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# Activation of the c-*myc* gene by translocation: A model for translational control

(cellular oncogene/non-Hodgkin lymphoma/multiple promoters/DNA sequence)

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**ABSTRACT** **We have shown that the human cellular oncogene c-*myc* is composed of three exons and is transcribed from two initiation sites separated by 175-base-pair DNA in HeLa cells. For both resulting mRNA species, exon 1 composes the 5′ untranslated region and the initiator methionine is located 16 base pairs downstream from the 5′ splice acceptor of exon 2. In a non-Hodgkin lymphoma, Manca, harboring a t(8;14) translocation, c-*myc* gene is broken within intron 1, and its exons 2 and 3 are translocated to a site between the heavy chain joining region cluster and $C_\mu$-coding DNA segment of the immunoglobulin heavy chain locus. The translocated c-*myc* gene is transcribed from points within intron 1 but is apparently still translated from the same methionine codon as the mRNA from the unrearranged c-*myc* gene. The nucleotide sequence of the c-*myc* gene shows that a region of exon 1 is highly complementary to a region of exon 2. Thus the mRNA from the untranslocated c-*myc* gene, as opposed to that of the translocated c-*myc* gene, could form a stable stem–loop structure ($\Delta G^0 = -90$ kcal/mol; 1 cal = 4.184 J) where the initiator AUG would be located within the loop. In view of the bind-and-scan model for the initiation of eukaryotic translation, we propose that such a secondary structure will severely hinder the translation. We further propose that the c-*myc* gene is often activated by translocation through the escape from such a translational suppression.**

Avian myelocytomatosis virus, MC29, causes a wide range of tumors in chickens and transforms chicken fibroblasts and macrophages *in vitro*. The viral oncogene apparently responsible is v-*myc*. The v-myc protein is a fusion product of part of the major viral structural protein (gag) sequences and sequences transduced from the chicken genome (1, 2). The transduced sequences are derived from a cellular gene, c-*myc* (3–5).

Activation of chicken c-*myc* has been suggested as the means by which the nonacute avian leukosis virus induces neoplastic disease. By integrating close to the c-*myc* gene, the avian leukosis provirus can lead to enhanced levels of c-*myc* transcription (6). In addition, in a human cell line (HL60) derived from an acute promyelocytic leukemia, the human c-*myc* gene is amplified and there is a concomitant amplification of c-*myc* mRNA (7, 8). Amplification of the c-*myc* gene has also been observed in a human colon carcinoma cell line (9).

Nonrandom chromosomal translocations have been observed in a wide variety of vertebrate neoplasms (10–12). These observations, together with the demonstration of c-*myc* activation by avian leukosis virus integration, have led to the suggestion that c-*onc* genes might be activated by specific translocation events (6, 11, 12). In support of this idea, recent studies have shown that c-*myc* is translocated in certain lymphoid neoplasms of both mice and humans. In particular, murine c-*myc* on chromosome 15 is recombined into the heavy chain locus of mouse immunoglobulin genes (on chromosome 12) in BALB/c plasmacytomas characterized by t(12;15) translocations (13–17). The human c-*myc* gene has been mapped to a site on chromosome 8 (q24) (16, 17) that corresponds to the break point associated with translocations in Burkitt and other non-Hodgkin lymphomas. In a majority of Burkitt lymphomas characterized by t(8;14) (q24;q32) translocations, the c-*myc* gene on chromosome 8 is recombined into the immunoglobulin heavy chain locus on chromosome 14 (16, 18, 19). In other Burkitt lymphomas the translocations t(8;22) (q24;q11) and t(2;8) (p12;q24) may recombine the c-*myc* gene close to the λ and κ immunoglobulin light chain loci, respectively. Do these translocations directly activate the oncogenic potential of the c-*myc* gene? If so, what is the mechanism for the activation?

In this communication the structure and expression of a normal and a translocated human c-*myc* gene are compared. This comparison led to the hypothesis that the expression of c-*myc* gene product is ordinarily suppressed at the level of translation and that this suppression is removed as a result of c-*myc* translocation.

## MATERIALS AND METHODS

The Manca cell line (SK-DHL2A) was derived from a patient with non-Hodgkin lymphoma. The line is pseudodiploid, carrying a single copy of the characteristic t(8;14) translocation (20). Cells were kindly provided by Bayard Clarkson (Memorial Sloan–Kettering Cancer Center). The isolation of cosmid clones cU2.3 and cAH1, which contain the translocated and untranslocated versions of the human c-*myc* gene, respectively, will be discussed elsewhere. Nucleotide sequences were determined according to the method of Maxam and Gilbert (21). Sequence homologies were analyzed by the computer program SEQ (22). Glyoxal RNA gels, blotting of RNA to nitrocellulose filters, hybridization, and removal of the probes from the nitrocellulose filters were done according to Thomas (23). The nuclease S1 protection experiments were done by a slightly modified method of Berk and Sharp (24, 25). A 348-base-pair (bp) *Hga* I/*Sma* I fragment and a 171-bp *Taq* I/*Sma* I fragment labeled at the 5′ ends by T4 polynucleotide kinase were used as probes after strand separations (see Fig. 1).

