$pV\cos^{-7} + T24$ -ras		0/12	2/12	1/2	1/2	
pV-Lmyc-S	0/12	_	0/12	_	_	
T24-ras	0/12	0/9	2/12	0/2	0/2	
None		—	_	0/2	0/3	
	1000bp					
	18 		R I	pL-myc ψ - S		
	I 8		L L	pL-myc√-S pV-Lmyc-S		
	L R I	OH	и В	pV-Lmyc-AS		
		Pricos7 Vector				

The data for the first three columns are presented as the number of plates with positive foci compared to the total number of plates into which each particular transfection was finally divided; due to the way the cells were passaged after transfection, each plate in the final passage has independent transformants. At 2 weeks after transfection each positive plate had 1-5 foci, whereas at 4 weeks each positive plate had 5-20 foci; presumably some of these were due to metastases. Cell lines derived from c-myc/ras foci (two lines examined) or L-myc/ras foci (four lines examined) were assayed for both colony formation in soft agar and the ability to cause tumors in young syngeneic rats; each line assayed for tumorigenicity was injected in two rats. As a nontransfected control for these assays (bottom line) we used REFs propagated in culture. Both the colonies in soft agar and the tumors in the rats appeared and grew at approximately the same rate for either the L-myc/ras or c-myc/ras transformants. These tumors were noted in all cases within 1 week of cell injection, grew rapidly, and resulted in the death of the host animal within 2-3 weeks.

The L-myc pVcos7 expression constructs are shown below the table. L-myc and L-myc ψ inserts were subcloned between two directly repeated Moloney leukemia virus LTR sequences in the transcriptional orientation shown. pVL-myc-S and pVL-myc-AS constructs contained the L-myc gene in the same or opposite transcriptional orientation as the flanking LTRs, respectively. pL-myc construct contained the L-myc subcloned into the pUC18 plasmid vector and did not contain neighboring LTR sequences. pVL-myc ψ -S contained the L-myc ψ gene in the same transcriptional orientation as the flanking LTRs. pT24-ras construct contains the 6.2-kb BamHI insert harboring the activated (Val-12) human H-ras oncogene.

sponds to the structure of the L-myc cDNA (Figs. 1B and 2). The L-myc ψ gene contains initiation and termination codons in the same relative positions as the L-myc gene; however, the L-myc ψ gene contains numerous inphase termination codons due to insertions and deletions that result in frameshift mutations. Nucleotide sequence homology between L-myc transcribed regions and the corresponding L-myc ψ regions is approximately 90%. However, restriction map comparisons between the L-myc ψ and L-myc 3' untranslated regions reveal that the L-myc ψ gene contains an additional 850 bp within the 2.4-kb region not subjected to direct nucleotide sequence analysis (Fig. 1B, 2.4-kb region is indicated as a series of Ns) and suggests that the L-myc ψ gene has undergone extensive alterations in this region (Fig. 2, dashed portion of exon 3). A consensus polyadenylation signal is followed by a small poly(A) stretch starting at position 1991 which does not exist in the L-myc 3'flanking sequence. Immediately flanking the poly(A) tract, there exists a 9-bp sequence that is directly repeated precisely 5' to where the 5' homology between the L-myc and L-myc ψ ceases. The repeat sequences, AGAAATAAA and AGAAGTAAA, occur at positions 482 and 1996, respectively. Direct repeats are a sequence motif common to many processed pseudogenes and may be related to the mechanism(s) responsible for pseudogene reintegration in the genome (for review, see Vanin 1984). The L-myc ψ gene does not exist in the mouse genome (data not shown) and does not function in the cotransformation assay (see above). Thus, the L-myc ψ gene appears to possess all of the structural features of a processed pseudogene: It is a processed, nonfunctional Figure 5. Expression of introduced ras and myc genes in the transfected REF cell lines. Ten micrograms of total RNA, prepared from indicated lines, was used in each lane. REF refers to RNA isolated from normal rat embryo fibroblasts. H510 refers to RNA isolated from a human SCLC. All of the L-myc/ras transformants listed in Table 1 expressed the introduced L-myc construct (not shown); the highest and lowest L-myc-expressing transformants are shown. Duplicate filters were probed with a ³²P-labeled 500-bp PstI fragment containing the human c-myc exon 2 (A), a 1.6-kb HindIII fragment containing the Ha-ras gene (B), or a 450bp Nael fragment containing human L-myc exon 2. The introduced c-myc gene was driven from the simian virus 40 promoter linked upstream to the second exon, therefore c-myc RNA expressed from the construct is smaller than the normal endogenous c-myc mRNA.



