**The EMBO Journal** vol.4 no.13B pp.3727–3733, 1985

# Truncation of exon 1 from the c-*myc* gene results in prolonged c-*myc* mRNA stability

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Communicated by T.H.Rabbitts

**The human c-*myc* gene consists of three exons transcribed from two distinct promoters and the function of the first, non-coding exon is unknown. In COLO 320 cells, there co-exist normal and truncated (i.e., lacking exon 1) c-*myc* genes, both of which are transcribed. Studies on the turnover of c-*myc* mRNA show that the normal mRNA has an *in vivo* half-life of ~30 min which is approximately similar to the turnover time of the mRNA in lymphoblastoid cells. However, the truncated mRNA was found to be substantially more stable. This observation was also made with a Burkitt's lymphoma cell line which has a translocated, truncated c-*myc* gene. Therefore truncation of the c-*myc* gene can cause the mRNA to be more stable than the full size product suggesting that this can be a crucial factor in the activation of the c-*myc* oncogene, by exon 1 loss, in chromosomal translocation. The results also suggest a role for exon 1 in the c-*myc* mRNA degradative mechanism.**

*Key words:* c-*myc* gene/exon 1/mRNA turnover/translocation

## Introduction

The human and mouse c-*myc* genes have three exons, of which the first is a non-coding exon ~550 bases in length, while exons 2 and 3 encode for the c-*myc* protein (Colby *et al.*, 1983; Hamlyn and Rabbitts, 1983; Marcu *et al.*, 1983). In Burkitt's lymphoma (BL) and in mouse myeloma, chromosomal translocation brings the c-*myc* proto-oncogene into the region of the immunoglobulin locus which is thought to result in activation of the c-*myc* gene as an oncogene. How this is achieved is largely obscure at present owing to the variety of different breakpoints which occur both upstream and downstream of the c-*myc* (Bernard *et al.*, 1983; Croce *et al.*, 1983; Dalla-Favera *et al.*, 1983; Erickson *et al.*, 1983; Davis *et al.*, 1984; Hollis *et al.*, 1984; Rabbitts *et al.*, 1984). It is probable, however, that disruption of c-*myc* metabolism occurs after translocation. Recently, data on mitogen stimulation of B cells has demonstrated that the transcription of the c-*myc* gene is rapidly induced (Kelly *et al.*, 1983) but it has also been shown that c-*myc* mRNA and protein are expressed throughout the cell cycle (Hann *et al.*, 1985; Thompson *et al.*, 1985; Rabbitts *et al.*, 1985). These results suggest that expression of the c-*myc* gene is normally under stringent control and that translocation effects this control.

At present the function for the unusual, large non-coding exon 1 sequence of c-*myc* is unknown. It has been proposed that it may act as the site of binding for a repressor molecule (Dunnick *et al.*, 1983; Leder *et al.*, 1983; Rabbitts *et al.*, 1984) or that it may be involved in control of protein translation (Saito *et al.*, 1983). A third possibility, which we explore in this paper, is that this sequence is involved in the degradative process of c-*myc* mRNA. This possibility is consistent with the observation that in BL and mouse myeloma translocations the c-*myc* gene is often either truncated in such a way that exon 1 is lost or severely shortened (Crews *et al.*, 1982; Croy *et al.*, 1983; Dalla-Favera *et al.*, 1983) or that multiple mutations are introduced into this sequence (Rabbitts *et al.*, 1984; Taub *et al.*, 1984). Recently evidence has accumulated that the c-*myc* mRNA has a short half life *in vivo* (Dani *et al.*, 1984; McCormack *et al.*, 1984) suggesting the possibility that sequences in the mRNA (such as exon 1) might be involved in determining the stability of the mRNA.

With these general considerations in mind we have studied c-*myc* mRNA turnover in various cells. The results show that in COLO 320 cells, where both normal and truncated c-*myc* genes are expressed, the truncated mRNA has a significantly longer half-life than the normal mRNA from the same cells. This conclusion is supported by the observation of increased half-life of truncated mRNA derived from a BL cell line. These observations suggest that a crucial effect of translocation can be increased c-*myc* mRNA stability (with consequential effects on protein availability) by exon 1 loss.