## RESULTS

**Human c-*myc* Gene Consists of Three Exons.** A two-exon structure for both the chicken (3–5) and human (26, 27) c-*myc* genes has been deduced by comparing restriction maps and nucleotide sequences with those of the v-*myc* gene of MC29 vi-

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Abbreviations: bp, base pair(s); kb, kilobase(s).

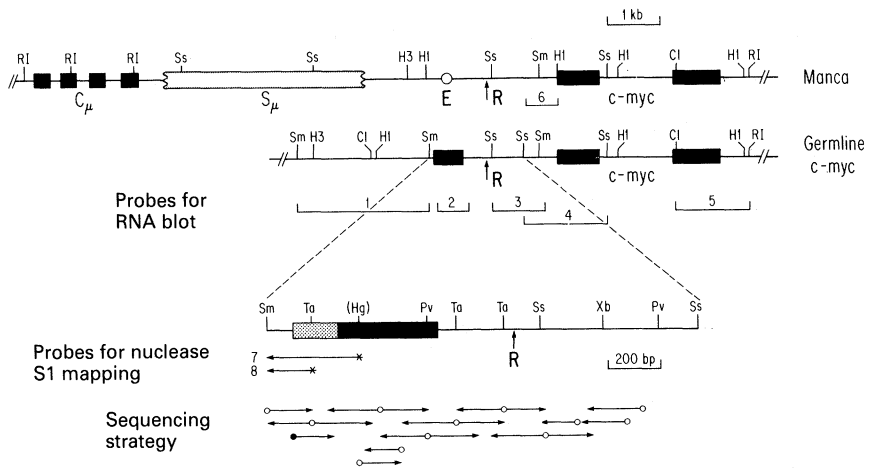


FIG. 1. Restriction maps of the germ-line c-*myc* gene and the rearranged c-*myc* gene in a non-Hodgkin lymphoma, Manca. Solid boxes are exons and the hatched box is the switch region ($S_\mu$) of immunoglobulin $\mu$ heavy chain. The region around the first exon of the c-*myc* gene is enlarged in the lower panel. The dotted area in exon 1 indicates the sequence expressed only in the longer mRNA. Horizontal arrows in "Sequencing strategy" show the direction and extent of sequencing. Circles at the origins of these arrows indicate 5′- (○) and 3′- (●) end labeling. E, transcription enhancer element; R, rearrangement point in Manca. Restriction sites: Bg, *Bgl* II; Cl, *Cla* I; H1, *Hpa* I; H3, *Hin*dIII; Hg, *Hga* I; Pv, *Pvu* II; RI, *Eco*RI; Sm, *Sma* I; Ss, *Sst* I; Ta, *Taq* I; Xb, *Xba* I. Not all *Hga* I sites are shown. Probes used for RNA blot hybridizations (Fig. 2) are as follows: 1, 2.5-kb *Sma* I/*Sma* I; 2, 550-bp *Taq* I/*Taq* I; 3, 1.1-kb *Hgi*AI/*Hgi*AI; 4, 2.2-kb *Sst* I/*Sst* I; 5, 1.4-kb *Cla* I/*Eco*RI; 6, 600-bp *Rsa* I/*Hpa* I. Probes used for nuclease S1 mapping (Fig. 4) are as follows: 7, 348-base [5′-$^{32}$P]*Hga* I/*Sma* I; 8, 171-base [5′-$^{32}$P]*Taq* I/*Sma* I.

rus. However, more recent sequence analysis of a human c-*myc* cDNA clone (28) suggests the presence of a third exon upstream of these two exons. To establish the exon–intron structure of the human c-*myc* gene, a series of DNA fragments (Fig. 1, probes 1–5) was dissected from a genomic c-*myc* gene clone cAH1 (unpublished results) and used as hybridization probes for RNA blotting analysis of the c-*myc* mRNA from HeLa cells (Fig. 2). Probes 4 and 5, which contain the previously deduced 5′ and 3′ exons of the c-*myc* gene, respectively, both hybridized to a mRNA of about 2.3 kilobases (kb). In addition, probe 2, which contains a 550-bp sequence located 1.6 kb upstream of the 5′ exon, hybridized to RNA of the same apparent size. Because probe 3 (a 1.1-kb fragment occurring between the probe 2 and the previously defined 5′ exon) did not hybridize to the 2.3-kb RNA, the RNA sequence detected by probe 2 must have been transcribed from an additional exon—probably the one suggested by Watt *et al.* (28).

