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Cyclic AMP downregulates c-*myc* expression by inhibition of transcript initiation in human B-precursor Reh cells

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Abstract

In the human pre-B cell line Reh, activation of the cyclic AMP signal transduction pathway induces a rapid, transient 10-fold down-regulation of steady-state c-*myc* mRNA. We have investigated the mechanisms involved in this cAMP-mediated regulation of c-*myc* expression. Forskolin did not alter c-*myc* mRNA stability. Initiation of c-*myc* transcripts was strongly inhibited after 1 h of forskolin treatment, as measured by nuclear run-on assays. Reinitiation of c-*myc* transcription was apparent after 3–4 h, and full transcriptional elongation was detected after 8 h of forskolin treatment. These data suggest that cyclic AMP downregulates c-*myc* expression by inhibition of transcriptional initiation.

Key words: c-*myc*; Transcriptional inhibition; cAMP; B lymphocyte

1. Introduction

Cyclic AMP functions as a negative regulator of proliferation in a variety of cell types including lymphoid cells [1–4]. We have previously shown that activation of the cAMP signal transduction pathway by forskolin results in a strong G_1 arrest in the human B-precursor cell line Reh [3,5,6]. Concurrent with the growth arrest, the steady-state level of c-*myc* mRNA is transiently down-regulated 10-fold between 1 and 4 h and returns to control levels within 24 h after forskolin treatment [3,7].

The c-*myc* proto-oncogene is a nuclear phosphoprotein, the function of which is not yet fully understood. Extensive evidence suggests important roles for c-*myc* as a regulator in cell proliferation and cell differentiation (reviewed in [8]). Recent studies suggest that c-*myc* functions as a transcriptional regulator [9–11], although target genes for c-*myc* action have yet to be defined. Many studies support the notion that tight regulatory control of c-*myc* expression is required for normal cell function (discussion in [8]). c-*myc* gene expression has been shown to be regulated by mechanisms acting at the levels of transcriptional initiation, transcriptional elongation, and mRNA stability (for reviews see [8,12]).

At present, the exact mechanism for how the cAMP signal transduction pathway down-regulates c-*myc* gene expression is poorly understood. Several studies have reported that the cAMP analog dibutyryl-cAMP reduces the steady-state level of c-*myc* mRNA during HL-60 differentiation [13–15]. Schwartz and co-workers have shown that treatment with PGE_2, cholera toxin and isoproterenol all reduce the level of c-*myc* mRNA in GM-CSF treated HL60 cells [16]. In fibroblasts, forskolin down-regulates the c-*myc* message and inhibits PDGF-induced proliferation [17]. In none of these studies was the mechanism for cAMP-dependent reduction investigated in sufficient detail. This study was initiated to gain more insight into the mechanism(s) for cAMP-mediated regulation of steady-state c-*myc* mRNA expression at the molecular level. We report here that the level of c-*myc* mRNA is transiently down-regulated by a cAMP-induced block in initiation of transcription, rather than by a transcriptional elongation block mechanism.

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Abbreviations: cAMP, cyclic AMP, adenosine 3′:5′-cyclic monophosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TFGβ, Transforming Growth Factor β.

2. Materials and methods

2.1. Cells

The human precursor B cell line Reh (derived from an acute lymphoblastic leukaemia) [18] was kindly provided by M.F. Greaves (Imperial Cancer Research Fund Laboratories, London). Cells were maintained as described [6]. Cells were treated with 100 μM forskolin (Calbiochem) where indicated in the text.

2.2. RNA isolation

Total RNA was isolated from cultures of 20–50 × 10^6 cells by the guanidine isothiocyanate/CsCl gradient purification [19] or by the LiCl/urea method [20].

2.3. Northern analysis

Twenty μg of total RNA was size-fractionated in 1% agarose/2.2 M formaldehyde gels and transferred to BIOTRANS nylon filters (ICN Biomedicals Inc., USA) or to Hybond N filters (Amersham). Blots were hybridized with ^{32}P-labeled human genomic c-*myc* exon 3 [21], chicken β-actin [22] or human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [23] cDNA fragments (10^6 cpm/ml) in solution containing 50% formamide, 5 × SSC, 5 × Denhardt's, 50 mM sodium phosphate, pH 6.5, 0.1% SDS and 250 μg/ml sonicated salmon sperm DNA. Blots were washed to a stringency of 0.1 × SSC, 0.1% SDS at 50°C and subjected to autoradiography at −70°C using Amersham MP films and intensifying screens.