gene containing a small poly(A) stretch that is flanked by directly repeated sequences. Our preliminary mapping data indicate that L-myc ψ is located on human chromosome X (R. DePinho et al., in prep.); previous studies have assigned the L-myc gene to human chromosome 1 (Nau et al. 1985).

Discussion

L-myc is the third member of the myc oncogene family

Our results clearly demonstrate that the human L-myc gene represents an additional functional member of the myc-family of cellular oncogenes. This conclusion is based on the observation that the L-mvc gene and gene product share oncogenic activities and structural features with the other well-characterized myc-family members. The common and divergent structural elements described in this report could provide direction in the preliminary dissection of potential functional domains in the L-myc and other myc proteins. For example, c-myc encodes a nuclear protein that has DNAbinding capacity in vitro (Donner et al. 1982; Persson and Leder 1984; Watt et al. 1985), a property attributed to the abundance of basic amino acids at its carboxyl terminus (Persson and Leder 1984). This structural feature is also present in the putative L-myc gene product; thus, the L-myc protein may also be a nuclear protein with capacity to bind nucleic acids. In general, we have also found that most c-myc protein domains necessary for cotransformation activity, nuclear targeting, and retention in the nucleus are well conserved in the L-mvc protein. Moreover, extremely well-conserved residues that we have identified within these potential functional domains may further delineate critical residues involved in these properties. However, not all of the c-myc domains are conserved in L-myc; one region that appears important for nuclear targeting in c-myc is not conserved in the putative L-myc gene product. Despite the striking degree of conservation of various sequences and aspects of function (e.g., activity in the REF cotransformation assay), the conservation of the c-, N-, and L-myc genes throughout vertebrate evolution as distinct sequences suggests unique physiological activities (see below for further discussion of this point). In this context, the potential roles of the extremely divergent regions that appear at common positions within the various myc proteins or regions conserved only between N- and c-myc need to be assessed.

The mouse and human L-myc untranslated regions are well conserved. This remarkable degree of sequence conservation in regions that are not under any selective pressure at the protein level is consistent with a role in the regulation of gene expression. In contrast to the remarkable evolutionary conservation within a given myc-family gene, untranslated sequences among the different myc family members are totally divergent from each other. The different myc-family genes have very distinct expression patterns (Alt et al. 1986; Zimmerman et al. 1986); in this context, it is conceivable that evolutionarily conserved flanking sequences unique to each family member somehow could be involved in mediating differential expression.

L-myc transforming activity

A large body of evidence has suggested that progression to malignancy is a multistep process (for review, see Weinberg 1985). Thus, the c-myc gene has been linked with immortalization activity (Mougneau et al. 1984; Ruley et al. 1984) while the action of Ha-ras has been correlated with transformation activity (Land et al. 1983; Ruley et al. 1983). Additional studies, however, indicate that c-myc expression constructs can induce