No additional exon was found in the 2.5-kb region immediately upstream of the area covered by probe 2. These results suggest that the human c-*myc* gene is composed of three exons. Hereafter, these exons will be called exons 1, 2, and 3 from 5′ to 3′.

To confirm that the RNA sequence detected by probe 2 indeed represents a single exon, we determined the DNA sequence of the region covered by this probe and its immediate flanking region, using the strategy indicated in Fig. 1. The sequence is shown in Fig. 3 together with part of the exon 2 sequence previously determined (27). Comparison of the genomic sequence with the cDNA sequence (28) allows the 3′ boundary of exon 1 to be assigned to nucleotide 657. In the region upstream of this splice site the entire 5′ portion of the cDNA sequence of Watt *et al.* (28) is accounted for by a continuous stretch of the genomic DNA sequence presented here, except for 3 single bp insertions or deletions. This indicates that no additional introns split the c-*myc* gene, at least not in the region covered by the cDNA clone. The reasons for the discrepancies between the genomic DNA and cDNA sequences are unknown, but at least some may be attributed to human polymorphism.

**Two Major Transcription Initiation Sites in the c-*myc* Gene.** To accurately localize the 5′ end of exon 1 on the genomic DNA sequence we carried out nuclease S1 protection experiments using probes composed of the two genomic DNA fragments thought to span the 5′ boundary of exon 1 (Fig. 1, probes 7 and 8). The results (Fig. 4) indicate that two alternative sites define the 5′ boundaries of exon 1, one at nucleotide position 104 and the other at position 279 (Fig. 3).

Upstream of each of these sites are "TATA" sequences, characteristic of many eukaryotic promoters (29). No sequence characteristic of splice acceptor sites (30) precedes either of the two boundaries (note that the conserved A-G dinucleotide alone is not a sufficient condition). The sizes of the c-*myc* mRNAs [2,200 bp and 2,030 bp plus poly(A)] predicted by summing the sizes of the three exons match well with the sizes of the mRNA detected by RNA blotting (2.3 kb). In fact, close inspection of the RNA blot data (Fig. 2) indicates that the 2.3-kb band is a doublet. We thus conclude that the c-*myc* gene is transcribed from at least two start sites in HeLa cells. The sequences covered by the two probes used in the nuclease S1 protection experiment overlap with the 5′ end of the cDNA sequence (28).

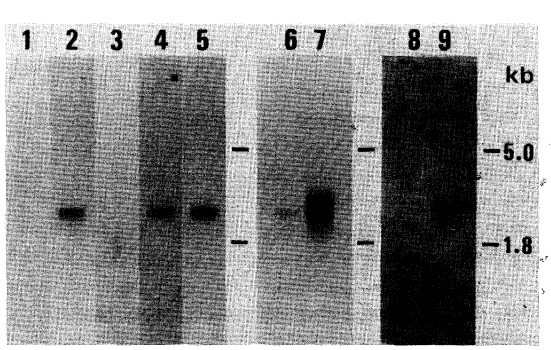


FIG. 2. Lanes 1–5: RNA blot hybridization showing the locations of c-*myc* exons and introns. Three micrograms of HeLa poly(A)$^+$ RNA was transferred to a nitrocellulose filter after electrophoresis through an agarose gel. The same filter was hybridized to probes 1–5 (shown in Fig. 1), one probe at a time and after completely washing off the previous probe. Size markers are 28S and 18S mouse rRNA. Lanes 6–9: RNA blot hybridization of HeLa (lanes 6 and 8) and Manca (lanes 7 and 9) poly(A)$^+$ RNA (1 $\mu$g) to the exon 3 probe (probe 5 in Fig. 1) (lanes 6 and 7) or the intron 1 probe (probe 6 in Fig. 1) (lanes 8 and 9).

