The Effect of Cyclic-AMP on the Regulation of c-*myc* Expression in T Lymphoma Cells

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Abstract

Myc is implicated in the control of growth in a variety of cell types. I investigated c-*myc* gene expression in several lymphoid cell lines to determine the response to cyclic AMP. Cyclic AMP causes a precipitous decline in c-*myc* message concentration that precedes G1 cell cycle arrest in wild type S49 cells but not in KIN$^-$ cells that lack cAMP dependent PKA activity. In wild-type S49 cells washout of cyclic AMP restores c-*myc* message levels within 2 h but does not relieve the G1 arrest until 10 h later. Transcription runoff studies demonstrate inhibition of both transcriptional initiation and prolongation of initiated transcripts. However, the degree of inhibition is insufficient to explain the absence of detectable *myc* message suggesting that the predominant effect of cyclic AMP is to destabilize the c-*myc* message. In contrast to wild-type cells, the "Deathless" mutant S49 cell line is viable when arrested in G1 by exposure to cyclic AMP and has preserved c-*myc* expression. Thus, in S49 cells down regulation of c-*myc* expression appears to be associated with loss of viability rather than G1 cell cycle arrest. Interestingly, CEM human T lymphoma cells do not arrest in G1 phase when exposed to cyclic AMP in spite of losing detectable c-*myc* gene expression. This suggests that in some T lymphoma cells c-*myc* gene expression may not be necessary for cell cycle progression and proliferation. (*J. Clin. Invest.* 1995. 95:1490–1496.) Key words: S49 • CEM • cell cycle • protein kinase A • G1

Introduction

The c-*myc* proto-oncogene encodes a 64-kD protein that, when heterodimerized with a related protein MAX, is transcriptionally active, binding to the consensus sequence CACGTG (1, 2). This transcriptional activity is thought to relate to the putative role of c-*myc* in cell cycle progression and cell proliferation (3). *Myc* gene expression is under complex regulation (reviewed in reference 4) with initiation at three different promoter initiation sites (P0, P1, P2) multiple promoter elements that may regulate transcription (5, 6, 7) transcriptional attenuation after the first exon and rapid message turnover (8, 9). More recently c-*myc* has been implicated in an opposite role—that of programmed cell death (apoptosis). This effect, but not its mitogenic activity, can be blocked by the bc1-2 gene product (10, 11).

Cyclic AMP also has a dual role. In many cell types it is mitogenic, presumably through its ability to induce phosphorylation and activation of the cyclic AMP response element binding protein CREB (12, 13). In other cell types it is inhibitory causing G1 cell cycle arrest and cytotoxicity (14). Both of the properties require protein kinase A activity. Most recently, the inhibitory activity of cyclic AMP has been linked the glucocorticoid response (15). One of the effects of cyclic AMP in some cell types is to attenuate c-*myc* expression.

The purpose of this study is to characterize c-*myc* gene expression in response to cyclic AMP in several T lymphoid cell lines. The specific hypothesis I investigated is whether the cyclic AMP inhibition of cell cycle progression and cytotoxicity correlates with diminished c-*myc* gene expression.

Methods

Cell culture. S49 mouse T lymphoma cells were grown in DME in the presence of 10% heat-inactivated horse serum in a humidified atmosphere containing 10% CO_2 at 37°C. CEM human T lymphoma cells were grown in RPMI media in the presence of 10% heat-inactivated fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37°C. Experimental cultures were used during logarithmic growth at a density of 0.5–2.0 × 10^6 cells/ml.

RNA extraction. Total cellular RNA was extracted from cells using guanidine precipitation as previously described (16).

Northern blot analysis. RNA (20 μg/lane) was fractionated onto a 1% agarose/6% formaldehyde gel by electrophoresis then transferred to a Hybond-N+ (Amersham, Arlington Heights, IL) nylon membrane by capillary action and fixed by incubation at 80°C for 2 h. Northern blots were prehybridized for 4–6 h at 42°C in a solution containing 50% formamide, 5× Denhardt's, 5× SSC, 0.5% SDS, and 500 μg/ml denatured salmon sperm DNA. Hybridization was performed by the addition of 1.0 to 2.0 × 10^6 cpm/ml of ^{32}P-labeled DNA probed to the prehybridization mix and incubating for 18 h at 42°C. Blots were washed free of unbound probes by sequential exposure to 2× SSC, 0.1% SDS at 42°C for 30 min then 0.1× SSC, 0.1% SDS at 55–60°C for 30 min. After autoradiography at −70°C bound probes were removed by incubating the blots in 0.1× SSC, 0.1% SDS at 100°C for 15 min. The blots were then rehybridized to a different radiolabeled probe. Autoradiographic bands were quantitated by scanning densitometry using a Helena Cliniscan densitometer. In preliminary experiments a probe for the constitutively expressed enzyme GAPdH was used to quantitate RNA however, it was found to exhibit modest cell cycle variation. Additionally, each gel was stained with ethidium bromide to determine equal loading of samples and complete transfer to the nylon membrane.