transformation in immortalized cell lines (Keath et al. 1984; Kelekar and Cole 1986) and that Ha-ras can immortalize primary cells (Spandidos and Wilkie 1984). Currently, it is not clear how the activities of these genes result in the observed spectrum of deregulated growth. We have demonstrated that the L-myc gene can cooperate with a mutant Ha-ras gene to malignantly transform rat embryo fibroblasts. Although we could not detect a difference in the malignant phenotype between the L-myc/ras transformants and the N- or c-myc/ras transformants, the L-myc/ras transformants did arise at a lower frequency. If the three myc genes encode proteins with identical transforming activities, the different efficiencies in the REF transformation assay might result from constructs with different capacities to produce sufficient levels of the respective myc proteins. However, in this regard, it is surprising that the L-myc gene was able to cooperate with ras in the absence of a retroviral enhancer because the N-myc gene seemed to require the presence of associated transcriptional enhancer elements. Alternatively, it is possible that the L-mvc gene product may not possess a transforming activity equivalent to that of the c- or N-myc proteins and/or may require additional genetic events to achieve the malignant phenotype; both these possibilities could result in diminished activity of L-myc in this assay.

We have demonstrated that the transforming activity of L-myc is not limited to the very restricted set of tumors in which the gene has been found to be amplified and overexpressed (to date, only in a subset of SCLC). The apparently limited involvement of L-myc in the genesis of naturally occurring tumors is also observed for N-myc (clearly implicated only in neuroblastomas and some retinoblastomas and SCLC), but contrasts with that of c-myc, which appears to participate in the neoplastic transformation of a much wider variety of cells (for review, see Alt et al. 1986). We have recently demonstrated that the restricted set of tumors in which the N- and L-myc genes are amplified and/or overexpressed correlates in part with the tissue- and stage-specific expression patterns of these genes during normal development (Nisen et al. 1986; Zimmerman et al. 1986). Given our current observation that L-myc, like N-myc, can participate in the in vitro transformation of other cell types (fibroblasts), which do not even express detectable levels of either gene, it seems possible that deregulated expression of these genes contributory to the development of naturally occurring tumors may occur preferentially in cell types that actively express the genes. Another, not mutually exclusive, possibility for the restricted contribution of N- and L-myc to natural tumors is that the activity of the different myc gene products may be effected in a tissue-specific fashion. It is notable that, to date, the only observed mechanism for activated expression of the N- and L-myc genes in naturally occurring tumors is by gene amplification (for review, see Alt et al. 1986) and that expression levels of Nor L-myc genes in REF transformants often approach those of some amplified tumors (e.g., Fig. 5). To achieve high-level amplification, a gene usually must give some selective growth advantage at low amplification (expression) levels, with the selective advantage increasing at higher levels (Schimke 1984; Alt et al. 1986). One possible rationalization for the apparently disparate transforming activities of the L- and N-myc genes in vivo as opposed to in vitro could be that the relatively high-level expression observed in nearly all in vitro transformants leads to a more degenerate transforming activity than would occur with small increases that initially occur in an amplification process (in vivo). In this regard, a threshold level of N-myc expression is necessary for regulation of MHC Class I gene expression (Bernards et al. 1986).

Methods

Genomic and cDNA cloning

Genomic DNA from human placenta was digested to completion with *Eco*RI and cloned into the *Eco*RI site of λ phage Charon 30 essentially as described previously (DePinho et al. 1985). This library was screened for clones that hybridized to exons 2 (400-bp *XhoI-Bam*HI fragment) and 3 (1100-bp *Eco*RI*-HincII fragment) under low stringency conditions as described by Legouy et al. 1987.

Primer-extension and oligo(dT)-primed cDNA libraries were prepared from cytoplasmic poly(A)⁺ RNA isolated from the H510 SCLC cell line (graciously provided by John Minna) and 1C.1, an L-myc-expressing REF transformant cell line (see text), as described previously (Kohl et al. 1986; Legouy et al. 1987). This library was screened for clones which hybridized to exons 1 and 2 L-myc-specific probes; probes are illustrated and described in Figure 2. Several clones were isolated and subcloned into the pUC18 plasmid vector for further analysis.

DNA sequencing

The nucleotide sequence of indicated regions of the cDNA and genomic clones was determined by the partial chemical degradation method of Maxam and Gilbert (1977).