## Results

### *c-myc mRNA stability in BL and lymphoblastoid cells*

Previously published experiments indicate that c-*myc* mRNA has a very short half-life *in vivo*, and it has been suggested that it may be as short as 10 min in some cell types (Dani *et al.*, 1984). We have analysed mRNA turnover, in the presence of actinomycin D concentrations which completely inhibit RNA synthesis, in a BL cell JI (which carries t2;8 but with an intact c-*myc* gene) and in a non-tumorigenic cell line DHLCL. Aliquots from exponentially growing cultures of these cells were removed immediately after addition of actinomycin D. Further aliquots were removed at the times indicated (Figure 1), RNA prepared and analysed by Northern filter hybridisation initially with a c-*myc* probe (Figure 1A) and then with an actin probe (Figure 1B). There are ~3–5 times higher levels of c-*myc* mRNA present in log phase JI cells than in DHLCL (Figure 1); the rate at which the c-*myc* mRNA decay takes place in these cell lines is, however, roughly equivalent occurring with a half-life of ~30 min. Interestingly, in the JI cells we could still detect c-*myc* mRNA 2 h after treatment with actinomycin D and, furthermore, there is an indication that the mRNA with prolonged stability originates from the P1 c-*myc* promoter. This apparent difference in mRNA stability may not be significant since it might reflect differential, initial levels of P1- and P2-derived mRNA. The possible significance of this observation is currently under investigation, but focussed our attention on the possible involvement of exon 1 in c-*myc* mRNA metabolism (see below).

### *Normal and truncated c-myc genes in COLO 320 cells*

We had previously observed that the colon carcinoma line COLO 320 produces c-*myc* mRNA of both normal size and also of ~1.9 kb (unpublished). COLO 320 cells contain amplified copies

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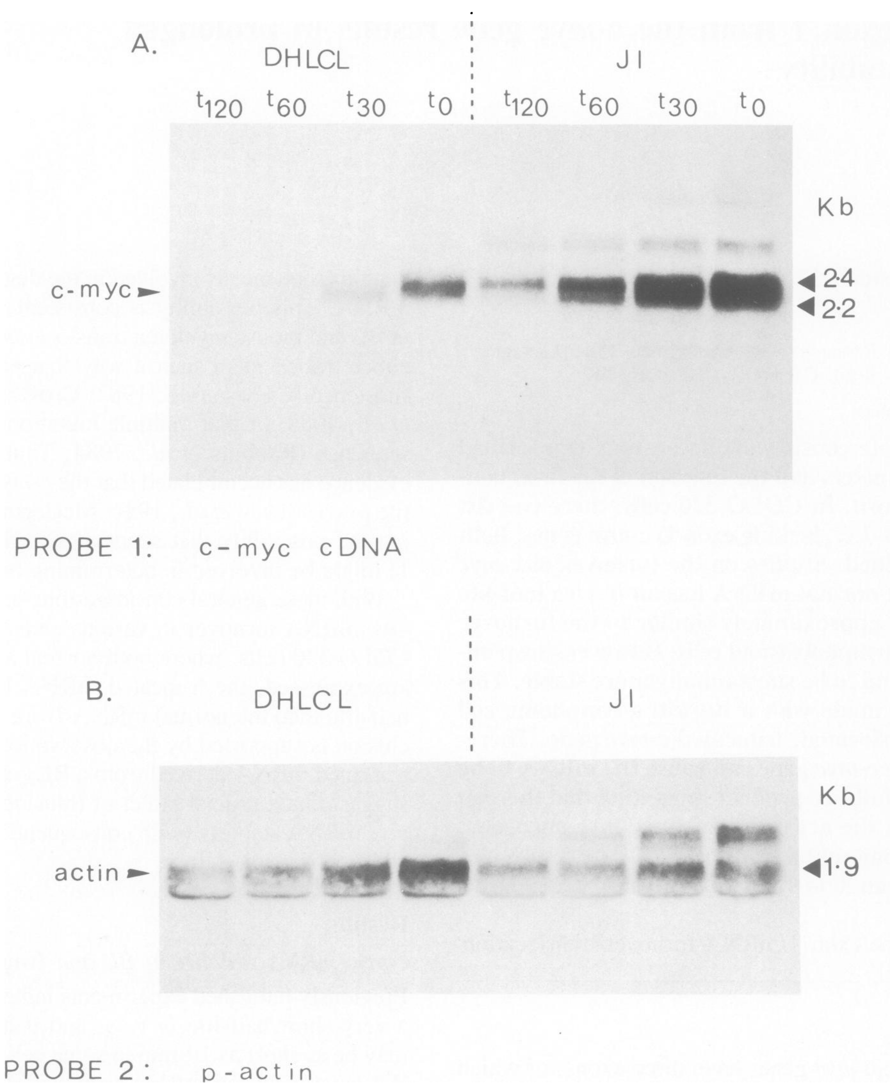


**Fig. 1.** Instability of c-*myc* mRNA in the BL and lymphoblastoid cells. JI cells or DHLCL were grown in log phase and aliquots were removed for RNA extraction ($t_0$). The remaining cells were incubated at 37°C in the presence of 5 μg/ml actinomycin D. After 30, 60 and 120 min RNA was prepared from aliquots of these cultures. 10 μg of each RNA sample was fractionated on 1.4% agarose gels followed by transfer to nitrocellulose filters. The filters were hybridised with either the pUCCDIA c-*myc* cDNA clone (Rabbitts *et al.*, 1983) (**panel A**) or the actin clone pRT3 (**panel B**).