Centrifugal elutriation. Cell cycle-specific populations were obtained by centrifugal elutriation as previously described (16). Briefly, 4 × 10^8 cells were loaded at 9.5 ml/min onto a Beckman J2-21 centrifuge equipped with a JE-6 elutriation rotor at a speed of 2,000 rpm. Fractions of 50–100 ml were removed as the flow rate was increased from 11.5 to 32 ml/min.

Cell cycle analysis. Cell suspensions were centrifuged and resuspended in a solution of cold (4°C) hypotonic staining solution (0.05

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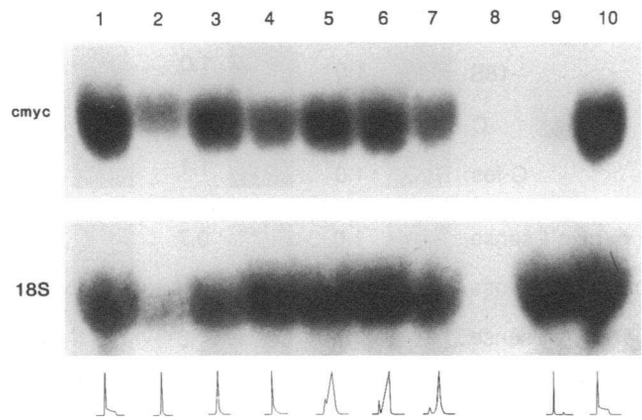


Figure 1. Cell cycle expression of the gene encoding c-*myc*. Exponentially growing S49 T lymphoma cells were separated by centrifugal elutriation as described in Methods. Fractions were analyzed by cytofluorimetry of propidium iodide-stained cells. Fractions representative of different states of the cell cycle were selected and total cellular RNA (20 μg/lane) extracted for Northern blot analysis of messenger RNA coding for c-*myc*. On the left (lane *1*) exponentially growing cells are ~ 30% in G1 phase in comparison to the first elutriation fraction shown in lane *2* with over 90% in G1; lane *3*, 70%; lane *7*, 3.4% G1, 31% S phase, and 66% G2/M. Lane *8* is blank. In the two lanes on the right, a cyclic AMP induced G1 cell-cycle-arrested population (lane *9*, 88% in G1) is compared with an exponentially growing population (lane *10*). Equivalent loading of each lane was determined by ethidium bromide staining.

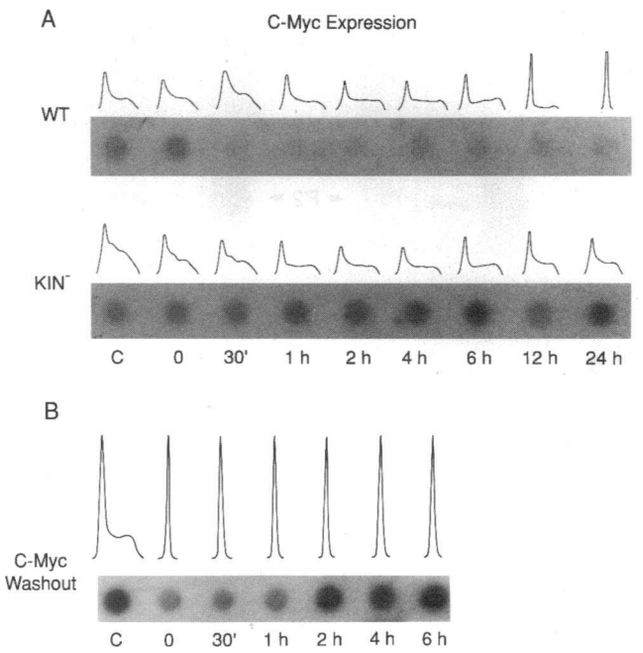


Figure 2. The effect of cyclic AMP on c-*myc* gene expression. Exponentially growing wild type S49 cells (*A*) were exposed to dibutyryl cyclic AMP (mM) for the times indicated and cells were examined by cytofluorimetry and northern dot blot analysis. The same analysis was performed for a PKA deficient S49 cell line, KIN$^-$ shown in the middle panel. In the *B* wild-type S49 cells were exposed to dibutyryl cyclic AMP (1 mM) for 24 h (time 0) and compared with exponentially growing cells (*c*) and time points after removal of cyclic AMP.

mg/ml propidium iodide in 0.1% sodium citrate and 0.1% Triton X-100) for 15 min. Flow cytometry was carried out (Becton-Dickinson, Rutherford, NJ) on an EPICS V analyzer (Coulter) equipped with a multiparameter data acquisition system (Coulter).