Analysis of RNA and DNA

DNA and RNA preparation, restriction endonuclease digestions, probe preparation by the nick-translation method, blotting procedures, and hybridization conditions were performed as described previously (DePinho et al. 1985). Reduced-stringency hybridizations were performed in 35% formamide, $5 \times$ SSCPE, 10% dextran sulfate, $1 \times$ Denhardt's solution, and 100 µg/ml salmon sperm DNA; washing was conducted at 56°C in $2 \times$ SSC and 0.1% SDS; all other conditions follow standard procedures as described previously (DePinho et al. 1985).

Preparation and transfection of REFs

Early-passage cultures of REFs were prepared as described elsewhere (Yancopoulos et al. 1985) from 12- to 14-day Fischer rat embryos. REFs were initially plated at near-confluent density and allowed to grow to confluence. To prepare for transfection, REFs were then replated after 1–2 days at 1.5×10^6 cells per 10-cm plate in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. DNA was introduced into REFs by calcium phosphate precipitation as described previously (Andersson et al. 1979) using 30 µg of human placental carrier DNA with 2 µg of each of the appropriate plasmid DNAs per plate. The cultures were refed 6–9 hr after transfection and split 18 hr after the transfection into either three (ex-

periments 1 and 2) or four (experiment 3) plates. Assuming that limited cell division occurs in the 24 hr after the transfection, each plate after this final passage contains independent transformants. This was verified by DNA analysis of isolated transformants (data not shown). The various plasmids used in the REF transfections are as follows: pT24-*ras* contains the mutant Ha-*ras* oncogene with (experiments 2 and 3) and without (experiment 1) a neomycin resistance gene (Fasano et al. 1983; provided by M. Goldfarb); the pKO-*myc* plasmid, kindly provided by Daniel Birnbaum, is a c-*myc* expression construct in which transcription of the second and third exons of c-*myc* is driven from a simian virus 40 promoter; the L-*myc* constructs are described below and illustrated in Table 1, bottom.

Assay for colony formation in soft agar

A single-cell suspension of approximately 1×10^4 cells in 5 ml of 0.28% agar was overlaid onto a 60-mm culture dish containing a 0.7% agar base. All agar suspensions were made in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. Duplicate plates were prepared for each tested line. Normal REFs propagated in culture served as a non-transformed control for these studies.

Assay for tumorigenicity

Cell lines propagated from transformed foci (see text) were assayed for tumorigenicity by subcutaneously injecting 1×10^6 to 5×10^6 cells in 0.4 ml of PBS into 5- to 10-day-old syngeneic Fischer rats. Each line tested was injected into at least two rats. A nontransfected control was provided by propagating normal REFs in culture until sufficient numbers of cells were attained; four rats each injected with 2×10^7 normal REFs served as controls. The rats were followed until death, or for 4 weeks in the case of the normal REF injections.

Computer analysis of DNA and protein sequence

Computer analyses of the L-myc sequence were performed on an IBM-AT personal computer using the Microgenie Sequence Analysis Program (SciSoft, Inc.).

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References

Abrams, H.D., L.R. Rohrschneider, R.N. Eisenman. 1982. Nuclear location of the putative transforming protein of avian myelocytomatosis virus. *Cell* **29**: 427–439.

