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Serum-Inducible Expression of Transfected Human c-*myc* Genes

ADAM RICHMAN AND ADRIAN HAYDAY*

Department of Biology, Yale University, New Haven, Connecticut 06511-8112

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Activation of the c-*myc* proto-oncogene is implicated in the initiation or progression of many vertebrate cancers. In nontransformed cells, the expression of c-*myc* is induced by growth factors. Studies have indicated that such induction is effected by multiple mechanisms. To study regulation of c-*myc* expression, a transfection system has been developed in which introduced c-*myc* genes exhibit serum-responsive activity. The responsiveness assayed is not mediated by increased transcription initiation. Rather, it is effected at a point(s) between transcription and stabilization of the RNA.

The c-*myc* proto-oncogene is the prototypic member of the conserved multigene *myc* family that includes the proto-oncogenes N-*myc* and L-*myc* (53). The gene products of this family exhibit sequence motifs common to proteins involved in cellular differentiation. These include the products of the *achaete-schute* and *daughterless* loci of *Drosophila melanogaster* and the *myoD* gene of vertebrates (10, 34, 45, 49). Whereas the activated c-*myc* oncogene is implicated in the initiation or progression of a wide range of vertebrate malignancies (11, 13), there is now much experimental evidence that proper regulation of c-*myc* proto-oncogene expression is important in the normal growth and differentiation of vertebrate cells (1, 12).

Ordinarily, expression of both c-*myc* mRNA and protein is low in resting cells and is increased shortly after administration of serum growth factors (7, 9, 15, 19, 24, 25). The importance of c-*myc* induction is suggested by the capacity of constitutively expressed c-*myc* genes to partially substitute for the addition of growth factors in stimulating proliferation of quiescent cells (1). c-*myc* gene expression is controlled by multiple mechanisms, including transcription initiation (19, 36, 42), attenuation (5, 35), and stability of c-*myc* mRNA (7, 14, 26, 27, 36, 37). Previous studies using various cell types have shown that a combination of these and other mechanisms act to modulate c-*myc* expression in response to a physiological stimulus (19, 36, 42). A detailed understanding of how mitogens may regulate c-*myc* activity requires a transfected cell system in which the expression of introduced c-*myc* genes is regulated in response to serum growth factors. This paper describes such a system.

MATERIALS AND METHODS

DNA cloning. The cloning and analysis of c-*myc* from normal human cells have been described previously (40). An *Eco*RI fragment containing the normal allele was inserted in both orientations into the vector pSV2.*gpt* (33), which encodes the *Escherichia coli gpt* gene (conferring resistance to mycophenolic acid) under the control of the simian virus (SV40) early promoter (Fig. 1).

Cells and tissue culture. FR3T3 rat fibroblasts (41) were grown on Nunc tissue culture dishes in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum (FCS). Cells were starved by maintaining confluent cultures in Dulbecco modified Eagle medium plus 1% FCS for 4 days. For serum stimulation, starved cultures were incubated for 1 h in medium containing 15% FCS. Human lymphoma cell lines A. W. Ramos and Manca (52) were grown in RPMI medium containing 10% FCS and 2-mercaptoethanol.

Isolation of transfected cell lines. Subconfluent cultures of FR3T3 cells were transfected with plasmid DNA by the calcium phosphate method (18, 50). Stable cell lines were derived from mycophenolic acid-resistant colonies expanded after isolation within cloning cylinders. No attempt was made to establish clonal cell lines by limiting dilution. Stably transfected cells could be maintained in the absence of selective medium.

Tumorigenicity testing. Subconfluent cells were washed, trypsinized, and suspended in phosphate-buffered saline. A total of 1×10^5 to 2×10^6 cells were injected subcutaneously into young male syngeneic Fisher rats. The animals were monitored for tumor development over a 3-month period.

Southern analysis. For each sample, 10 μg of *Eco*RI-digested genomic DNA was electrophoresed through 0.7% agarose, transferred to nitrocellulose (43), and hybridized to a nick-translated (39) human c-*myc* probe, a 1.4-kilobase-pair (kbp) *Cla*I-*Eco*RI fragment containing exon 3 and 3′ untranslated sequences (40).