Nuclear runoff transcription assay. The nuclear runoff analysis was carried out as previously described (16). Nuclei were isolated on a sucrose cushion using the method described by Marzluff (17). DNA (2 μg) for each of the probes was spotted on Hybond-N+ filters (Amersham) using a slot blot apparatus (Schleicher and Schuell, Keene, NH), except for 18 S, where only 200 ng was used. Densitometry was performed to quantitate the results.

RNase protection analysis. Total cellular RNA was analyzed by the RNase protection assay as described by Zinn (18). Samples (10 μg of total RNA plus RNA probe) were denatured at 85° for 10 min, followed by hybridization at 56°C for 12–14 h. RNase treatment with 10 μg/ml RNase A and 500 u/ml T1 (Sigma Chemical Co.) for 1 h at 33°C was followed by addition of 10 μg/ml proteinase K for 10 min at 37°C. RNase resistant fragments were analyzed on 5% polyacrylamide sequencing gel electrophoresis.

Materials. Plasmid pSV c-*myc* was obtained from American Type Culture Collection (ATCC cat no. 41029) Rockville Md. Single stranded M13 plasmids containing exon 1 and 2 sequences in the sense and antisense orientation were a gift of David Bentley (ICRF, London, UK). Plasmid probes were labeled with (^{32}P) dCTP by random priming (Amersham, Multiprime Kit) (19). The remainder of the materials were commercially obtained (Sigma Chemical Co., St. Louis, MO).

Results

Cell cycle regulation of Myc gene expression. S49 mouse T lymphoma cells are transformed and originate from a mineral oil induced tumor in a BALB/c mouse. They have a doubling time of 14 h and during exponential growth ~ 30% of cells are in G1 phase, 40% in S phase and 30% in G2/M phase. Cells may be fractionated by centrifugal elutriation as shown in Fig. 1 and samples assayed for c-*myc* gene expression by northern blot analysis. Exponentially growing S49 cells (lanes *1* and *10*) have large amounts of c-*myc* messenger RNA that are easily detectable on Northern blots. There appears to be some cell cycle variability in the amount of c-*myc* message. However, when compared with the intensity of 18 S messenger RNA the variability in densitometric quantitation is ~ 50% which is the same as the variation amongst samples from exponentially growing cells. Thus, there is no evidence for cell cycle variation in c-*myc* messenger RNA concentration in S49 cells. Under most circumstances *Myc* protein levels are proportional to c-*myc* messenger RNA concentrations (4).

Effect of cyclic AMP on c-myc message level. Agents that elevate cyclic AMP generate G1 cell cycle arrest in S49 cells. This is reversible for at least 48 h but longer exposure results in cell death. Cyclic AMP treated cells are arrested in G1 phase and have undetectable levels of c-*myc* message (Fig. 1, lane *8*) which is less than that of G1 elutriated cells (Fig. 1, lane *2*). Densitometric quantitation indicates a 5–10-fold reduction in c-*myc* message. Time course Northern blot experiments (Fig. 2) reveal that c-*myc* message disappears in wild-type S49 cells within 30 min of exposure to dibutyryl cyclic AMP ~ 6 h before the first significant alteration in cell cycle distribution. Furthermore, in wild-type S49 cells, G1 arrested by exposure to dibutyryl cyclic AMP for 24 h, washout experiments reveal a return of c-*myc* gene expression within 2 h which is 10 h before the cells begin to exit from G1. By contrast, KIN$^-$ S49 cells which lack PKA activity are unaffected by dibutyryl cyclic