- Adams, J., A. Harris, C. Pinkert, L. Corcoran, W. Alexander, S. Cory, R. Palmiter, and R. Brinster. 1985. The c-myc oncogene driven by Ig enhancers induces lymphoid malignancy in transgenic mice. Nature 318: 533-538.
- Alitalo, K., G. Ramsay, J.M. Bishop, S. Pfeifer-Ohlsson, W.W. Colby, and A.D. Levinson. 1983. Identification of nuclear proteins encoded by viral and cellular myc oncogenes. Nature 306: 274-277.
- Alt, F.W., R.A. DePinho, K. Zimmerman, E. Legouy, K. Hatton, P. Ferrier, A. Tesfaye, G.D. Yancopoulos, and P. Nisen. 1986. The human myc-gene family. Cold Spring Harbor Symp. Quant. Biol. 51: 931-941.
- Alt, F., E. Harlow, and E. Ziff. 1987. Nuclear oncogenes. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (In press).
- Andersson, P., M.P. Goldfarb, and R.A. Weinberg. 1979. A defined subgenic fragment of in vitro synthesized Moloney sarcoma virus DNA can induce cell transformation upon transfection. *Cell* 16: 63-75.
- Battey, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder. 1983. The human c-myc oncogene: Structural consequences of translocation into the IgH locus in Burkitt lymphoma. Cell 34: 779-787.
- Bernard, O., S. Cory, S. Gerondakis, E. Webb, and J.M. Adams. 1983. Sequence of the murine and human cellular myc oncogenes and two modes of myc transcription resulting from chromosome translocation in B lymphoid tumours. *EMBO J.* 2: 2375-2383.
- Bernards, R., S.K. Dessain, and R.A. Weinberg. 1986. N-myc amplification causes down-modulation of MHC Class I antigen expression in neuroblastoma. *Cell* **47**: 667-674.
- Coppola, J.A. and M.C. Cole. 1986. Constitutive c-myc oncogene expression blocks MEL cell differentiation but not commitment. Nature 320: 760-763.
- DePinho, R.A., K. Kruger, N. Andrews, S. Lutzker, D. Baltimore, and F.W. Alt. 1985. Molecular basis of heavy-chain class switching and switch region deletion in an Abelson virus-transformed cell line. *Mol. Cell. Biol.* 4: 2905-2910.
- DePinho, R.A., E. Legouy, L.B. Feldman, N.E. Kohl, G.D. Yancopoulos, and F.W. Alt. 1986. Structure and expression of the murine N-myc gene. Proc. Natl. Acad. Sci. 83: 1827– 1831.
- DePinho, R.A., L. Mitsock, K. Hatton, P. Ferrier, K. Zimmerman, E. Legouy, A. Tesfaye, R. Collum, G. Yancopoulos, P. Nisen, R. Kriz, and F. Alt. 1987. myc family of cellular oncogenes. J. Cell. Biochem. 33: 257-266.
- Dmitrovsky, E., W.M. Kuehl, G.F. Hollis, I.R. Kirsh, T.P. Bender, and S. Segal. 1986. Expression of a transfected human c-myc oncogene inhibits differentiation of a mouse erythroleukemia cell line. Nature 322: 748-750.
- Donner, P., I. Greiser-Wilke, and K. Moelling. 1982. Nuclear localization and DNA binding of the transforming gene product of avian myelocytomatosis virus. *Nature* 296: 262– 266.
- Fasano, O., E. Taparowsky, J. Fiddes, M. Wigler, and M. Goldfarb. 1983. Sequence and structure of the coding region of the human H-ras-1 gene from T24 bladder carcinoma cells. J. Mol. Appl. Genet. 2: 173-180.
- Hann, S.R., H.D. Abrams, L.R. Rorshneider, and R.N. Eisenman. 1983. Proteins encoded by v-myc and c-myc oncogenes: Identification and localization in acute leukemia virus transformants and bursal lymphoma cell lines. Cell 34: 789-798.
- Hann, S.R. and R.N. Eisenman. 1984. Proteins encoded by the human c-myc oncogene: Differential expression in neoplastic cells. Mol. Cell. Biol. 4: 2486-2497.