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SmaI                                                                    TATA box                100
CCCGGGTTCC CAAAGCAGAG GGCGTGGGGG AAAAGAAAAA AGATCCTCTC TCGCTAATCT CCGCCCACCG GCCCTTTATA ATGCGAGGGT CTGGACGGCT

 CAP site 1                                                                    TaqI              200
GAGGACCCCC GAGCTGTGCT GCTCGCGGCC GCCACCGCCG GGCCCCGGCC GTCCCTGGCT CCCCTCCTGC CTCGAGAAGG GCAGGGCTTC TCAGAGGCTT

                                        TATA box                                CAP site 2      300
GGCGGGAAAA AGAACGGAGG GAGGGATCGC GCTGAGTATA AAAGCCGGTT TTCGGGGCTT TATCTAACTC GCTGTAGTAA TTCCAGCGAG AGGCAGAGGG

                                                                                                400
AGCGAGCGGG CGGCCGGCTA GGGTGGAAGA GCCGGGCGAG CAGAGCTGCG CTGCGGGCGT CCTGGGAAGG GAGATCCGGA GCGAATAGGG GGCTTCGCCT

                                                                                                500
CTGGCCCAGC CCTCCCGCTG ATCCCCCAGC CAGCGGTCCG CAACCCTTGC CGCATCCACG AAACTTTGCC CATAGCAGCG GGCGGGCACT TTGCACTGGA

                                                                                                600
ACTTACAACA CCCGAGCAAG GACGCGACTC TCCCGACGCG GGGAGGCTAT TCTGCCCATT TGGGGACACT TCCCCGCCGC TGCCAGGACC CGCTTCTCTG

          PvuII                                  Splice site                                   700
AAAGGCTCTC CTTGCAGCTG CTTAGACGCT GGATTTTTTT CGGGTAGTGG AAAACCAGGT AAGCACCGAA GTCCACTTGC CTTTTAATTT ATTTTTTTAT

                    TaqI                                                                        800
CACTTTAATG CTGAGATGAG TCGAATGCCT AAATAGGGTG TCTTTTCTCC CATTCCTGCG CTATTGACAC TTTTCTCAGA GTAGTTATGG TAACTGGGGC

                                                                                                900
TGGGGTGGGG GGTAATCCAG AACTGGATCG GGGTAAAGTG ACTTGTCAAG ATGGGAGAGG AGAAGGCAGA GGGAAAACGG GAATGGTTTT TAAGACTACC

                                  Rearrangement point
 TaqI                               in "Manca"                                                 1000
CTTTCGAGAT TTCTGCCTTA TGAATATATT CACGCTGACT CCCGGCCGGT CGGACATTCC TGCTTTATTG TGTTAATTGC TCTCTGGGTT TTGGGGGGCT

                                  SstI                                                          1100
GGGGGTTGCT TTGCGGTGGG CAGAAAGCCC CTTGCATCCT GAGCTCCTTG GAGTAGGGAC CGCATATCGC CTGTGTGAGC CAGATCGCTC CGCAGTCGCT

                                                                                                1200
GACTTGTCCC CGTCTCCGGG AGGGCATTTA AATTTCGGCT CACCGCATTT CTGACAGCCG GAGACGGACA CTGCGGCGCG TCCCGCCCGC CTGTCCCCGC

                                                                                    XbaI        1300
GGCGATTCCA ACCCGCCCTG ATCCTTTTAA GAAGTTGGCA TTTGGCTTTT TAAAAAGCCA TAATACAAGT TAAAACCTGG GTCTCTAGAG GTGTTAGGAC

                                                                                                1400
GTGGTGTTGG GTAGGCGCAG GCAGGGGAAA AGGGAGGCGA GGATGTGTCC GATTCTCCTG GAATCGTTGA CTTGGAAAAA CCAGGGCGAA TCTCCGCACC

                                                                                                2200
CAGCCCTGAC TCCCCTGCCG CGGCCGCCCT CGGG.....................................TAGC TCTGCAAGGG GAGAGGTTCG GGACTGTGGC

                                                                                 Splice site   2297
GCGCACTGCG CGCTGCGCCA GGTTTCCGCA CCAAGACCCC TTTAACTCAA GACTGCCTCC CGCTTTGTGT GCCCCGCTCC AGCAGCCTCC CGCGACG

                                                    TaqI                                        2378
ATG CCC CTC AAC GTT AGC TTC ACC AAC AGG AAC TAT GAC CTC GAC TAC GAC TCG GTG CAG CCG TAT TTC TAC TGC GAC GAG
Met Pro Leu Asn Val Ser Phe Thr Asn Arg Asn Tyr Asp Leu Asp Tyr Asp Ser Val Gln Pro Tyr Phe Tyr Cys Asp Glu

                                          PstI                                                  2459
GAG GAG AAC TTC TAC CAG CAG CAG CAG CAG AGC GAG CTG CAG CCC CCG GCG CCC AGC GAG GAT ATC TGG AAG AAA TTC GAG
Glu Glu Asn Phe Tyr Gln Gln Gln Gln Ser Glu Leu Gln Pro Pro Ala Pro Ser Glu Asp Ile Trp Lys Lys Phe Glu