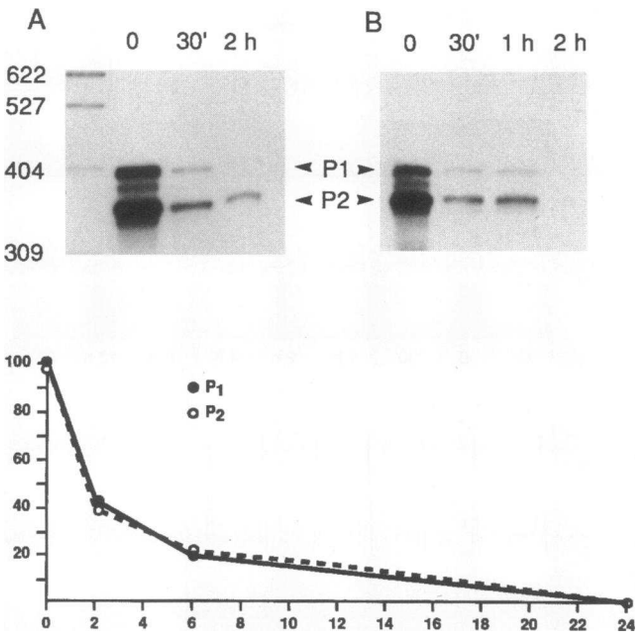


Figure 3. Analysis of c-*myc* mRNA after exposure of cells to actinomycin D or cyclic AMP. Total cellular RNA was extracted from exponentially growing S49 cells or cells treated with actinomycin D (5 μg/ml) (*A*) or dibutyryl cyclic AMP (1 mM) (*B*) for the times shown. RNA was analyzed by RNase protection using α-^{32}P UTP-labeled RNA probe encompassing both *Myc* promoters P1 and P2. The protected fragments were analyzed by electrophoresis in a denaturing 5% polyacrylamide gel. The bottom graph is an analysis of c-*myc* mRNA by radioactive scanning of a gel of an RNase protection assay after CEM cells were exposed to dibutyryl cAMP (1 mM).

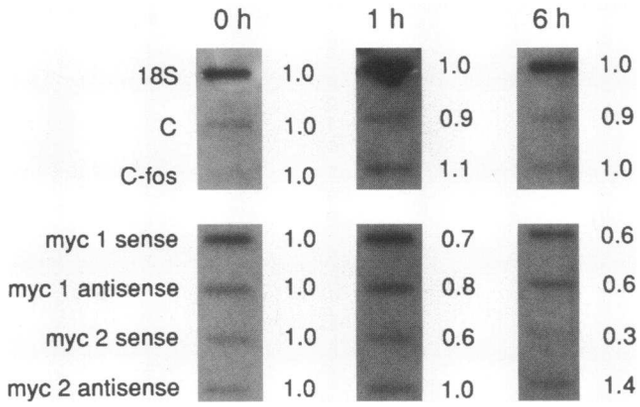


Figure 4. Transcription runoff assay. Transcription runoff analysis was performed as indicated in the Methods Section. Nuclei were isolated after exposure to dibutyryl cyclic AMP (1 mM) for the times indicated. Each slot represents 2 μg of cDNA except for 18S which is 200 ng per slot. Densitometric quantitation was performed by normalizing the absorbance to the 18S band for each strip and subsequently the absorbance of the exponential band was set at 1.0. Control cDNA is the Bluescript vector without an insert. A c-*fos* probe is also shown for comparison.

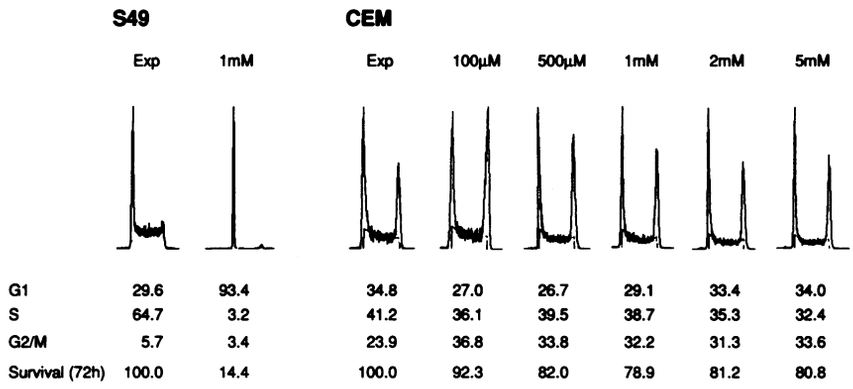
AMP both in terms of cell cycle distribution and c-*myc* message levels. Other S49 cell lines with different mutations segregate into these two classes depending on whether they possess PKA activity or not (data not shown). In other studies we have shown that (IL-2 dependent) mouse T cell clones of both helper and suppressor/cytolytic phenotype are inhibited by cyclic AMP (19a).