- Hopp, T.P. and K.R. Woods. 1981. Prediction of protein antigenic determinants from amino acid sequences. *Proc. Natl. Acad. Sci.* 78: 3824–3828.
- Ikegaki, N., J. Bukovsky, and R.H. Kennett. 1986. Identification and characterization of N-myc gene product in human neuroblastoma cells by monoclonal antibodies with defined specificities. Proc. Natl. Acad. Sci. 83: 5929-5933.
- Jakobovits, A., M. Schwab, J.M. Bishop, and G.R. Martin. 1985. Expression of N-myc in teratocarcinoma stem cells and mouse embryos. Nature 318: 188-191.
- Keath, E.J., P.G. Caimi, and M.D. Cole. 1984. Fibroblast lines expressing activated c-myc oncogenes are tumorigenic in nude mice and syngeneic animals. *Cell* **39**: 339–348.
- Kelekar, A. and M.D. Cole. 1986. Tumorigenicity of fibroblast lines expressing the adenovirus E1A, cellular p53, and normal c-myc genes. *Mol. Cell. Biol.* 6: 7–14.
- Kelly, K. and U. Siebenlist. 1986. The regulation and expression of c-myc in normal and malignant cells. Annu. Rev. Immunol. 4: 327-338.
- King, M.W., J.M. Roberts, and R.N. Eisenman. 1986. Expression of the c-myc proto-oncogene during development of Xenopus laevis. Mol. Cell. Biol. 6: 4499-4508.
- Kohl, N.E., N. Kanda, R.R. Schreck, G. Bruns, S.A. Latt, F. Gilbert, and F.W. Alt. 1983. Transposition and amplification of oncogene-related sequences in human neuroblastomas. *Cell* 35: 359-367.
- Kohl, N.E., E. Leguoy, R.A. DePinho, R. Smith, C. Gee, and F.W. Alt. 1986. Human N-myc is closely related in organization and nucleotide sequence to c-myc. Nature 319: 73-77.
- Lachman, H.M., G. Cheng, and A.I. Skoultchi. 1986. Transfection of mouse erythroleukemia cells with *myc* sequences changes the rate of induced commitment to differentiation. *Proc. Natl. Acad. Sci.* 83: 6480–6484.
- Land, H., L.F. Parada, and R.A. Weinberg. 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* **304**: 596–601.
- Legouy, E., R.A. DePinho, K. Zimmerman, R. Collum, G.D. Yancopoulos, L. Mitsock, R. Kriz, and F.W. Alt. 1987. Structure and expression of the murine L-myc gene. EMBO J., (In press).
- Minna, J.D., J.F. Battey, B.J. Brooks, F. Cuttitta, A.F. Gazdar, B.E. Johnson, D.C. Ihde, A.M. Lebacq-Verheyden, J. Mulshine, M.M. Nau, H.K. Oie, E.A. Sausville, E. Seifter, and M. Vinocour. 1986. Molecular genetic analysis reveals chromosome deletion, gene amplification, and autocrine growth factor production in the pathogenesis of human lung cancer. Cold Spring Harbor Symp. Quant. Biol. 51: 843–853.
- Mougneau, E., L. Lemieux, M. Rassoulzadegan, and F. Cuzin. 1984. Biological activities of v-myc and rearranged c-myc oncogenes in rat fibroblast cells in culture. *Proc. Natl. Acad. Sci.* 81: 5758-5762.
- Mount, S.M. 1982. A catalog of splice junction sequences. Nucleic Acids Res. 10: 459-472.
- Nau, M., B. Brooks, J. Battey, E. Sausville, A. Gasdar, I. Kirsh, O. McBride, V. Bertness, G. Hollis, and J. Minna. 1985. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. Nature 318: 69-73.
- Nisen, P.D., K.A. Zimmerman, S.V. Cotter, F. Gilbert, and F.W. Alt. 1986. Enhanced expression of the N-myc gene in Wilms tumors. *Cancer Res.* **46**: 6217–6222.
- Persson, H. and P. Leder. 1984. Nuclear localization and DNA binding properties of a protein expressed by human c-myc oncogene. Science 225: 718-721.
- Persson, H., H.E. Gray, F. Godeau, S. Braunhut, and A.R. Bellve. 1986. Multiple growth associated nuclear proteins immuno-

precipitated by antisera raised against human c-myc peptide antigens. Mol. Cell. Biol. 6: 942-949.