RNA analysis. Total cellular and cytoplasmic RNA samples were prepared as described previously (29). RNA samples were quantitated by spectrophotometric analysis and ethidium staining of agarose gels (not shown). RNase protection assays were performed essentially as described previously (54), using high-specific-activity T7 RNA polymerase (Promega Biotec) transcripts as probes (the specificity of the probes is demonstrated by the failure of the human probe to detect rat *myc* transcripts from line gpt4 and by the failure of the rat probe to detect human *myc* transcripts from the human Burkitt's lymphoma cell line A. W. Ramos [see Fig. 4]). RNase-digested products were fractionated on polyacrylamide-urea sequencing gels. For S1 nuclease protection assays, probe preparation, annealing, S1 enzyme digestion, and analysis of protected products were performed as described previously (21). For Northern (RNA) analysis, samples were electrophoresed, transferred to nitrocellulose as previously described (8), and hybridized to nick-translated probes: human c-*myc* *Cla*I-*Eco*RI and a rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA clone (17).

Determination of cellular DNA content. Fibroblast cell cultures were trypsinized, fixed in paraformaldehyde, stained with propidium iodide (C. D. Gregory and A. B. Rickinson, personal communication), and analyzed on a Becton Dickinson FACS analyzer.

* Corresponding author.

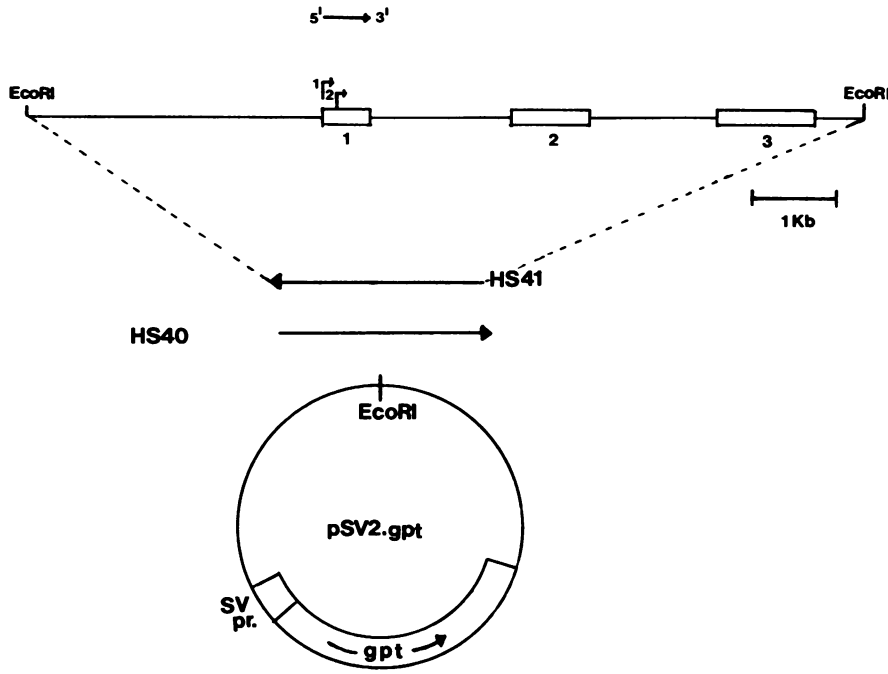

FIG. 1. Structure of human c-*myc* gene and constructs. ▭, c-*myc* exons. The normal c-*myc* gene is represented by an ~10.4-kbp *Eco*RI genomic fragment (the 5' *Eco*RI site was created in vitro). The two normal c-*myc* promoters P1 and P2 are shown. Plasmids pHS40 and pHS41 were constructed by insertion of the fragment, in the indicated orientations, into the *Eco*RI site of the vector pSV2.*gpt* (33). Plasmid pSV2.*gpt* contains the bacterial *gpt* transcription unit driven by the SV40 early-region promoter-enhancer (SV pr.).