2.4. mRNA stability

The stability of c-*myc* mRNA was measured as the rate of RNA degradation in the presence of RNA synthesis inhibitors. Actinomycin D (Boehringer) or DRB (5′,6-Dichloro-1-D-ribofuranosyl-Benzimidazole, Calbiochem) were used at 10 μg/ml or 0.1 mM, respectively. Cells were pre-treated with forskolin for 1 h, after which Actinomycin D or DRB was added to block RNA synthesis. Cells were subsequenctly harvested after appropriate time points. Total RNA was extracted and subjected to Northern analysis (20 μg/lane). An additional series of total RNA from forskolin treated cells (30 μg/lane) was loaded on the gel due to low levels of the c-*myc* message. Suitably exposed autoradiograms were scanned with a Molecular Dynamics laser densitometer, and analyzed by Imagequant (TM) software. The levels of c-*myc* and GAPDH mRNA were calculated relative to controls. Densitometric scanning analysis should be interpreted in light of their semiquantative nature.

2.5. RNase protection assay

Experiments were conducted essentially as described elsewhere [24]. A uniformly [^{32}P]UTP-labeled antisense myc 1 exon RNA transcript was generated from a c-*myc* exon 1 *Xho*I–*Pvu*II fragment subcloned into Bluescript KS- (Stratagene Inc., USA) using T7 polymerase (Epicentre Technologies Inc., USA). Five μg total RNA from Reh cells, or 5 μg control tRNA, were hybridized with the antisense RNA transcript (5 × 10^5–1 × 10^6 cpm). Digestion and electrophoresis in 4% polyacrylamide/7.7 M urea gels were done exactly as described [24]. ØX174 *Hae*III fragments were 3′-labeled with [^{32}P]ddATP using a 3′ labeling kit (Amersham) and run as standards in the gel. Autoradiography was performed as described above.

2.6. Nuclear run-on transcription assay

Experiments were performed generally as described [25]. Nuclei were prepared by mild lysis with NP40 as follows: 20 × 10^6 cells per timepoint were washed in PBS, and pelleted at 500 × *g* for 5 min. The cell pellet was resuspended in 450 μl hypotonic buffer (10 mM Tris- HCl, pH 7.6, 10 mM NaCl, 3 mM $MgCl_2$). NP40 was added to 0.05%, the tubes were immediately mixed by inversion and centrifuged at 200 × *g* for 5 min. Nuclei were carefully resuspended in 90 μl glycerol buffer [25]. Transcription buffer [25] with 220 μCi [^{32}P]UTP (800 Ci/mmol) was added, and elongation of nascent transcripts allowed to proceed for 35 min at 30°C. Elongation was stopped by addition of 50 μl (50 U) DNase RQ (Promega Biotech) for 1 h. The nuclei were lysed and digested with 200 μl of proteinase K solution (3 μg/μl) proteinase K (Boehringer), 20 mM Tris-HCl, pH 7.5, 50 mM EDTA, 1% SDS) for 30 min at 50°C. Yeast tRNA (250 μg) was added and the labeled RNA was purified by phenol/chloroform extraction, precipitated twice with 10 M NH_4Ac and 96% ethanol at room temperature, washed in ice-cold 70% ethanol and resuspended in H_2O. Filter strips with appropriate probes (10 μg per slot, see below) were pre-hybridized in 50% formamide, 20 mM sodium phosphate, pH 6.5, 1 M NaCl, 1% SDS, 1 × Denhardt's, 10 μg/ml yeast tRNA, 50 μg/ml heparin (Novo Nordisk, Denmark), for 4 h. Equal amounts of denatured labeled nascent RNA, typically 5–10 × 10^6 Cerenkov units, were added to each filter strip and hybridization proceeded for 36–48 h at 42°C. The filters were washed twice in 2 × SSC, 0.1% SDS for 10 min at 65°C, once in 2 × SSC (subsequent washes at room temperature), once in 2 × SSC with 10 μg/ml RNase A for 30 min and 2–3 brief washes in 2 × SSC. Autoradiography was done by standard procedures at −70°C.

2.6.1. Filter preparation

Linearized plasmid probes were denatured at 95°C for 10 min, and immediately cooled on ice. An equal volume of ice-cold 20 × SSC was added, and the probes were applied to a Hybond N filter (Amersham) with the aid of a slot blot manifold (Schleicher & Schuell Minifold II), and crosslinked to the filters by UV light.