- Proudfoot, N.J. and G.G. Brownlee. 1976. 3' non-coding region sequences in eukaryotic messenger RNA. Nature 263: 211-214.
- Ralston, R. and J.M. Bishop. 1983. The protein products of the *myc* and *Myb* oncogenes and adenovirus E1A are structurally related. *Nature* **306**: 803-806.
- Ramsay, G., G.I. Evan, and J.M. Bishop. 1984. The protein encoded by the human proto-oncogene c-myc. Proc. Natl. Acad. Sci. 81: 7742-7746.
- Ruley, H.E. 1983. Adenovirus early region 1A enables viral cellular transforming genes to transform primary cells in culture. *Nature* **304**: 602-606.
- Ruley, H.E., J.F. Moomaw, and K. Maruyama. 1984. Avian myelocytomatosis virus myc and adenovirus early region 1A promote the *in vitro* establishment of cultured primary cells. *Cancer cells* 2: 481–486. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Schimke, R.T. 1984. Gene amplification in cultured animal cells. Cell 37: 705-713.
- Schwab, M., K. Alitalo, L. Klempnauer, H. Varmus, J. Bishop, F. Gilbert, G. Brodeur, M. Goldstein, and J. Trent. 1983. Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumor. *Nature* 305: 245-248.
- Schwab, M., H.E. Varmus, and J.M. Bishop. 1985. The human N-myc gene contributes to tumorigenic conversion of mammalian cells in culture. Nature 316: 160-162.
- Sheiness, D. and J.M. Bishop. 1979. DNA and RNA from uninfected vertebrate cells contain nucleotide sequences related to the putative transforming gene of avian myelocytomatosis virus. J. Virol. 31: 514-521.
- Slamon, D.J., T.C. Boone, R.C. Seeger, D.E. Keith, V. Chazin, H.C. Lee, and L.M. Souza. 1986. Identification and characterization of the protein encoded by the human N-myc oncogene. Science 232: 768-772.
- Spandidos, D.A. and I.M. Wilkie. 1984. Malignant transformation of early passage rodent cells by a single mutant human oncogene. *Nature* 310: 469–475.
- Spector, D.L., R.A. Watt, and N.F. Sullivan. 1987. The v- and c-*myc* proteins colocalize *in situ* with small nuclear ribonucleoprotein particles. Oncogene 1: 5-12.
- Stanton, L., M. Schwab, and J.M. Bishop. 1986. Nucleotide sequence of the human N-myc gene. Proc. Natl. Acad. Sci. 83: 1772-1776.
- Stone, J., T. DeLange, G. Ramsay, E. Jakobovits, J.M. Bishop, H.E. Varmus, and W. Lee. 1987. Definition of regions in human c-myc involved in transformation and nuclear localization. Mol. Cell. Biol. 7: 1697-1709.
- Van Beneden, R.J., D.K. Watson, T.T. Chen, J.A. Lautenberger, and T.S. Papas. 1986. Cellular myc (c-myc) in fish (rainbow trout): Its relationship to other vertebrate myc genes and to the transforming genes of the MC29 family of viruses. Proc. Natl. Acad. Sci. 83: 3698-3702.
- Vanin, E.F. 1984. Processed pseudogenes: Characteristics and evolution. Biochim. Biophys. Acta 782: 231-241.
- Watt, R.A., A.R. Shatzman, and M. Rosenberg. 1985. Expression and characterization of the human c-myc DNA-binding protein. Mol. Cell. Biol. 5: 448-456.
- Weinberg, R.A. 1985. The action of oncogenes in the cytoplasm and nucleus. *Science* 230: 770-776.
- Yancopoulos, G.D., P.D. Nisen, A. Tesfaye, N.E. Kohl, M.P. Goldfarb, and F.W. Alt. 1985. N-myc can cooperate with ras to transform normal cells in culture. Proc. Natl. Acad. Sci. 82: 5455-5459.

Zimmerman, K.A., G.D. Yancopoulos, R.G. Collum, R.K. Smith, N.E. Kohl, K.A. Denis, M.M. Nau, O.N. Witte, D. Toran-Allerand, C.E. Gee, J.D. Minna, and F.W. Alt. 1986. Differential expression of myc family genes during murine development. Nature 319: 780-783.



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