Nuclear Run-on assays. Isolation of nuclei and run-on transcription were performed by using a modification of the procedure of Linial et al. (28). Briefly, $>5 \times 10^7$ cells were washed, lysed in buffer containing Nonidet P-40, and Dounce homogenized, and the nuclei were treated with RNase A for 30 min. RNasin was added, and the nuclei stored at −70°C. For run-on analyses, the nuclei were thawed on ice, and transcription was allowed to proceed in the presence of [^{32}P]UTP. Labeled transcripts were hybridized to immobilized DNA probes for 48 h at 65°C. Filters were washed at 65°C in 2× SSC (SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate for 1 h and then in 0.1× SSC–0.1% sodium dodecyl sulfate for an additional 1 h. Exposure times were 2 to 7 days at −70°C. The human c-*myc* probes used in these experiments are single-stranded M13 clones kindly provided by M. Groudine and described elsewhere (4, 5). The gapdh and gpt probes are double-stranded plasmids. Single-stranded M13 DNA probes (0.6 to 1.0 μg) or double-stranded plasmid DNA (2.0 μg) was slot blotted onto nitrocellulose after alkaline denaturation at 65°C.

RNA stability determinations. Actinomycin D (Boehringer Mannheim Biochemicals) was added to cells at a final concentration of 5 μg/ml. Total cellular RNA was extracted from treated cultures at various time intervals after inhibition of transcriptional activity and analyzed by RNase protection assays.

RESULTS

Recombinant vectors HS40 and HS41, containing normal human c-*myc* genes (Fig. 1), were introduced into early-passage FR3T3 rat fibroblasts (41). FR3T3 cells arrest growth at confluence in low concentrations of serum and are not transformed by activated c-*myc* alleles (32). As is often the case after calcium phosphate transfection (50), cell lines were derived that had assimilated low (hf.1) or high (hf.10) copy numbers of the exogenously added *myc* sequences (Fig. 2). Stably transfected cell lines exhibiting *Eco*RI fragments diagnostic of full-length (10.4 kbp) c-*myc* inserts (Fig. 2) were studied further. Lines gpt3 and gpt4, transfected by the pSV2.*gpt* parent vector alone, were analyzed as controls.

All transfectants examined in detail behaved like the parental early-passage FR3T3 cells by arresting growth at confluence and by maintaining a 2*n* DNA content during extended periods of serum starvation (Fig. 3). None of the lines examined produced tumors during a 3-month period after subcutaneous injection into young syngeneic Fisher rats (data not shown).

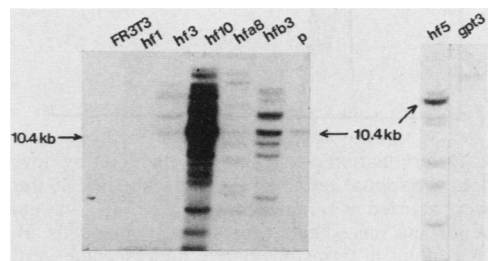

FIG. 2. Southern analysis of human c-*myc* sequences in transfected cell lines. *Eco*RI-digested genomic DNA was analyzed from parental cell line FR3T3, pHS40 transfectants (lanes hf1, hf3, hf10, and hf5), pHS41 transfectants (lanes hfa8 and hfb3), and a control pSV2.*gpt* transfectant (lane gpt3). Lane p is a control *Eco*RI digest of 10 pg of pHS40 plasmid DNA. Under conditions of reduced stringency, the endogenous rat *myc* loci of ~18 kbp are evident (lane gpt3) (44). The probe was a human c-*myc* *Cla*I-*Eco*RI fragment (see Materials and Methods).