of the c-*myc* gene associated in separate cell lines with either double minute (DM) chromosomes or homogeneously staining regions (HSR) (Alitalo *et al.*, 1983). The RNA analysis suggested that these cells might contain both normal and truncated c-*myc* gene copies, both of which are transcriptionally active. To investigate this possibility, COLO 320 DM and COLO 320 HSR genomic DNA was analysed by filter hybridisation with c-*myc* probes. The two types of COLO 320 DNA were digested with *Bam*HI, fractionated and transferred to cellulose nitrate filters. The filters were first hybridised with a probe containing only exon 1 of the c-*myc* gene (see legend to Figure 2A). Both DM and HSR cell lines hybridised the normal size *Bam*HI restriction fragment of ~30 kb indicating the presence of amplified but complete copies of the c-*myc* gene in both lines (Figure 2A). The same filters were subsequently rehybridised with a probe containing only c-*myc* exon 2 (see legend to Figure 2B). In this case, in addition to the 30 kb *Bam*HI fragment, we observed a new band, only in COLO 320 DM, of ~18 kb. Since we did not detect this band with the exon 1 probe, the 18-kb restriction fragment must correspond to amplified c-*myc* genes which have also undergone a deletion (truncation) of exon 1 (Figure 2B). A more precise localisation of the position of the truncation within the intron of the c-*myc* gene was determined by comparative Southern filter hybridisation of COLO 320 HSR and DM DNAs digested with various enzymes and probed with c-*myc* exon 2 clone (Figure 3). The enzyme *Bgl*II cleaves the c-*myc* gene just on the 3′ side of exon 2, thus providing a constant 3′ end for the fragment generated by double digestion between *Bgl*II and either *Kpn*I, *Xba*I, *Sac*I or *Pvu*II. The normal-sized respective restriction fragments (a, b, c, d and e illustrated in Figure 3B) are present in both COLO HSR and DM DNA. In accord with the data shown in Figure 2, a new restriction fragment, hybridising with the *myc* exon 2 probe, was observed in each case with COLO 320 DM DNA. The presence of a new, 3.4-kb *Sac*I fragment hybridising with the exon 2 probe shows that the site of rearrangement in COLO 320 DM occurs within the normal 1.5-kb *Sac*I fragment shown in Figure 3B, within the intron between exons 1 and 2. This situation is analogous with the truncated c-*myc* genes sometimes observed after translocation in BL and mouse myeloma. In addition it should be noted that one of