2.6.2. Probes

The following human c-*myc* genomic fragments were transferred into Bluescript KS- to ensure an identical hybridization background from the vector: exon 1 (*Xho*I–*Pvu*II; 446 bp of myc1PsS [26]); exon 2 (*Sac*I–*Sac*I; 1,533 bp of genomic c-*myc* clone [27]); exon 3 (*Cla*I–*Bcl*I; 609 bp fragment of pMC41-RC [21]). Chicken β-actin cDNA (*Pst*I–*Pst*I; 1.2 kb from pA1 [22]) was ligated into Bluescript KS-.

3. Results

To examine whether c-*myc* mRNA was down-regulated by a decrease in mRNA stability, we measured the mRNA degradation rate after addition of RNA synthesis inhibitors in control and forskolin-treated cells. Reh cells were pretreated with 100 μM forskolin for 1 h. Actinomycin D was added to a final concentration of 10 μg/ml (sufficient for complete inhibition of transcriptional activity), and RNA was isolated from control and forskolin-treated cells after 0, 20, 40 and 60 min and subjected to Northern analysis as shown in Fig. 1A. The amount of c-*myc* and GAPDH mRNA at each timepoint was plotted relative to the levels of specific message at the time of addition of Actinomycin D (0 timepoint) (Fig. 1B). Fig. 1 shows that c-*myc* mRNA stability was not dramatically decreased by forskolin treatment. Similar effects on c-*myc* mRNA stability were obtained using 5′,6-Dichloro-1-D-ribofuranosyl-benzimidazole (DRB) instead of Actinomycin D, or 8-(4-chlorophenylthio)-adenosine 3′:5′-cyclic monophosphate (8-cpt cAMP) instead of forskolin (data not shown). Relative to control cells, the steady-state c-*myc* mRNA level in forskolin treated cells was reduced to 40% within 1 h (Fig. 1A, lanes 1 and 5) and to 10% after 3–4 h [3]. Assessment of mRNA stability after treatment with forskolin for longer than one hour was therefore not possible, due to the rapid decline of the c-*myc* message.

Nuclear run-on experiments were performed to determine whether c-*myc* was down-regulated at the level of transcriptional initiation or elongation. The kinetics of regulation of steady-state c-*myc* mRNA and β-actin mRNAs by forskolin is shown in a Northern blot in Fig. 2A for comparison with the nuclear run-on data. Nuclei were isolated for in vitro elongation of RNA transcripts from control cells and from cells treated with 100 μM forskolin for 1, 4 or 8 h. In our hands, Reh cell nuclei were unusually fragile in NP40 lysis buffer, and the procedure for preparation of nuclei was therefore modified (see Section 2). We observed a 10-fold increase in [^{32}P]UTP incorporation when nuclei were prepared ac-