Cyclic AMP versus actinomycin D. We compared the inhibition of expression of the c-*myc* gene by dibutyryl cyclic AMP with the RNA polymerase inhibitor actinomycin D. Initial studies indicated that the c-*myc* half-life using both of these inhibitors is ~ 20 min which correlates with published estimates of the c-*myc* messenger RNA half-life. We then used the RNase protection assay to determine if there is any difference in promoter use with these two agents. As shown in Fig. 3 both actinomycin D (*A*) and cyclic AMP (*B*) cause a prompt decrease in expression of the gene from both the P1 and P2 promoter sites. Densitometric analysis confirms a half-life of ~ 20 min with either of these agents.

The effect of cyclic AMP on transcription of c-myc. Transcriptional regulation of c-*myc* was analyzed by transcription run off assays using labelled nuclei from treated and untreated S49 cells and immobilized probes for both exon 1 and 2 of the c-*myc* gene in the sense and antisense orientation (Fig. 4). At 30 min of exposure there was no significant difference from control untreated nuclei (data not shown) however by 1 h there was a modest (30%) decline in transcription of exon 1 in the sense orientation and a 40% decline in the transcription of exon 2 in the sense orientation indicating a modest decline in transcriptional initiation and a slight block to transcriptional elongation. Attenuation became more pronounced at 6 h of exposure to cyclic AMP. There was also a modest decline in the antisense transcription from exon 1 but not from exon 2.

cAMP in S49 versus CEM Cells. We investigated cyclic AMP sensitivity in human T lymphoblast cell lines and determined that MOLT 4 cells like S49 cells are inhibited by cyclic AMP (data not shown). By contrast, CEM cells like KIN$^-$ are resistant to agents that elevate intracellular cyclic AMP including dibutyryl cyclic AMP, RO-20-1724 (a phosphodiesterase inhibitor) (data not shown) and forskolin, an activator of adenylcyclase. As shown in Fig. 5 S49 cells are arrested in the G1 phase of the cell cycle by 1 mM dibutyryl cyclic AMP for 24 h. While completely viable at 24 h their survival at 72 h is profoundly reduced. By contrast, CEM cells have virtually no alteration in cell cycle distribution and virtually no reduction in survival at concentrations of dibutyryl cyclic AMP necessary to arrest S49 cells (100μM).

c-myc in S49 versus CEM Cells. Surprisingly, CEM cells appear to regulate c-*myc* message in much the same manner as S49 cells. The amount of c-*myc* message is increased in CEM cells relative to S49 cells but the half-life, as measured by actinomycin D treatment, is only slightly prolonged in CEM cells by Northern blot analysis (Fig. 6 *C*). Cyclic AMP has similar effects on c-*myc* message in S49 versus CEM cells again demonstrating a half-life of 20 to 40 min. (Fig. 6 *A*). Pretreatment with cycloheximide (3 h) increases the half-life suggesting protein synthesis is necessary to transduce part of the cyclic AMP effect (Fig. 6 *B*). A very interesting phenomena occurs when dibutyryl cyclic AMP is added to actinomycin D treated cells. In S49 cells the combination of actinomycin D and cyclic AMP has little effect or perhaps shortens the half-life of c-*myc* messenger RNA whereas in CEM cells it vastly prolongs the c-*myc* message half life (Fig. 6 *D*). When the half-life of c-*myc* messenger RNA is determined by the RNase protection assay the rate of diminution is somewhat slower in CEM cells than S49 cells (Fig. 3, graph at the bottom) probably



	S49		CEM					
G1	29.6	93.4	34.8	27.0	26.7	29.1	33.4	34.0
S	64.7	3.2	41.2	36.1	39.5	38.7	35.3	32.4
G2/M	5.7	3.4	23.9	36.8	33.8	32.2	31.3	33.6
Survival (72h)	100.0	14.4	100.0	92.3	82.0	78.9	81.2	80.8

Figure 5. Cell cycle distribution and cAMP sensitivity of S49 and CEM cells. Exponentially growing S49 and CEM cells were exposed to varying concentrations of dibutyryl cyclic AMP and harvested at 24 h for cytofluorimetry and 72 h for cell viability. Cytofluorimetric cell cycle distribution is shown above the proportion of cells in each cell cycle phase and the survival at 72 h compared with exponentially growing cells. S49 cells doubling time is 12–14 h whereas CEM cells double every 24 h which lengthened to 29 h when cultured in media containing 5 mM dibutyryl cyclic AMP.

because the assay is more sensitive. In most experiments the c-*myc* expression in CEM cells treated with dibutyryl cAMP is undetectable at 24 h by RNase protection when the cells are still proliferating.