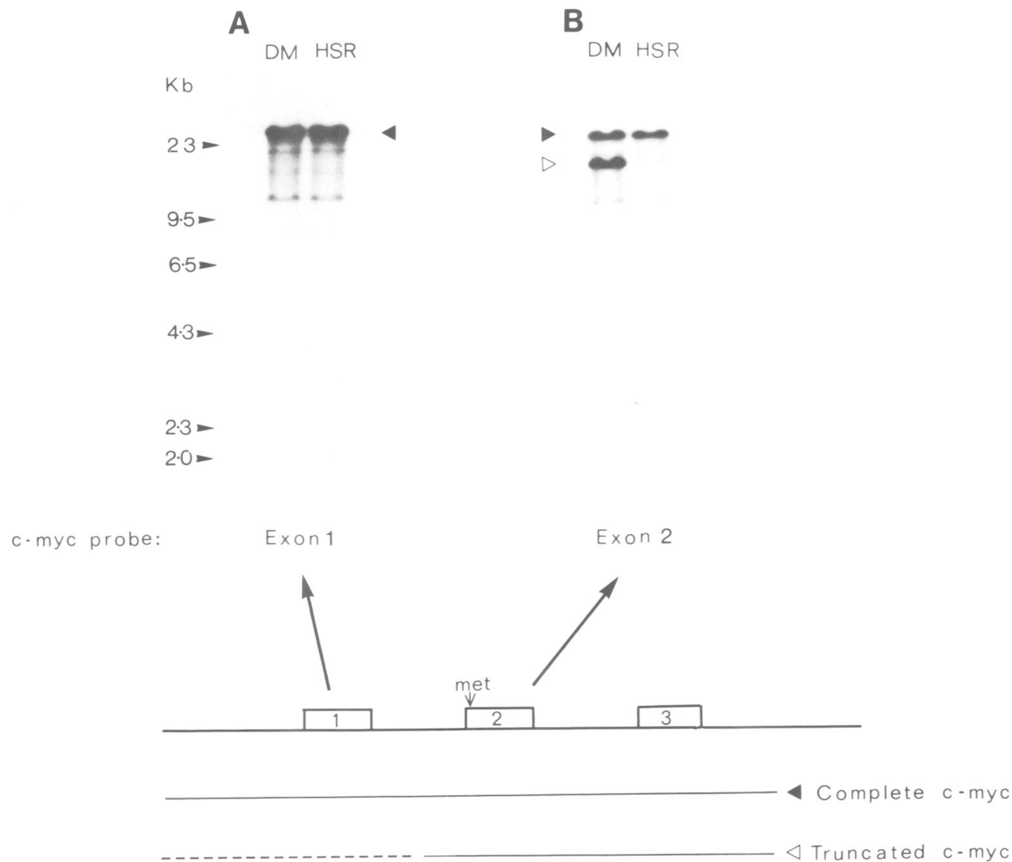


**Fig. 2.** Detection of c-*myc* genes in genomic DNA from COLO 320 DM and HSR. 10 μg genomic DNA prepared from COLO 320 DM or HSR was digested completely with *Bam*HI and fractionated on 0.8% agarose gel. After transfer to cellulose nitrate, the filters were hybridised first with a c-*myc* exon 1 probe (pUCLYXh16) (Rabbitts *et al.*, 1984) **(panel A)** and, following autoradiography, reprobed with an exon 2 clone (M131.5Sac) (Hamlyn and Rabbitts, 1983) **(panel B)**. Fragment sizes were estimated by co-electrophoresis of λ phage DNA cut with *Hin*dIII. The lower panel is a diagrammatic illustration of the *myc* gene structures and the closed arrows **(panels A and B)** indicate the complete c-*myc* gene while the open arrow **(panel B)** indicates the truncated gene.

the two, major cryptic promoter transcription starts identified within the intron 1 of the c-*myc* gene (Hayday *et al.*, 1984) coincides with the upstream *Sac*I restriction site and thus cannot be involved in production of the truncated, 1.9-kb mRNA in these cells, which probably initiates just 5′ to exon 2 since the size of the mRNA is only just sufficient to include exons 2 and 3 of the gene (alternatively, RNA splicing of a small upstream segment onto exon 2 cannot be excluded).

*Transcription of c-myc genes in COLO 320 cells*

The occurrence of the two types of c-*myc* genes in one cell type thus provided the opportunity to study c-*myc* mRNA stability in a single cell population in which both normal (exons 1, 2 and 3) and truncated (exons 2 and 3) genes were actively transcribed, and therefore to critically test the possibility that the presence of exon 1 influences c-*myc* mRNA stability. An experiment analogous to that described in Figure 1 was carried out with COLO 320 DM and HSR cells. RNA was prepared from cells before and after actinomycin D treatment to monitor c-*myc* turnover in the absence of RNA synthesis. The RNA samples were fractionated onto cellulose nitrate filters which were hybridised sequentially with a c-*myc* exon 1 probe (Figure 4A), a c-*myc* exon 2 probe (Figure 4B) and an actin probe to normalise the quantity of RNA in each sample (Figure 4C). In a manner analogous to the experiment in Figure 2, the exon 1 probe only detects full-size mRNA derived from the c-*myc* gene; this probe detects 2.4- and 2.2-kb mRNAs which decay, in the presence of actinomycin D, with a half-life of ~30 min in both cell lines (Figure 4A). The mRNA product of the truncated gene in COLO 320 DM is not detected with the exon 1 probe but is detected, along with the full-size mRNA, using the exon 2 probe (Figure 4B). The hybridisation characteristics of the exon 2 probe to normal and truncated c-*myc* mRNA in COLO 320 DM cells shows that the latter is present at a higher steady-state level. Furthermore, the ratio of hybridising mRNA, in COLO 320 DM, detected with exon 1 or exon 2 probes shows that the truncated c-*myc* mRNA [which is truncated in the region of 70 bp from the start of exon 2 (K.Alitalo, personal communication)] was much more stable than normal *myc* mRNA throughout the time period of the experiment (Figure 4B). In COLO 320 HSR, on the other hand, where only normal genes are present, the two probes detect the same *myc* mRNA which therefore have identical turnover. In several experiments, we have found little evidence for turnover of actin mRNA and in the experiment shown in Figure 4 the actin mRNA is essentially stable. Quantitative comparison of truncated c-*myc* and actin mRNA levels in COLO 320 DM show that a small decline of c-*myc* mRNA occurs relative to actin mRNA (taking into account variations in absolute RNA yield).

*Truncated c-myc mRNA stability in BL cells*

The existence of a transcribed truncated c-*myc* gene in COLO 320 cell parallels that in a proportion of BL and mouse myeloma cells which carry translocated genes. The observation that loss of exon 1 affects the stability of c-*myc* mRNA in COLO 320 prompted an investigation of the analogous situation in the BL cell line, BL 29, which has an actively transcribed truncated (translocated) c-*myc* gene, and in which the normal gene is silent (Bernard *et al.*, 1983). The BL 29 and DHLCL cells were grown