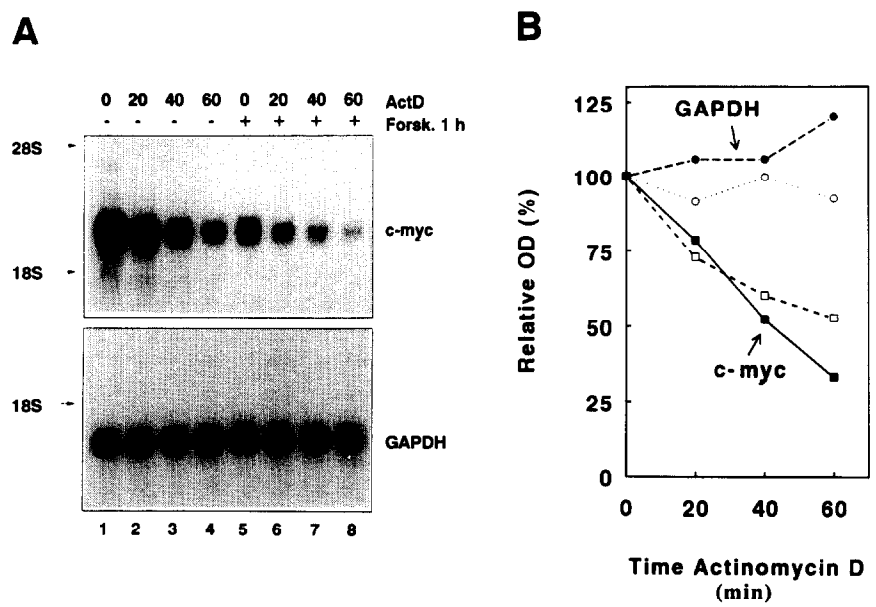

Fig. 1. Analysis of c-*myc* RNA stability. (A) Actinomycin D (10 μg/ml) was added to control cells (−) and cells pretreated with 100 μM forskolin for 1 h (+). Cells were removed after 0, 20, 40 and 60 min (as indicated at the top), total RNA was extracted by the guanidine isothiocyanate/CsCl method and subjected to Northern analysis as described in Section 2. 20 μg total RNA was loaded in each lane. The blot was hybridized with a ^{32}P-labeled c-*myc* exon 3 probe (upper panel), stripped and rehybridized with a ^{32}P-labeled GAPDH probe (lower panel) as indicated. The mobility of ribosomal 28S and 18S RNAs are indicated. (B) Suitably exposed autoradiograms of the filters shown in panel (A) were analyzed by laser densitometry as described in Section 2. Relative levels of c-*myc* and GAPDH mRNA were plotted as function of time: c-*myc* mRNA in control (■) and forskolin-treated (□) cells; GAPDH mRNA in control (○) and forskolin-treated (●) cells.

cording to the described modifications (data not shown). The diagram in Fig. 2B shows the positions of the probes used for detection of various regions of c-*myc* transcripts. Probes a, b and c correspond to exon 1, 2 and 3, respectively. Within 1 h of forskolin treatment, the hybridization signals detected by all three c-*myc* probes were strongly suppressed (Fig. 2C, lanes 1 and 2), suggesting a block in transcriptional initiation. No elevated exon 1/exon 2 signal ratio typical of an elongation block was observed, even when the signal ratio was adjusted for the difference in the number of incorporated UTPs detected by the exon 1 and exon 2 probes. This result suggests that the cAMP signal transduction pathway down-regulates c-*myc* expression by inhibiting initiation of c-*myc* transcripts.

We also investigated the kinetics of c-*myc* RNA re-expression at the transcriptional level. Transcriptional activity was measured by nuclear run-on experiments with nuclei from cells treated with forskolin for 4 or 8 h (Fig. 2C). After 4 h, initiation of nascent RNA transcript was evident (exon 1 probe), and after 8 h, c-*myc* transcriptional elongation proceeded as in control cells (Fig. 2C, lanes 1 and 4). The timepoint for reinitiation of c-*myc* transcription was determined in more detail with cells treated with 100 μM forskolin for 1, 2, and 3 h. The resulting autoradiogram (Fig. 2D) shows that reinitiation of c-*myc* transcription in exon 1 was not detected before 3 h of forskolin treatment. We did not observe a pronounced increase in the nascent exon 1/exon 2 transcript ratio, which would suggest a transcriptional elongation block mechanism acting concurrent with reinitiation of c-*myc* transcription. This possibility can not be excluded, however, as a slight increase in the exon 1/exon 2 signal ratio in nuclei from cells treated with forskolin for 3 and 4 h is reproducibly detected (Fig. 2C,D). The difference in exposure times of the autoradiography films (2 and 7 days, respectively) accounts for the apparent discrepancies between hybridization signals in Fig. 2 parts C and D. We conclude that c-*myc* transcription is reinitiated between 3 and 4 h and that full transcriptional activity is restored after 8 h of forskolin treatment.

Differential promoter usage has been described as an important mechanism for induction of a transcriptional elongation block in the c-*myc* gene [12]. The ratio of transcripts initiated from the P1 and P2 promotors has been shown to correlate with regulation of transcriptional read-through at the distal end of c-*myc* exon 1 [12]. We examined the trancriptional usage of c-*myc* promotors P1 and P2 by RNase protection assay. An exon 1 antisense *Xho*I–*Pvu*II probe distinguishes between P1 (445 nt) and P2 (350 nt) derived c-*myc* transcripts (see diagram in Fig. 3B). This probe does not distinguish between P0- and P1-derived transcripts, but mRNA transcribed from the P0 promoter is usually less than 5% of total c-*myc* mRNA [12]. Fig. 3A shows that c-*myc* P2-derived transcripts are initiated at a P1/P2 ratio of 1:2 to 1:3 in Reh cells (Fig. 3A, lane C) in accordance with previous data for lymphoid cells [28,29]. Furthermore, the P1/P2 transcript ratio was unaltered by forskolin