                                                                                                2540
CTG CTG CCC ACC CCG CCC CTG TCC CCT AGC CGC CGC TCC GGG CTC TGC TCG CCC TCC TAC GTT GCG GTC ACA CCC TTC TCC
Leu Leu Pro Thr Pro Pro Leu Ser Pro Ser Arg Arg Ser Gly Leu Cys Ser Pro Ser Tyr Val Ala Val Thr Pro Phe Ser

                                                                                                2615
CTT CGG GGA GAC AAC GAC GGC GGT GGC GGG AGC TTC TCC ACG GCC GAC CAG CTG GAG ATG GTG ACC GAG CTG CTG .....
Leu Arg Gly Asp Asn Asp Gly Gly Gly Gly Ser Phe Ser Thr Ala Asp Gln Leu Glu Met Val Thr Glu Leu Lue .....
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FIG. 3. Nucleotide sequence of exon 1 of the human c-*myc* gene and the flanking region. Also shown is the 5′ end of exon 2 whose sequence was determined by Colby *et al.* (27). The intron and the 5′ untranscribed region are indicated by italics. The predicted amino acid sequence is shown under the nucleotide sequence. The upward arrow indicates the break point in the translocation in Manca. Downward arrows indicate the transcription initiation sites or splice sites. The two sequences in exon 1 and exon 2 that are complementary to each other are underlined.

Thus, all the above results indicate that no additional introns split the c-*myc* gene in the 5′ region, confirming that this gene is composed by three exons. Because the cDNA reported by Watt *et al.* (28) starts at nucleotide 118, the corresponding mRNA is likely to have been transcribed from start site 1.

**Exon 1 Does Not Code for Protein.** The first exon has no ATG codon in any of the three reading frames, while it has termination codons in all of the three reading frames (Fig. 3). These observations strongly indicate that exon 1 of the human c-*myc* gene has no protein-coding capacity. The first ATG codon appears in the second exon and is followed by a long coding frame showing a strong homology to the v-*myc* gene of MC29 virus (27).

**The Translocated c-*myc* Gene Is Often Devoid of Exon 1 and Its Transcription Initiates Within Intron 1.** In several Burkitt lymphomas harboring t(8;14) translocations the break points on the side of the c-*myc* gene have been mapped within 1–2 kb 5′ of exon 2 (19). As shown in Fig. 1, and as will be described in detail elsewhere, the break point of the translocated c-*myc* gene in a non-Hodgkin lymphoma line (Manca) has been mapped to within intron 1 at a point 291 bp downstream of the 3′ end of exon 1. Thus this c-*myc* gene is devoid of the entire exon 1 as well as the associated transcriptional promoters. To define the intron–exon structure of the translocated c-*myc* gene RNA blot analyses of the Manca RNA were carried out using exon 3 and intron 1 probes (probes 5 and 6, respectively, in Fig. 1). RNA from HeLa cells was used as a control for the transcription of

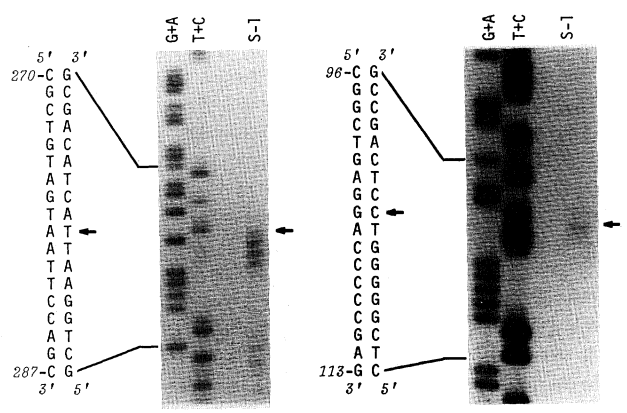


FIG. 4. Nuclease S1 map of the transcription initiation sites of the germ-line c-*myc* gene. One microgram of HeLa mRNA was hybridized individually to probe 7 (*Left*) or 8 (*Right*) (see Fig. 1). The protected sequence was electrophoresed through urea/acrylamide gel. For the size marker, the G+A and T+C sequence ladders of the probes were used. The possible initiation sites are indicated by arrows.

an untranslocated c-*myc* gene. As shown in Fig. 2 (lanes 6–9), the exon 3 probe detected the 2.3-kb RNA in HeLa cells and the 2.6- and 2.2-kb RNA in Manca cells. As expected, the 2.3-kb HeLa RNA was not detectable with the intron 1 probe. By contrast, both the 2.6- and 2.2-kb Manca RNAs hybridized with the intron 1 probe, suggesting that the sequence in this intron is part of an exon in the translocated c-*myc* gene. That this exonic sequence is in fact contiguous with the exon 2 sequence in the mRNA derived from the translocated c-*myc* gene has been shown by nucleotide sequence analysis of a cDNA clone isolated from Manca cells (unpublished data). Furthermore, nuclease S1 protection experiments with Manca RNA indicate that new transcription start sites located within intron 1 are used for the transcription of the translocated c-*myc* gene. Despite the alteration in the mRNA, the translation initiation site is conserved on translocation because no ATG codon is present between the new transcription start sites and the ATG codon located in the 5′ region of exon 2 (ref. 27; unpublished results). Similar features of a translocated c-*myc* gene in the mouse plasmacytoma system were recently described by Stanton *et al.* (31).