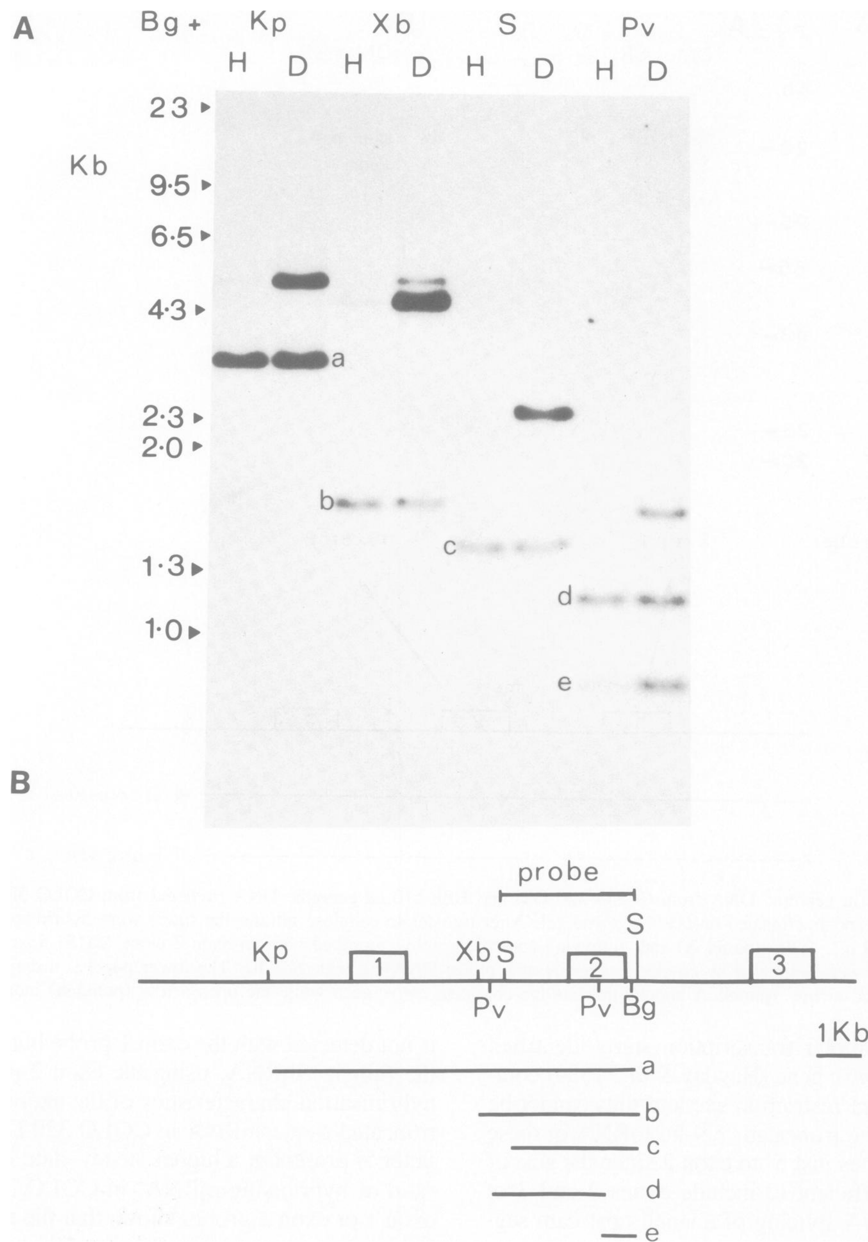


**Fig. 3.** Mapping site of c-*myc* gene truncation in COLO 320 DM DNA. 10 μg aliquots of COLO 320 HSR or DM DNA were digested with *Bgl*II plus *Kpn*I, *Xba*I, *Sac*I or *Pvu*II followed by fractionation on 0.8% agarose. The denatured DNA was transferred to cellulose nitrate and the filter hybridised with M13JI1.5 Sac [c-*myc* genomic exon 2 probe containing 1.5 kb *Sac*I cloned in M13 (Hamlyn and Rabbitts, 1983)]. This probe is indicated in the lower panel B. **(A)** Autoradiograph of hybridised filter. Sizes shown are from co-electrophoresis of λ DNA cut with *Hin*dIII and φX174 DNA cut with *Hae*III. **(B)** Diagram of the c-*myc* gene (showing the three exons) with only the relevant restriction enzyme sites marked. Kp = *Kpn*I, Xb = *Xba*I, S = *Sac*I, Bg = *Bgl*II, Pv = *Pvu*II, H = HSR, D = DM.

in exponential culture, treated with actinomycin D to suppress RNA synthesis and samples removed at zero time, 30 min, 60 min and 120 min. RNA was extracted and analysed by Northern filter hybridisation (Figure 5). The DHLCL cell line, as shown before, showed a rapid turnover of detectable c-*myc* mRNA (Figure 5A) whilst the level of actin mRNA remained fairly constant. The c-*myc* mRNA transcribed from the truncated gene in the BL 29 cell line, however, showed very little turnover during this experiment (Figure 5B and C) as we had observed with the truncated c-*myc* mRNA of COLO 320 (Figure 4). The size of the c-*myc* mRNA transcribed from the translocated gene in BL 29 is unexpectedly large (~2.7 kb) as previously shown (Bernard *et al.*, 1983) and, in addition, a larger band of ~3.5 kb is apparent. Both of these RNA species, however, exhibit a stable profile in the actinomycin chase. Therefore, c-*myc* mRNA transcribed from a (truncated) translocated gene in either a BL cell line or in COLO 320 showed extended half-lives compared with normal mRNAs. It is reasonable, therefore, to conclude that exon 1 itself has a post-transcriptional role in the process of *myc* mRNA degradation. The presence of this sequence in the normal c-*myc* mRNA would appear, at least partly, to account for its rapid turnover.