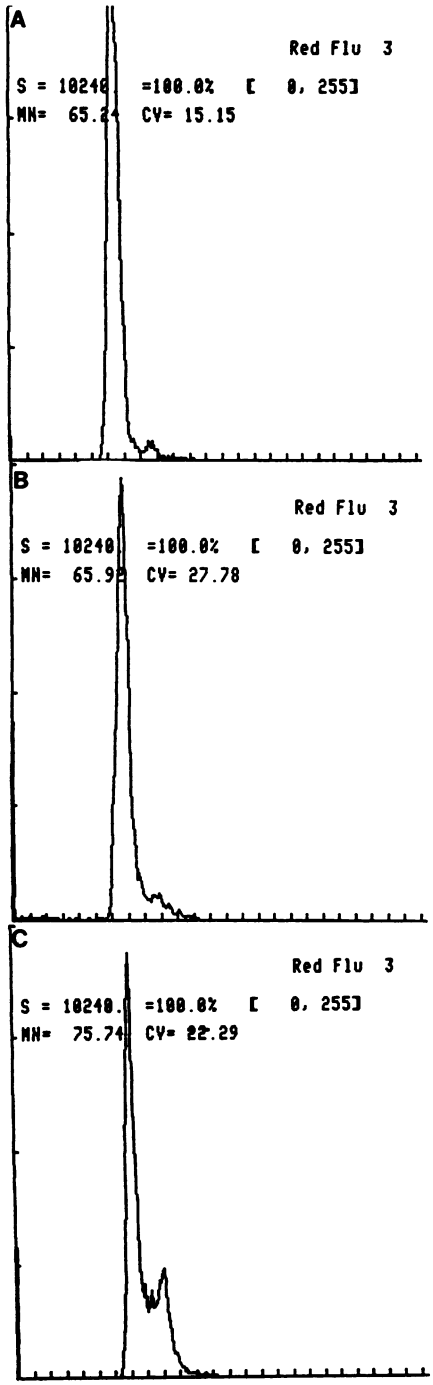

FIG. 3. Determination of cellular DNA content by flow cytometry. Cultures of parental cell line FR3T3 (A) and pHS40 transfectant hf10 (B) were starved at confluence. Culture r2 (C) was analyzed at subconfluence and served as a control for cycling cells. Horizontal axis, DNA content in terms of relative fluorescence: vertical axis, cell number.

Expression of the transfected c-*myc* genes in response to serum growth factor deprivation and subsequent serum stimulation was studied in all cell lines. Total cellular or cytoplasmic RNA was prepared from confluent cultures after 4 days in 1% serum (serum starved) or at 1 h after treatment of such cells with medium containing 15% serum (serum-stimulated). RNase protection analyses (54) showed that the levels of human c-*myc* mRNA were modulated in response to serum in all lines (Fig. 4). Induction of c-*myc* mRNA was quantitated by scanning densitometry (Table 1). The induction of human c-*myc* expression was similar whether measured in total or cytoplasmic RNA (Fig. 4 and Table 1). The serum inducibility of transfected genes was comparable to that shown by the endogenous rat c-*myc* genes (Fig. 4 and Table 1). Growth factor-responsive expression of c-*myc* occurred in the presence of cycloheximide (data not shown). Furthermore, the inducible expression of the transfected genes exhibited kinetics of induction similar to that previously reported for the growth factor response of endogenous *myc* genes: a significant increase in RNA levels, peaking between 1 and 2 h after serum addition (see Fig. 7, lanes 11 to 14).

TABLE 1. Quantitation of c-*myc* serum induction[a]

c-*myc* locus	Cell line	Fold induction[b] at promoter:	
		P1	P2
Human	hf10	6.5	8.1
	hfb3(T)	ND	21.5
	hfb3(C)	ND	12.8
	hf1	ND	13.8
Rat	hfb3(T)		6.6
	hfb3(C)		12.8
	FR3T3		2.1
	gpt4		3.7
	hf1		4.6
	hf10		1.9

[a] Autoradiograms of RNase protection assays shown in Fig. 4 were quantitated by scanning densitometry. Human and rat c-*myc* induction levels were analyzed in the same experiment.