## DISCUSSION

The data presented here indicate that the human c-*myc* gene comprises three exons, transcribed from two initiation sites separated by 175 bp in HeLa cells. For both resultant mRNA species, exon 1 lacks a methionine codon and is therefore noncoding. Instead, the initiator methionine is located 16 bp downstream from the 5′ splice acceptor of exon 2. In a non-Hodgkin lymphoma, Manca, harboring a t(8;14) translocation, the c-*myc* gene is broken within intron 1, and its exons 2 and 3 are translocated to a site between the $J_H$ cluster and $C_\mu$-coding segments of the immunoglobulin heavy chain locus. This translocated c-*myc* gene is therefore devoid of exon 1 and is transcribed from points normally located within intron 1 of the c-*myc* gene. Despite this, the mRNA from the translocated c-*myc* gene is apparently translated from the same methionine codon as the mRNA from the unrearranged c-*myc* gene.

Does the lack of expression of exon 1 have an important effect *per se* in cells with such c-*myc* translocations? Examination of the c-*myc* sequence (Fig. 3) reveals a region of exon 1 (nucleotides 283–350) to have high complementarity to a region of exon 2 (nucleotides 2,456–2,526) (Fig. 5A). A stem–loop secondary structure for the human c-*myc* RNA may therefore be proposed. The standard free energy change ($\Delta G^0$) of −90 kcal/mol (1 cal = 4.184 J) predicted for such a structure (32) would be sufficient to maintain it under physiological conditions. The initiator AUG would then be located within the loop (Fig. 5 *B* and *C*). According to the "bind-and-scan" model for eukaryotic translation, the 40S ribosome subunit binds the 5′-terminus of mRNA and migrates toward the initiator AUG (33). This binding and migration is severely hindered when secondary structure is introduced into mRNA; reduction of secondary structure has the opposite effect (34). The negative effect of base pairing on the translational efficiency of prokaryotic RNA has also been demonstrated (35, 36). Therefore, the initiator AUG in the human c-*myc* RNA can be adjudged to be inaccessible to efficient initiation of translation.

By contrast, the lack of exon 1 in the c-*myc* RNA from the translocated gene means that such a stem–loop structure cannot be formed for this RNA. The transcript from the translocated c-*myc* gene is therefore quite likely to be translated at a much higher efficiency than the transcript from the unrearranged c-*myc* gene. The translocation may therefore result directly in overproduction of the *myc* gene product.

To date, precise sites of c-*myc* rearrangements have been mapped for only a few Burkitt lymphomas and non-Hodgkin lymphomas (ref. 19; unpublished results; this study). Among these, at least three (Lou, W1, and Manca) have rearrangement sites within exon 1 or intron 1. Similarly, in the three murine plasmacytomas (J558, M167, and M603) for which *myc* rearrangements have been documented at the nucleotide level, either a complete exon 1 or the normal transcriptional promoter is lost (19, 31). In short, the loss of exon 1 may be a common feature of human Burkitt lymphomas and murine plasmacytomas in which c-*myc* is rearranged. Therefore, the overproduction of the *myc* gene product via a translational mechanism may be a common feature for cells harboring these translocations. This