## Discussion

### *Exon 1 and c-myc mRNA turnover in vivo*

The results of this paper show that the COLO 320 DM cell line has both normal and truncated amplified c-*myc* genes and that

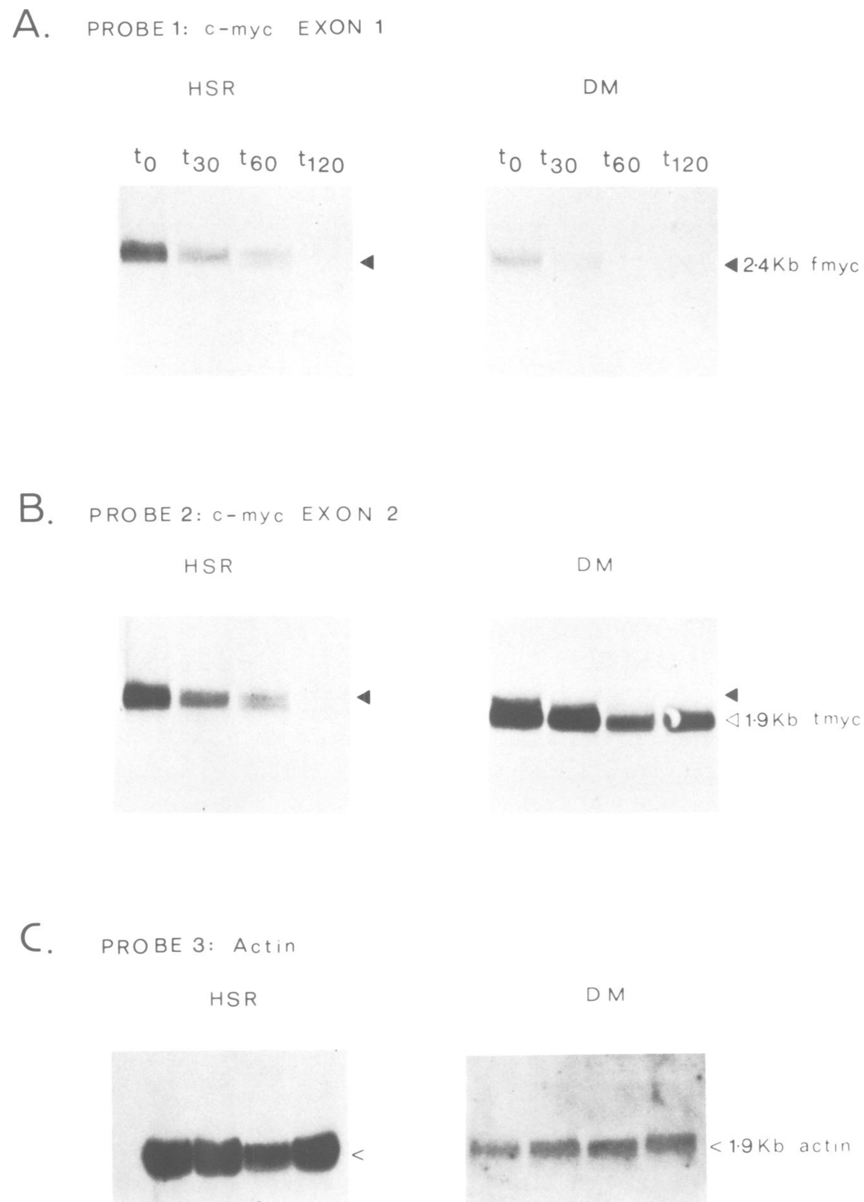


**Fig. 4.** Turnover of c-*myc* mRNA in COLO 320 cells. COLO 320 cells (DM and HSR) were grown in log phase and aliquots removed from RNA preparation ($t_0$). Actinomycin D (5 $\mu$g/ml) was added and further RNA preparations made at 30, 60 and 120 min. All RNA were fractionated and transferred to cellulose nitrate. The filters were hybridised sequentially (autoradiographs being prepared after each hybridisation) with: **panel A,** c-*myc* exon 1 clone (pUCLYXh16); **panel B**, exon 2 clone (M131.5Sac); **panel C**, p-actin. fmyc indicates full length *myc* mRNA and tmyc indicates truncated mRNA.