S49 wild type versus the "Deathless" mutant. We also compared these cell lines to the mutant S49 cell line called Deathless (D^-) that will arrest in G1 phase (Table I) but which is viable during prolonged exposure to agents that elevate cyclic AMP (Table II). Deathless has similar viability to cyclic AMP elevation as S49 cells that have defects in the PKA phosphorylation pathway. Interestingly, all of the S49 derived cell lines (wild type, KIN^- and D^-) are sensitive to dexamethasone whereas, our CEM cells are not (20). Comparing c-*myc* expression in S49 wild-type versus Deathless during cyclic AMP induced cell cycle arrest reveals a sharp decline in c-*myc* messenger RNA concentrations during G1 cell cycle arrest in wild-type cells, whereas, c-*myc* expression from both the P1 and P2 promoter persisted for at least 24 h in Deathless (Fig. 7).

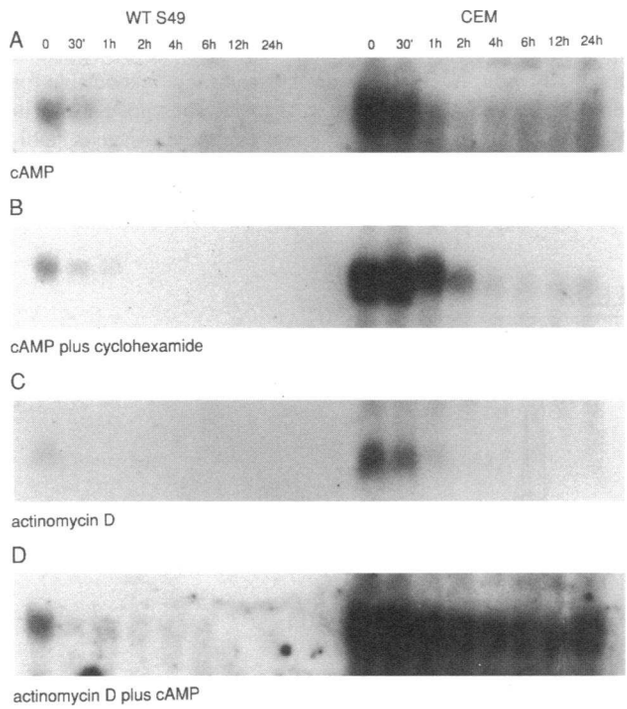


Figure 6. c-*myc* Gene Expression in S49 versus CEM T lymphoma cells exposed to cyclic AMP, actinomycin D and cycloheximide. Northern blot analysis was performed on S49 or CEM cells at time points indicated after exposure to dibutyryl cyclic AMP (1 mM) alone (*A*) or after pre-exposure (3 h) to cycloheximide (*B*) actinomycin D (5 μg/ml) alone (*C*) or with dibutyryl cyclic AMP (*D*). Equivalent loading and transfer was determined by hybridization with an 18 S mouse RNA probe filtered by densitometry. There was < 10% variation in 18S RNA hybridization by densitometry.

Discussion

Earlier observations on the effect of cyclic AMP on S49 T lymphocytes suggested that ribonucleotide reductase, a late G1 message, was transcriptionally inactivated by cyclic AMP (16). In preliminary studies, I investigated other genes expressed earlier in G1 phase to determine if events prior to the expression of ribonucleotide reductase are affected by cyclic AMP. The survey included c-*fos,* v-ki-*ras, egr*-1, c-*myb* and c-*mos.* There was no detectable expression of egr-1 in S49 cells and there was no difference in expression of v-ki-*ras,* c-*myb* or c-*mos* between exponentially growing S49 cells and cyclic AMP induced G1 arrested S49 cells. Others have shown a decrease in c-Ki-*ras* and c-*myb* as well as c-*myc* in glucocorticoid treated cells (21). There is a 50% decline in c-*fos* message during cyclic AMP induced G1 arrest. These results contrast with the dramatic decline in c-*myc* message during G1 arrest. Since *Myc* protein is also short lived (22), these observations led us to the hypothesis that cyclic AMP cell cycle arrest is the result of inhibition of c-*myc* gene expression. The c-*myc* protein product is thought to be a transcriptional activator (at least when heterodimerized with MAX) and necessary for proliferation. Indeed, in some cell lines excessive expression of c-*myc* is associated with increased viability and constitutive expression of ornithine decarboxylase mRNA (23). This hypothesis might, in turn, account for the decreased transcription of the genes encoding the two subunits of ribonucleotide reductase. This hypothesis

Table I. Percent of Cells in G1 Phase

	Control	dbcAMP (1 mM)
CEM	57.0	61.5
S49WT	54.2	85.2
D^-	37.5	84.2
KIN^-	38.2	37.9

Table II. Survival at 72 h (EC_{50})

	dbcAMP	Dexamethasone	Forskolin
	μM	*nM*	*μM*
CEM	1,800	>7,200	87
S49WT	70	38	8
D^-	450	14	50
KIN^-	780	23	300

was investigated by analyzing the mechanism of cyclic AMP inhibition of c-*myc* gene expression in a variety of different T lymphoma cell lines.