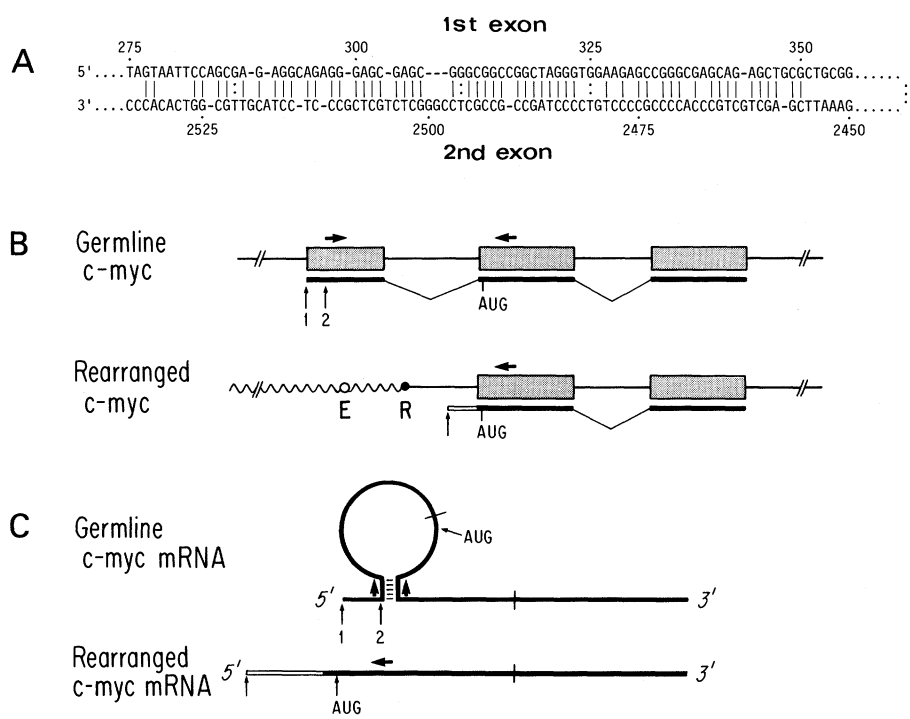


FIG. 5. (*A*) Internal sequence complementarity seen in exon 1 and exon 2 of the germ-line (untranslocated) c-*myc* gene. The nucleotide numbers are as in Fig. 3. The hydrogen-bond forming bases are connected by bars. G·T pairs, which in RNA are allowed to make weak hydrogen bonds as G·U pairs, are indicated by dots. Deletions indicated by dashes are included to maximize the complementarity. (*B*) Schematic representations of the transcription and splicing of the germ-line (untranslocated) and translocated c-*myc* genes. The germ-line c-*myc* gene is transcribed from one of the two initiation sites 5′ of exon 1; the translocated c-*myc* gene is transcribed from initiation sites within the intron. Only one of the initiation sites in intron 1 is shown. (*C*) Possible secondary structures of c-*myc* RNA. The germ-line c-*myc* RNA can form a stem–loop structure, with the initiation AUG codon within the loop. The c-*myc* mRNA from the translocated c-*myc* gene has a different 5′ structure and cannot form the stem–loop structure.

mechanism may also play a role in avian leukosis virus-induced B-cell lymphomas. The vast majority of proviral integrations in these tumors is located within a region that would correspond to intron 1 of human c-*myc* (i.e., 0–1 kb upstream of exon 2 of chicken c-*myc*) (refs. 5, 37–39; unpublished data). Although the precise boundaries of exon 1 in the chicken c-*myc* gene have not been defined, sequencing data have revealed a region within the putative exon 1 that would form a stable stem–loop structure with sequence in exon 2 (unpublished data), in a manner analogous to that described here for the human c-*myc* gene.

There may be cases in which c-*myc* rearrangements occur at some distance from the c-*myc* gene (e.g., see ref. 19) and in which it is therefore not easy to apply the model for c-*myc* overproduction presented here. In these cases, overproduction of c-*myc* may be due to transcriptional effects (14, 40–42). In the case described here, Manca, the rearranged c-*myc* gene is brought close to sequences that, like their murine counterparts (43, 44), have profound tissue-specific transcription-enhancing activity. The possible effect of these sequences on the transcription of the altered c-*myc* gene is being examined. It may be possible to find other such enhancers in the neighborhood of other translocated c-*myc* genes.

In summary, there clearly exist multiple mechanisms by which the c-*myc* gene can be activated in cancerous cells. These include its amplification in promyelocytic leukemia and its transcriptional activation by nearby viral elements in avian leukosis virus-induced lymphomas. We wish to add to this list its activation by a translational mechanism that occurs as the result of the disruption of the gene during translocation in both mouse and human.