both are actively transcribed. We cannot be sure that individual cells exhibit these phenomena, but in any case the experiments described here relate to c-*myc* mRNA in one cell population. The turnover of normal size c-*myc* mRNA in COLO 320 HSR and in DM cells was found to be roughly equivalent to that observed in, for example, DHLCL (Figures 1 and 4). It is likely, therefore, that amplification *per se* in these tumours is sufficient to exert the oncogenic effect of the c-*myc* gene. The data shown in Figure 4 demonstrate that the c-*myc* mRNA is rendered more or less stable when transcribed in the absence of exon 1. Since this analysis was carried out with RNA prepared from a single cell type, containing both normal and truncated mRNA simultaneously, it obviates the need for a comparison of mRNA in different cell lines. Further, RNA prepared from a BL cell line with a translocated, truncated c-*myc* gene (Bernard *et al.*, 1983) showed similar stable properties. These results seem, therefore, to suggest a role for exon 1 sequences in the post-transcriptional process of mRNA degradation. Such a role is distinct from a possible role in transcriptional control but these two mechanisms are not mutually exclusive. It remains formally possible that the new, transcribed material which replaces exon 1 (when truncated genes are transcribed from new promoters) has a positive effect of stabilising the c-*myc* mRNA. This is unlikely since two unrelated examples (COLO 320 and BL 29) exhibit the same effect with different segments of DNA near the truncated genes. Further, although BL 29 *myc* mRNA contains considerable new RNA sequence, COLO 320 DM truncated mRNA is only sufficiently large to possess ~100 bp of additional sequence. It is more likely, therefore, that exon 1 itself is involved in the degradative process. If sequence information of exon 1 in mRNA dictates,

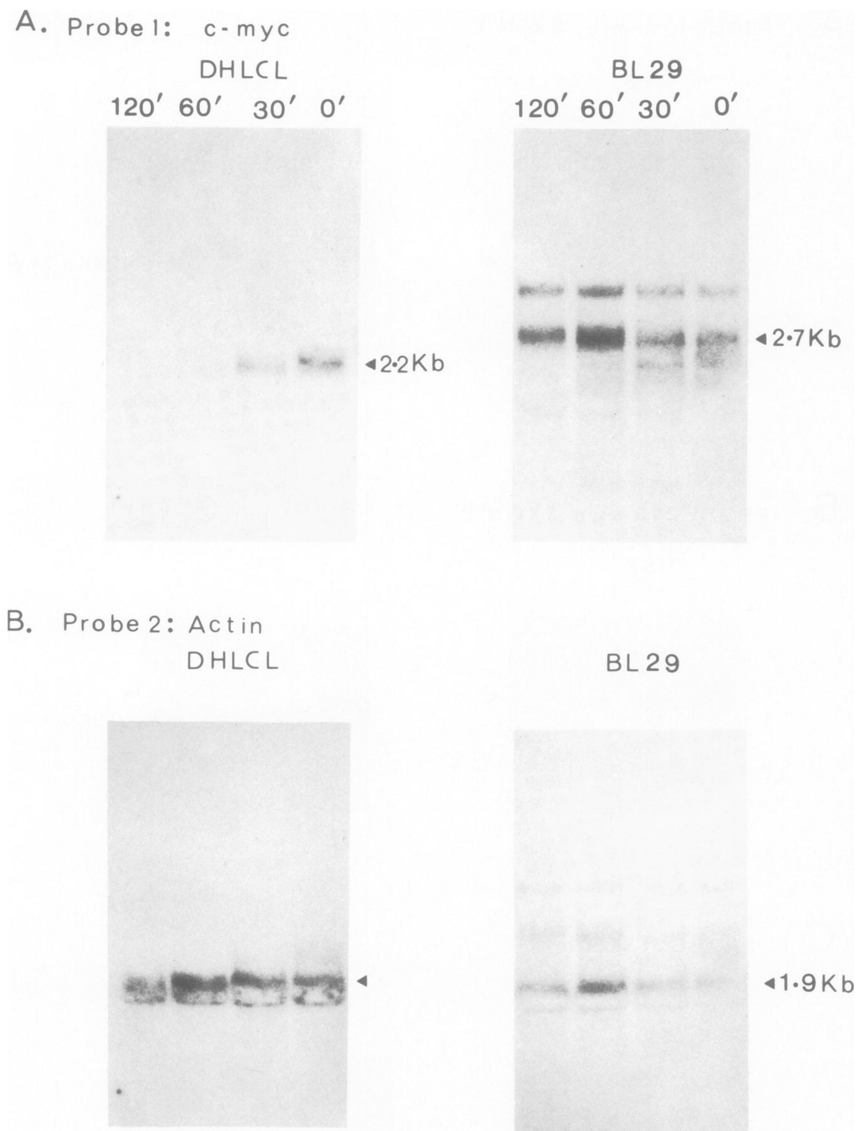


**Fig. 5.** Turnover of c-*myc* mRNA in the BL cell line BL 29. BL 29 and DHLCL cells were grown in exponential phase. Actinomycin D was added to 5 μg/ml and aliquots of cells removed for RNA preparation at $t_0$, $t_{30}$, $t_{60}$ and $t_{120}$ min. RNAs were fractionated on 1.4% agarose and transferred to cellulose nitrate. Filters were hybridised with pUCCDIA (a full length c-*myc* cDNA probe, Rabbits *et al.*, 1984) or p-actin (an actin cDNA probe kindly provided by Dr J.Rogers). **(A)** DHLCL. **(B)** BL 29.

even partly, stability *in vivo*, it is not at all clear to what extent sequences in exon 2 and/or 3 would also be necessary to cooperate with exon 1 in this process. However, it has been noted that potential hairpin structure could exist between part of exon 1 and exon 2 (Saito *et al.*, 1983). This putative structure is one possible recognition site for RNA degradation enzymes *in vivo*.