[b] Ratio, serum-stimulated/serum-starved cultures. Expressed in arbitrary units. ND, Not determined.

Quantitative S1 analysis using an end-labeled DNA probe (21) mapped the transcripts of the introduced genes to the normal human c-*myc* promoters, P1 and P2 (2, 40) (Fig. 5). A minor but consistent start site was noted 2 bases 3′ of P2 (arrow in Fig. 5). The serum-responsive expression of the transfected genes was observed with S1 probes specific both for the 5′ end of the gene (Fig. 5) and for intron 1-exon 2 boundary sequences (data not shown). Northern analysis using a human *myc* probe and high-stringency hybridization conditions confirmed that the transfected lines expressed inducible human c-*myc* mRNA of the expected size (2.2 to 2.4 kbp [2, 40]) (Fig. 6). Together, these data indicate that the inducible expression is reflective of the behavior of full-length c-*myc* transcripts and is unlikely to arise from subgenomic or attenuated transcripts (5, 6, 35). The latter are apparently highly labile and are readily not detected in FR3T3 cells (E. Gardiner and A. Hayday, unpublished observations).

The induction of c-*myc* genes was specific, as judged by the constitutive expression of the rat GAPDH gene (17) (Fig. 6). The specific serum stimulation of transfected *myc* gene expression was observed in every line of cells examined, regardless of gene copy number or site of integration, and was independent of the orientation of the *myc* gene on the transfecting vector DNA.

P2 usage was favored in the expression of both the transfected and endogenous genes (Fig. 4 and 7) (by contrast, P1-derived transcripts were frequently overrepresented in tumor cells such as A. W. Ramos [Fig. 7]). P1