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1. Bister, K., Hayman, M. J. & Vogt, P. K. (1977) *Virology* **82,** 431–448.
2. Alitalo, K., Bishop, J. M., Smith, D. H., Chen, E. Y., Colby, W. W. & Levinson, A. D. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 100–104.
3. Robins, T., Bister, K., Garon, C., Papas, T. & Duesberg, P. (1982) *J. Virol.* **41,** 635–642.
4. Vennstrom, B., Sheiness, D., Zabielski, J. & Bishop, J. M. (1982) *J. Virol.* **42,** 773–779.
5. Neel, B. G., Gasic, G. P., Rogler, C. E., Skalka, A. M., Ju, G., Hishinuma, F., Papas, T., Astrin, S. M. & Hayward, W. S. (1982) *J. Virol.* **44,** 158–166.
6. Hayward, W. S., Neel, B. G. & Astrin, S. M. (1981) *Nature (London)* **290,** 475–480.
7. Collins, S. & Groudine, M. (1982) *Nature (London)* **298,** 679–681.
8. Dalla-Favera, R., Wong-Staal, F. & Gallo, R. C. (1982) *Nature (London)* **299,** 61–63.
9. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E. & Bishop, J. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1707–1711.
10. Nowell, P. C. & Hungerford, D. A. (1960) *Science* **132,** 1497–1499.
11. Klein, G. (1981) *Nature (London)* **294,** 313–318.
12. Rowley, J. D. (1982) *Science* **216,** 749–751.
13. Shen-Ong, G. L. C., Keath, E. J., Piccoli, S. P. & Cole, M. D. (1982) *Cell* **31,** 443–452.
14. Adams, J. M., Gerondakis, S., Webb, E., Mitchell, J., Bernard, O. & Cory, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6966–6970.
15. Calame, K., Kim, S., Lalley, P., Hill, R., Davis, M. & Hood, L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6994–6998.
16. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
17. Neel, B. G., Jhanwar, S. C., Chaganti, R. S. K. & Hayward, W. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7842–7846.
18. Dalla-Favera, R., Bregni, M., Erikson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
19. Adams, J. M., Gerondakis, S., Webb, E., Corcoran, L. M. & Cory, S. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1982–1986.
20. Nishikori, M., Hansen, H., Jhanwar, S., Fried, J., Sordillo, P., Koziner, B., Lloyd, K. & Clarkson, B. (1983) *Cancer Genet. Cytogenet.*, in press.
21. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65,** 499–560.
22. Brutlag, D. L., Clayton, J., Friedland, P. & Kedes, L. H. (1982) *Nucleic Acids Res.* **10,** 279–294.
23. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
24. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12,** 721–732.
25. Weaver, R. F. & Weissmann, C. (1979) *Nucleic Acids Res.* **7,** 1175–1193.
26. Dalla-Favera, R., Gelmann, E. P., Martinotti, S., Franchini, G., Papas, T. S., Gallo, R. C. & Wong-Staal, F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6497–6501.
27. Colby, W. W., Chen, E. Y., Smith, D. H. & Levinson, A. D. (1983) *Nature (London)* **301,** 722–725.
28. Watt, R., Stanton, L. W., Marcu, K. B., Gallo, R. C., Croce, C. M. & Rovera, G. (1983) *Nature (London)* **303,** 725–728.
29. Goldberg, M. (1979) Dissertation (Stanford Univ., Stanford, CA).
30. Mount, S. M. (1981) *Nucleic Acids Res.* **10,** 459–472.
31. Stanton, L. W., Watt, R. & Marcu, K. B. (1983) *Nature (London)* **303,** 401–406.
32. Tinoco, I., Jr., Borer, P. N., Dengler, B., Levine, M. D., Uhlenbeck, O. C., Crothers, D. M. & Gralla, J. (1973) *Nature (London) New Biol.* **246,** 40–41.
33. Kozak, M. (1978) *Cell* **15,** 1109–1123.
34. Kozak, M. (1980) *Cell* **19,** 79–90.
35. Kozak, M. & Nathans, D. (1972) *Bacteriol. Rev.* **36,** 109–134.
36. Saito, H. & Richardson, C. C. (1981) *Cell* **27,** 533–542.
37. Rovigatti, U. G., Rogler, C. E., Neel, B. G., Hayward, W. S. & Astrin, S. M. (1982) in *Fourth Annual Bristol-Meyers Symposium on Cancer Research*, eds. Owens, A. H., Coffey, D. S. & Baylin, S. B. (Academic, New York) pp. 319–330.
38. Payne, G. S., Bishop, J. M. & Varmus, H. E. (1982) *Nature (London)* **295,** 209–214.
39. Fung, Y.-K. T., Crittenden, L. B. & Kung, H.-J. (1982) *J. Virol.* **44,** 742–746.
40. Marcu, K. B., Harris, L. J., Stanton, L. W., Erikson, J., Watt, R. & Croce, C. M. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 519–523.
41. Mushinski, J. F., Bauer, S. R., Potter, M. & Reddy, E. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1073–1077.
42. Maguire, R. T., Robins, T. S., Thorgeirsson, S. S. & Heilman, C. A. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1947–1950.
43. Gillies, S. D., Morrison, S. L., Oi, V. T. & Tonegawa, S. (1983) *Cell* **33,** 717–728.
44. Banerji, J., Olson, L. & Schaffner, W. (1983) *Cell* **33,** 729–740.