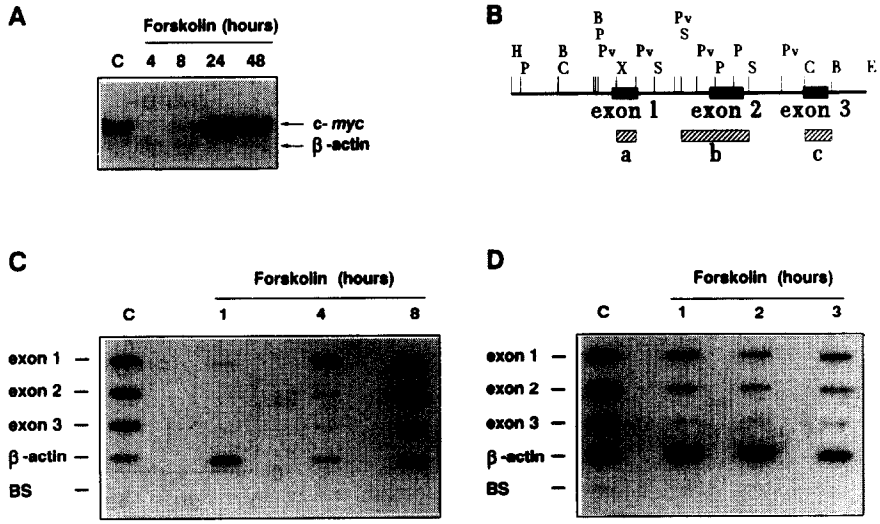

Fig. 2. Nuclear run-on analysis of c-*myc* transcription. (A) Total RNA was isolated by the LiCl/urea method from control cells (C) or cells treated with 100 μM forskolin for 4, 8, 24 or 48 h, respectively, and subjected to Northern analysis (5 μg/lane) as described in Section 2. Filters were hybridized with ^{32}P-labeled c-*myc* exon 3 *ClaI-EcoRI* or β-actin plasmid probes and subjected to autoradiography as described. Positions of c-*myc* and β-actin are indicated to the right. (B) Diagram of the 8 kb human c-*myc* gene (drawn to scale). Relevant probes and restriction sites are indicated: a, exon 1 *XhoI–PvuII*; b, exon 2 *SacI–SacI*; c, exon 3 *ClaI–BglI*. Restriction sites are: H, *Hin*dIII; E, *Eco*RI; P, *PstI*; Pv, *PvuII*; S, *SacI*; C, *ClaI* and B, *BclI*. (C) Nuclear run-on analysis of c-*myc* transcriptional activity in control and forskolin-treated cells. Nuclei were prepared from 30×10^6 control (C) Reh cells or cells treated with 100 μM forskolin for 1, 4 or 8 h. c-*myc* probes for exons 1, 2 and 3 are indicated in panel (B) as probes a, b and c, respectively. A chicken β-actin cDNA fragment in Bluescript KS- was used as control (β-actin) together with the empty vector Bluescript KS- (BS). Nuclear run-on experiments were performed as described, and detailed descriptions of the probes are given in Section 2. The autoradiograms are exposed for 2 days. Autoradiograms presented are representative for three experiments. (D) Nuclear run-on analysis of transcriptional activity of c-*myc* at 1, 2 and 3 h after forskolin addition. Nuclear run-on assay was performed as in (B), except that Reh cells were stimulated with 100 μM forskolin for 1, 2 and 3 h as indicated. Probes and other conditions were as in panel (B). The autoradiogram was exposed for 1 week.

treatment (Fig. 3A, lane F) supporting the nuclear run-on data in the notion that c-*myc* transcription is not down-regulated by the cAMP signal transduction pathway through a transcriptional elongation block mechanism.

4. Discussion

The c-*myc* gene is one of the few examples of genes (other than genes coding for components of the cAMP signal transduction pathway itself) that are negatively regulated at the transcriptional level by cAMP. Few studies have examined the effects of cAMP on the regulation of c-*myc* at the molecular level. The present study extends current data on transcriptional regulation of c-*myc* by cAMP. In the human B-precursor cell line Reh, we have shown that inhibition of c-*myc* transcript initiation occurs early (< 1 h) after forskolin treatment. Reinitiation of c-*myc* transcription is observed after 3–4 h and full transcriptional readthrough of the c-*myc* gene is restored after 8 h. Furthermore, we have shown that cAMP does not affect c-*myc* mRNA stability. Our results are generally in agreement with a recent report from Rock and colleagues that dibutyryl-cAMP down-regulated c-*myc* mRNA after 6 h by inhibition of transcriptional initiation in a murine CSF-1 dependent macrophage cell line, BAC1.2F5 [30], although our study detects a much earlier inhibitory effect on c-*myc* transcriptional initiation.