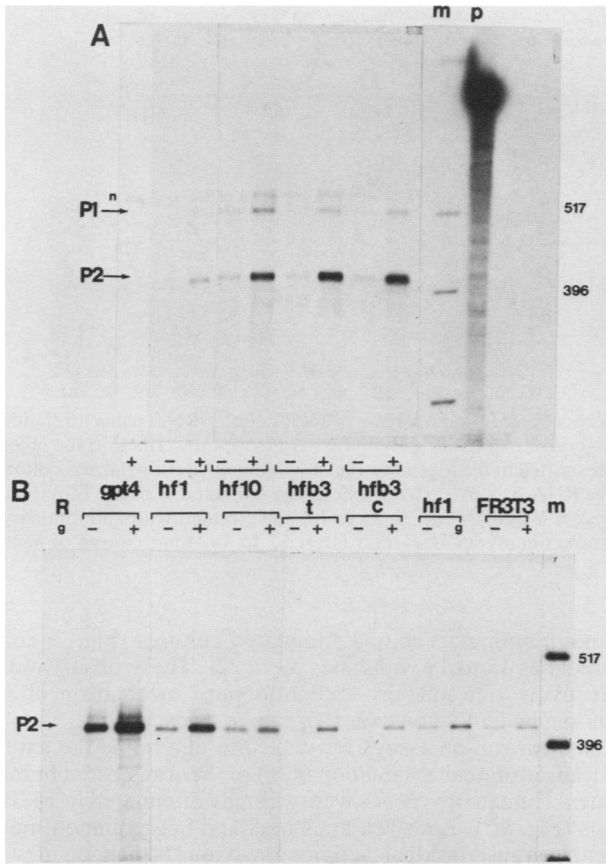

FIG. 4. Analysis of serum-regulated c-*myc* expression by RNase protection. Each assay contained 10 μg of RNA. Cell lines are described in the legend to Fig. 2. Lanes gpt4 represent a control line transfected with the pSV2.*gpt* vector. Total cellular RNA was isolated from confluent serum-starved (−) and serum-stimulated (+) cultures except for lanes designated hfb3 c, in which cytoplasmic RNA was assayed. R g, Total RNA from the human Burkitt's lymphoma cell line A. W. Ramos; hf1 g, total RNA from a subconfluent, growing culture. P1 and P2 designate protected products derived from the normal *myc* promoters. n, Nonspecific protection; m, end-labeled markers derived from a *Hin*fI digest of plasmid pAT153 (sizes [in nucleotides] of relevant fragments are given on the right); p, undigested human c-*myc* probe. (A) Protection of transfected human *myc* transcripts. An 876-nucleotide antisense probe specific for human c-*myc* mRNA was prepared by T7 polymerase transcription of a *Pst*I-*Sac*I fragment containing human c-*myc* exon 1 cloned in the Bluescript vector (Stratagene). (B) Transcripts arising from the endogenous rat c-*myc* genes analyzed by using an antisense probe produced by transcription of a 1.4-kbp fragment containing rat *myc* exon 1 cloned in pGem4 (Promega Biotec) (a gift of David Steffen).

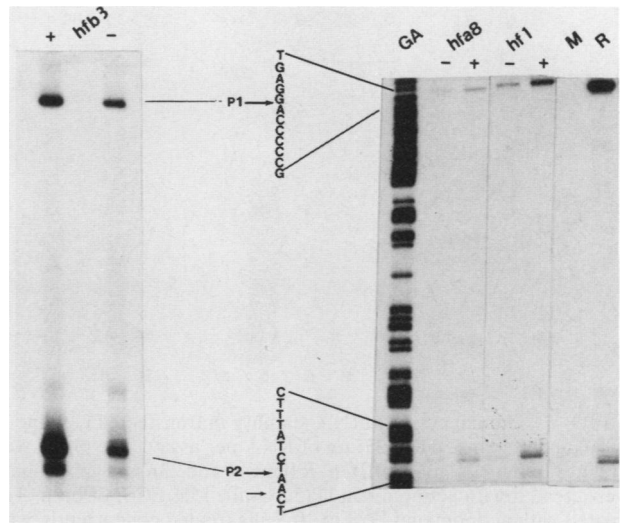

FIG. 5. S1 nuclease protection. The fibroblast clones analyzed are described in the legend to Fig. 2. Total RNA was isolated from confluent serum-starved (−) and serum-stimulated (+) cultures. M, RNA derived from the human Burkitt's lymphoma line Manca, which does not transcribe c-*myc* exon 1 and served as a negative control; R, Burkitt cell line A. W. Ramos, expressing full-length c-*myc* transcripts. Equal amounts of RNA were assayed in each lane. The human c-*myc* probe is a single-stranded ^{32}P-end-labeled *Sma*I-*Aha*II fragment of 354 nucleotides that extends across both P1 and P2, yielding protected fragments of 254 and 92 nucleotides, respectively. Reactions were electrophoresed in parallel with the products of a G+A sequencing reaction performed on the end-labeled probe DNA (GA) (31). Transcription initiation sites are indicated (P1, P2, and small arrow).

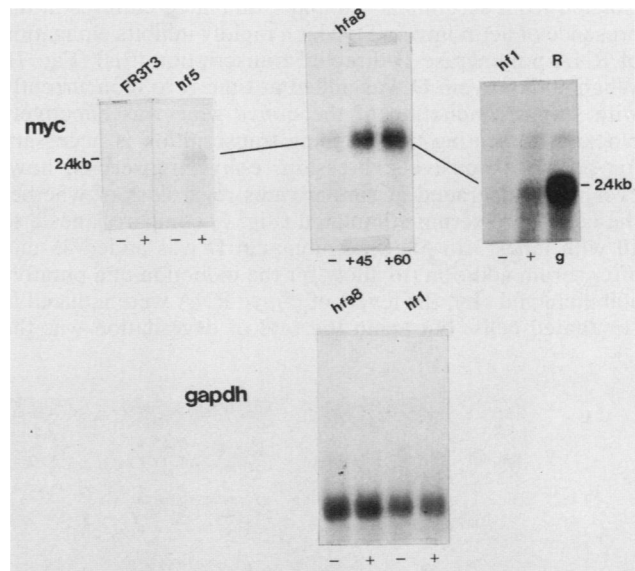

FIG. 6. Northern analysis. The fibroblast cultures analyzed are described in the legend to Fig. 2. R, A. W. Ramos cell line. Each lane contained 10 μg of total cellular RNA isolated from confluent serum-starved (−) or serum-stimulated (+) cultures. RNA was extracted from line hfa8 at both 45 and 60 min after serum addition. Filters were probed with a human c-*myc* *Cla*I-*Eco*RI fragment or a rat GAPDH cDNA clone.