The expression of c-*myc* is an attractive target for cyclic AMP cell cycle arrest since it is closely linked to lymphoid cell proliferation. For example, c-*myc* dysregulation is associated with transformation of lymphoid malignancies and c-*myc* is expressed early in the mitogenic response of lymphocytes. Suppression of c-*myc* expression by antisense oligo nucleotides can inhibit entry into S phase (24). More recently, it has become clear that uncontrolled c-*myc* expression, while hastening the cell cycle through G1 phase shortening, (25) leads to apoptosis. The mechanism of c-*myc* induced apoptosis is unknown but can be prevented by the *bcl*-2 gene that encodes a mitochondrial protein. The physiologic down-regulation of c-*myc* involves the FOS protein which binds to a negative regulatory sequence in the c-*myc* promoter (26, 27).

I confirmed the observations of others that the c-*myc* gene is expressed at relative stable levels through the cell cycle (28). However, the precipitous decline in c-*myc* gene expression before any change in cell cycle distribution, coupled with the return of c-*myc* expression soon after removal of cyclic AMP and before G1 exit suggests that c-*myc* gene expression might indeed be closely linked to G1 cell cycle arrest. This hypothesis is strengthened by the uniform correlation of persistent c-*myc* gene expression with cyclic AMP resistance in S49 T lymphoma mutant cell lines with deficient PKA activity.

Given the similarity of c-*myc* messenger RNA concentrations when cells are exposed to cyclic AMP or actinomycin D, I was surprised by the results of the transcription run-off studies. Based on previous studies by numerous investigators I expected that the mechanism of cyclic AMP inhibition of c-*myc* gene expression would be attenuation (29–33) which usually occurs at the end of the first exon (34) (that is unexpressed) although has been reported to occur between the P1 and P2 promoter (35, 36).

The transcription run-off studies however show no significant attenuation for several hours, which is well beyond the time period when c-*myc* message concentrations become undetectable. By 1 h there appears to be a 30% decline in transcriptional initiation and an additional 10% decrease in transcript elongation. At this point the message concentration is virtually undetectable suggesting that cyclic AMP affects c-*myc* message stability in S49 cells. Since c-*myc* mRNA stability may be altered by protein binding, PKA dependent phosphorylation could influence this effect. A similar effect has been noted in glucocorticoid treated T lymphoma cells (37).

The prolongation c-*myc* message with cycloheximide pretreatment is expected since a number of rapidly turned over transcripts are stabilized by protein synthesis inhibition (38). However, the divergent response of S49 cells and CEM cells to the combination of actinomycin D and dibutyryl cyclic AMP is unanticipated. In S49 cells the two agents may be additive which would be consistent with cyclic AMP induced message degradation whereas in CEM cells the effect is the opposite. Clearly, if cyclic AMP phosphorylates a protein responsible for c-*myc* degradation in S49 cells that event does not happen in CEM cells. In fact, under these conditions the message is stabilized.