It can not be excluded that forskolin exerts its effects through other mechanisms than the cAMP signal transduction pathway (see discussion in [31]). However, the generally accepted view is that the major target for forskolin is activation of adenylate cyclase with subsequent activation of cAMP-dependent protein kinase (cAK). Transient downregulation of c-*myc* mRNA in Reh cells and similar inhibitory effects on cell growth were obtained also with the cAMP analog 8-bromo-cAMP (unpublished observations). We have also recently transfected regulatory and catalytic subunits of cAK into Reh cells, and showed that enhanced levels of human Cα catalytic subunit potentiated the growth inhibitory effects of cAMP (Taskén, K., Andersson, K.B., Erikstein, B.K., Hansson, V., Jahnsen, T., and Blomhoff, H.K., submitted for publication). It is therefore unlikely that effects observed in our cell system are due to other effects of forskolin than activation of adenylate cyclase with subsequent action of cAMP-dependent protein kinase and downstream effectors.

The best studied mechanism for rapid transcriptional control of c-*myc* expression is the transcriptional elongation block (or attenuation) mechanism. Originally identified in differentiating HL60 cells [32], successive studies have shown that this elongation blockage mechanism is a common regulatory feature in several cell

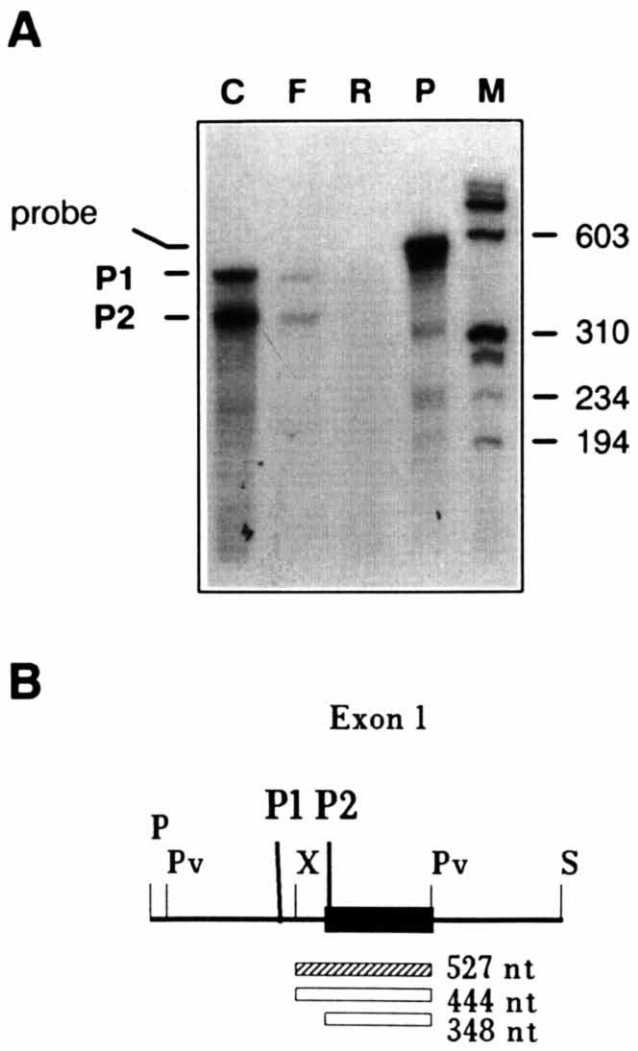

Fig. 3. RNase protection analysis of c-*myc* promoter usage in Reh cells. (A) Total RNA (5 μg) isolated from control Reh cells, or cells treated with 100 μM forskolin for 4 h, was hybridized to a ^{32}P-labeled c-*myc* exon 1 (*Xho*I–*Pvu*II) antisense probe. RNase protection assays were performed as described in Section 2. Lanes C, control cells; F, 4 h/ forskolin treatment; R, Probe hybridized with 5 μg tRNA, P: non-digested probe; M, 3′-labeled ØX174 *Hae*III fragments. The size of the ØX174 fragments are indicated to the right. Positions of undigested probe, P1 and P2 derived transcripts are indicated to the left. (B) Diagram of the relevant portion of the human c-*myc* gene showing the probe used for RNase protection assays in (A). Size of in vitro transcribed antisense probe (slanted rectangle) and expected protected fragments (open rectangles) are indicated. The size of in vitro transcribed probe includes the linker regions from the vector. The probe protects two fragments of 444 and 348 nt. which are derived from transcripts initiated at the P1 and P2 promoters, respectively. For restriction enzyme abbreviations, see legend to Fig. 2B.