*Loss of exon 1 in chromosomal translocations*

In so far as the COLO 320 DM cell line transcribes c-*myc* mRNA from a truncated gene, it mimics the c-*myc* gene in translocations present in some cases of BL and many mouse myelomas. The extended half-life *in vivo* of such truncated mRNAs offers one explanation for c-*myc* oncogenic activation in the subset of translocated c-*myc* genes which undergo truncation in BL. The c-*myc* gene is apparently set up to be rapidly modulated since both the mRNA and protein (Dani *et al.*, 1984; Hann and Eisenman, 1984; Rabbitts *et al.*, 1985) have a short half-life *in vivo*. In principle, therefore, cessation of RNA synthesis would be associated with a rapid loss of available c-*myc* protein. However, truncation of the *myc* gene and consequent rendering of the c-*myc* mRNA more stable would facilitate its availability for protein synthesis during longer periods after transcription has stopped. While in normal cells it may be necessary to provide external growth stimuli to induce c-*myc* mRNA appearance after each round of cell division, the prolongation of c-*myc* mRNA by stabilisation in BL may effectively 'pre-induce' these tumour cells to each division cycle.

This putative mechanism for activation of the c-*myc* gene is, however, unlikely to be the only pathway by which this can be achieved. For example, the BL cell line Ramos has a translocation which occurs ~300 bp upstream of exon 1 and very limited sequence alteration was found in exon 1 (Wiman *et al.*, 1984). However, putative chromosomal regions involved in control of c-*myc* gene transcription have been identified upstream of c-*myc* (Siebenlist *et al.*, 1984; Dyson and Rabbitts, 1985; Tuan and London, 1984) and at least three of these are removed by translocation in Ramos; this particular translocated gene may be expressed constitutively as a result. Similarly, examples of apparent

immunoglobulin enhancer control of translocated c-*myc* occur (Hayday *et al.*, 1984) as well as overproduction of c-*myc* mRNA resulting from gene amplification (Alitalo *et al.*, 1984; Little *et al.*, 1983). The net effect of these various situations would be to cause sufficient c-*myc* mRNA to exist at all times to support synthesis of an adequate quantity of c-*myc* protein to allow its function to be manifested in these tumour cells.

## Materials and methods

*Northern hybridisation: analysis of RNA*

RNA was prepared as described by Favaloro *et al.* (1980). For hybridisation analysis, 10 μg RNA samples were glyoxylated (Thomas, 1980), fractionated on 1.4% agarose gels and transferred to cellulose nitrate. The probes were labelled by nick-translation (sp. act. $\sim 1-2 \times 10^8$ c.p.m./μg) and hybridisation was conducted at 65°C using $10^6$ c.p.m./ml probe in 6 × SSC, 5% dextran sulphate, 0.4% Ficoll, bovine serum albumin (BSA) and polyvinylpyrolidine (PVP) (Denhardt, 1966) 0.1% SDS and 50 μg/ml sonicated denatured salmon sperm DNA. After 18 h, the filters were washed at 65°C in 0.1 × SSC 0.1% SDS to remove unhybridised probe prior to autoradiography.

mRNA turnover was assayed by preparing RNA from log phase cells after treatment with 5 μg/ml actinomycin D. Aliquots of cultures were removed prior to addition of actinomycin and at $t_{30}$, $t_{60}$ and $t_{120}$ min after actinomycin addition for RNA extraction as above.

*Analysis of DNA*

DNA was prepared from cells as described (Bentley and Rabbitts, 1981). 10 μg amounts were completely digested, fractionated on 0.8% agarose and transferred to cellulose nitrate (Southern, 1975). Filters were hybridised and washed as above before autoradiography.

*Cell lines used*

JI (Burkitt's lymphoma t2;8) (Bernheim *et al.*, 1981). DHLCL (EBV-transformed lymphoblastoid cell line). COLO 320 (small lung cell carcinoma) (Alitalo *et al.*, 1983).

## Acknowledgements

We wish to thank Dr J.Rogers for the actin clone, Dr A.Rickinson for DHLCL cells, Dr G.Lenoir for JI and BL 29 cells and Dr J.M.Bishop for COLO 320 cells.

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*Received on 16 September 1985; revised on 21 October 1985*