The observation that CEM cells respond to cyclic AMP by

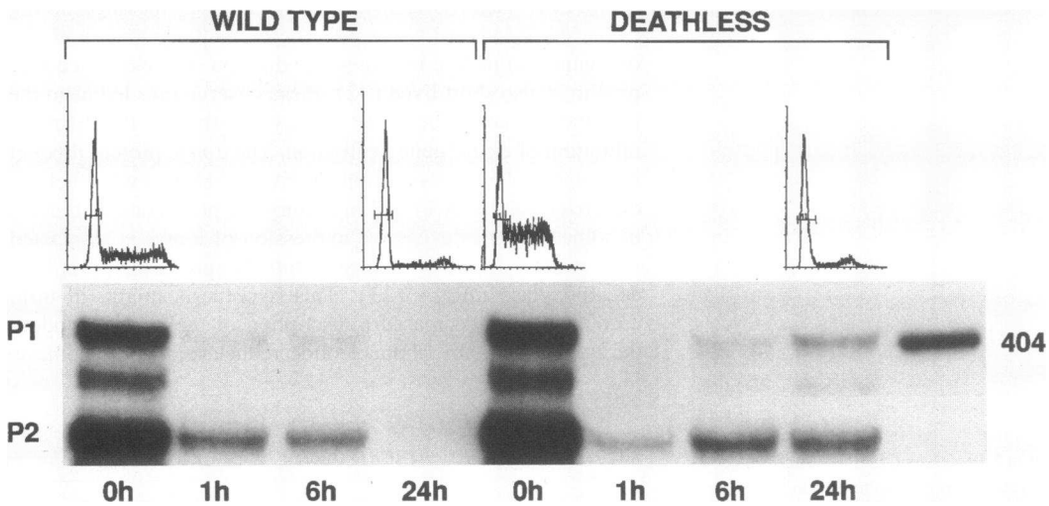


Figure 7. c-*myc* gene expression in wild-type S49 T lymphoma cells versus the mutant S49 cell line "Deathless" (D^-). Cultures containing 5 × 10^7 cells were exposed to dibutyryl cAMP (1 mM) for the times shown then harvested for RNA extraction. Aliquots were stained with propidium iodide for cytofluorimetry. The proportion of cells in G1 phase was 54% (wild type-0 h); 85% (wild type-24 h); 38% (Deathless-0 h); 84% (Deathless-24 h). The RNase protection assay was performed as described for Fig. 3. When adjusted for variation in loading there was a steep decline in c-*myc* expression in wild-type cells to undetectable levels at 24 h, whereas in Deathless cells, there is persistance of c-*myc* expression during G1 arrest.

diminishing c-*myc* gene expression while continuing to proliferate, calls into question the role of c-*myc* gene expression in T lymphocyte proliferation. Clearly, this line of T lymphoma cells appears to not require c-*myc* gene expression for continued proliferation, although we cannot exclude the possibility that very small amounts of c-*myc* messenger RNA persist and are adequate to permit proliferation. Conversely, continued expression of c-*myc* can occur under circumstances when the cells are growth arrested. For example, CEM cells exposed to cyclic AMP and actinomycin D continue to express c-*myc* but are growth inhibited (as in Fig. 6 *D*). Thus, c-*myc* expression appears to be neither necessary nor sufficient for CEM lymphocyte proliferation. Perhaps, there is some redundancy in the transcriptional activation that the *MYC* protein normally provides which would permit CEM cells to circumvent the cyclic AMP effect. Other members of the *myc* family, such as N-*myc,* which is known to be expressed in CEM cells (39), could provide this function. Further study will be necessary to determine if this hypothesis is valid. Alternatively, these CEM cells may have a block distal to the purported convergence point between cyclic AMP and glucocorticoid induced apoptosis (15) since this cell line is resistant to both agents (see Table II) although some CEM cell lines are sensitive to glucocorticoids (20). By contrast, Deathless is sixfold resistant to dbcAMP and Forskolin but sensitive to Dexamethasone (Table II). However, Deathless arrests in G1 phase as readily as wild-type S49 cells (Table I). Thus, G1 cell cycle arrest is not always associated with decreased c-*myc* expression and, conversely, decreased c-*myc* expression is not always associated with G1 cell cycle arrest.

While c-*myc* down-regulation and cyclic AMP cell cycle arrest could be mechanistically related in some cell types, it could be a protective rather than cytotoxic effect. Persistant c-*myc* expression is linked to apoptosis and, by contrast, cAMP G1 arrest is reversible (at least initially). It is possible that cAMP G1 arrest is a physiologic mechanism to down regulate lymphocyte proliferation and the down-regulation of c-*myc* expression prevents cell death and permits reversal. Preliminary data from our laboratory which supports this hypothesis is that constitutive expression of c-*myc* may be lethal to S49 cells. Analogous experiments in a human pre-B cell line Reh have also shown that constitutive expression of c-*myc* did not reverse cAMP-induced cell cycle arrest (40). Inducible expression of introduced vectors encoding the c-*myc* protein may help answer the question of whether cyclic AMP G1 cell cycle arrest in S49 cells is mechanistically linked to c-*myc* gene expression.

However, comparison of c-*myc* expression in S49 wild type cells with the Deathless mutant (41) clearly indicate that c-*myc* down regulation is not an absolute requirement for G1 cell cycle arrest in S49 cells. Furthermore, it suggests that c-*myc* down-regulation may be more closely associated with apoptosis than cell cycle arrest. In summary, the relationship of c-*myc* expression to cell cycle progression and viability is complex and appears to vary between cell types even within the lymphoid lineages. In S49 mouse T lymphoma cells cyclic AMP down-regulation of c-*myc* expression appears to be more closely related to a loss of viability than G1 cell cycle arrest.

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