systems (reviewed in [8]). In resting peripheral blood T lymphocytes, mitogens increase c-*myc* mRNA synthesis by a protein synthesis-dependent release from a transcriptional elongation block [33]. In Reh cells, cycloheximide delays the re-expression of c-*myc* mRNA after forskolin treatment (HKB, unpublished observations), suggesting that short-lived proteins are also required for renewed c-*myc* mRNA synthesis. The requirement for protein synthesis in reinitiation of c-*myc* transcription in Reh cells is paralleled by the release of the elongation block in T cells. In contrast to the T cell study however, our analysis of promotor usage and the nuclear run-on data in Reh cells supports the conclusion that renewal of c-*myc* RNA synthesis is due to transcript reinitiation rather than release from an elongation block.

TGFβ is the only other reported agent in addition to inducers or agonists of cAMP, that induces a similar transient down-regulatory effect on c-*myc* mRNA by blocking c-*myc* transcriptional initiation (< 1 h) in BALB/MK keratinocytes [34]. The cAMP and TGFβ signal transduction pathways that regulate c-*myc* expression by regulating transcriptional initiation activity, may define a separate major pathway for regulation of c-*myc* expression, distinct from signal inducers that regulate transcriptional attenuation.

Despite efforts from many groups in defining the exact mechanisms for regulating transcriptional initiation of the c-*myc* gene, this question is not resolved. However, several *cis*-acting elements in the promoters of the c-*myc* gene itself have recently been identified (discussed in [8]). Several studies provide evidence for the involvement of both the retinoblastoma gene product pRb and the transcription factor E2F in regulation of c-*myc* gene expression [34,35]. Both the E2F element 5′ to the P2 promoter and the TGFβ responsive element (TCE) 5′ to the P1 promoter have been shown to confer repression of transcript initiation in the c-*myc* promoter by overexpression of pRb. Since the c-*myc* promoter does not contain conventional cAMP-responsive elements, we have tested both c-*myc* P1 (189 bp) and P2 (165 bp) promoter fragments, and oligonucleotides for the TCE and E2F elements in electrophoretic mobility shift assays. We were unable to detect any changes in proteins binding to any of these elements in nuclear extracts from cells treated with forskolin for various time periods (K.B.A., data not shown). We speculate that a non-prototype cAMP-responsive element may lie at a greater distance from the c-*myc* promoter, or that cAMP-mediated events may affect other interactions than the DNA-binding properties of protein complexes binding to the c-*myc* promoter.

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References

[1] Boynton, A.L. and Whitfield, J.F. (1983) in Advances in Cyclic Nucleotide Research (Greengard, P. and Robinson, G.A., Eds.) pp. 192–294, Raven, New York.
[2] Muraguchi, A., Miyazaki, K., Kehri, J.H. and Fauci, A.S. (1984) J. Immunol. 133, 1283–1287.
[3] Blomhoff, H.K., Smeland, E.B., Beiske, K., Blomhoff, R., Ruud,

E., Bjøro, T., Pfeifer-Ohlsson, S., Watt, R., Funderud, S., Godal, T. and Ohlsson, R. (1987) J. Cell. Physiol. 131, 426–433.
[4] Skålhegg, B.S., Landmark, B., Døskeland, S.O., Hansson, V., Lea, T. and Jahnsen, T. (1992) J. Biol. Chem. 267, 15707–15714.
[5] Blomhoff, H.K., Ruud, E., Funderud, S., and Godal, T. (1986) Biochim. Biophys. Acta 887, 150–156.
[6] Blomhoff, H.K., Blomhoff, R., Stokke, T., DeLange Davies, C., Brevik, K., Smeland, E.B., Funderud, S. and Godal, T. (1988) J. Cell. Physiol. 137, 583–587.
[7] Lømo, J., Holte, H., DeLange Davies, C., Ruud, E., Laukas, M., Smeland, E.B., Godal, T. and Blomhoff, H.K. (1987) Exp. Cell Res. 137, 583–587.
[8] Marcu, K.B., Bossone, S.A. and Patel, A.J. (1992) Annu. Rev. Biochem. 61, 809–860.
[9] Amati, B., Dalton, S., Brooks, M.W., Littlewood, T.D., Evan, G.I. and Land, H. (1992) Nature 359, 423–426.
[10] Kretzner, L., Blackwood, E.M. and Eisenman, R.N. (1992) Nature 359, 426–429.
[11] Amin, C., Wagner, A.J. and Hay, N. (1993) Mol. Cell. Biol. 13, 383–390.
[12] Spencer, C.A. and Groudine, M. (1991) Adv. Cancer Res. 56, 1–48.
[13] McCachren, S.S.J., Nichols, J., Kaufman, R.E. and Niedel, J.E. (1986) Blood 68, 412–416.
[14] Trepel, J.B., Colamonici, O.R., Kelly, K., Schwab, G., Watt, R.A., Sausville, E.A., Jaffe, E.S. and Neckers, L.M. (1987) Mol. Cell Biol. 7, 2644–2648.
[15] Slungaard, A., Confer, D.L. and Shubach, W.H. (1987) J. Clin. Invest. 79, 1542–1547.
[16] Schwartz, E.L., Chamberlain, H. and Brechbühl, A.-B. (1991) Blood 77, 2716–2723.
[17] Heldin, N.-E., Paulsson, Y., Forsberg, K., Heldin, C.H. and Westermark, B. (1989) J. Cell Physiol. 138, 17–23.
[18] Rosenfeld, C., Goutner, A., Choquet, C., Venuat, A.M., Kayibanda, B., Pico, J.L. and Greaves, M.F. (1977) Nature 267, 841–843.
[19] Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistr 18, 5294–5299.
[20] Auffray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303– 314.
[21] Dalla-Favera, R., Gelmann, E.P., Martinotti, S., Franchini, G., Papas, T.S., Gallo, R.C. and Wong-Staal, F. (1982) Proc. Natl. Acad. Sci. USA 79, 6497–6501.
[22] Cleveland, D.W., Lopata, M.A., MacDonald, R.J., Cowan, N.J., Rutter, W.J. and Kirschner, M.W. (1980) Cell 20, 95–105.
[23] Tso, J.Y., Sun, X.H., Kao, T.H., Reece, K.S. and Wu, R. (1985) Nucleic Acids Res. 13, 2485–2502.
[24] Andersson, K.B., Deggerdal, A.D., Skjønsberg, C., Smeland, E.B. and Blomhoff, H.K. (1993) J. Cell. Physiol. 157, 61–69.
[25] Greenberg, M.E. and Ziff, E.B. (1984) Nature 311, 433–438.
[26] Chung, J., Sussman, D.J., Zeller, R. and Leder, P. (1987) Cell 51, 1001–1008.
[27] Battey, J., Moulding, C., Taub, R., Murphy, W., Stewart, T., Potter, H., Lenoir, G. and Leder, P. (1983) Cell 34, 779–787.
[28] Broome, H.E., Reed, J.C., Godillot, E.P. and Hoover, R.G. (1987) Mol. Cell Biol. 7, 2988–2993.
[29] Spencer, C.A., LeStrange, R.C., Novak, U., Hayward, W.S. and Groudine, M. (1990) Genes Dev. 4, 75–88.
[30] Rock, C.O., Cleveland, J.L. and Jackowski, S. (1992) Mol. Cell. Biol. 12, 2351–2358.
[31] Scott, J.D. (1991) Pharmacol. Ther. 50, 123–145.
[32] Bentley, D.L. and Groudine, M. (1986) Mol. Cell Biol. 6, 3481–3489.
[33] Lindsten, T., June, C.H. and Thompson, C.B. (1988) EMBO J. 7, 2787–2794.
[34] Pietenpol, J.A., Münger, K., Howley, P.M., Stein, R.W. and Moses, H.L. (1991) Proc. Natl. Acad. Sci. USA 88, 10227–10231.
[35] Hiebert, S.W., Chellappan, S.P. Horowitz, J.M. and Nevins, J.R. (1992) Genes Dev. 6, 177–185.