usage by transfected genes was consistently higher than that observed from the endogenous loci. This finding may reflect a partial deregulation of P1 activity by transfection. P1 expression remained serum responsive, however (Fig. 4, 5, and 7).

To determine whether the increased expression of the transfected human c-*myc* genes after serum stimulation was due to increased transcription initiation, nuclear run-on assays were performed on nuclei isolated from confluent, serum-starved, and serum-stimulated cultures (Fig. 8 and 9). There was substantial transcription of exon 1 sequences 4 days after serum starvation, which was not significantly

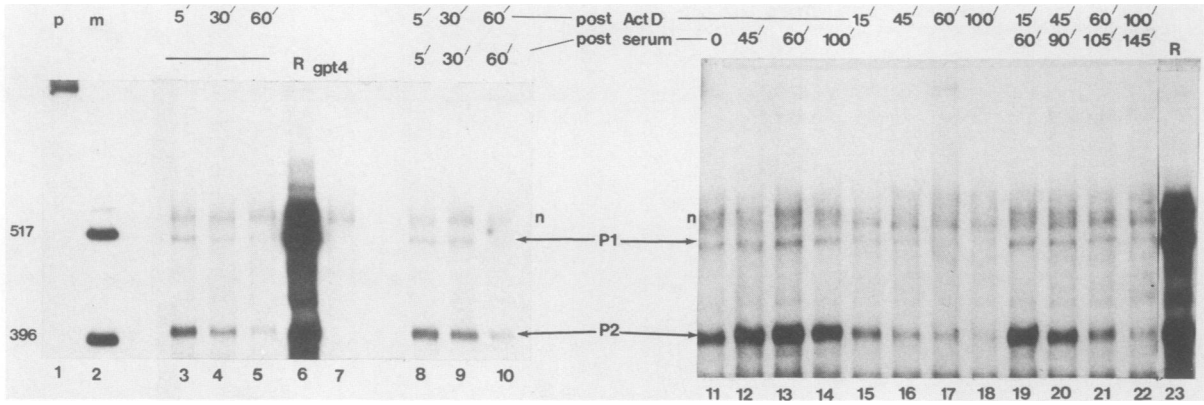

FIG. 7. Human c-*myc* mRNA stability in transfected cell line hfa8, by RNase protection analysis of total cellular RNA using 15 (lanes 3 to 10) and 10 (lanes 11 to 23) μg of RNA per assay. The probe was a human c-*myc* exon 1 T7 polymerase transcript described in the legend to Fig. 4. p (lane 1), m (lane 2), n, R (lanes 6 and 23), and gpt4 (lane 7) are as described in the legend to Fig. 4. Confluent serum-starved cultures were treated with actinomycin D (5 μg/ml), 15% FCS, or both. Total cellular RNA was extracted at the times indicated. Lanes: 3 to 5, cells treated with actinomycin D; 8 to 10, cells treated concurrently with actinomycin D and 15% FCS; 15 to 18, cells treated with actinomycin D only; 19 to 22, cells stimulated with 15% FCS, followed by treatment with actinomycin D 45 min later; 11 to 14, time course of serum stimulation after addition of 15% FCS.

increased by serum addition. Therefore, the serum induction of c-*myc* mRNA levels cannot be attributed to increased transcription initiation. These observations are consistent with data obtained by using c-*myc* promoter-driven reporter genes in FR3T3 cells (A. Zervos and A. Hayday, manuscript in preparation). By contrast, the transcriptional activity of the transfected *E. coli gpt* gene, directed by the SV40 enhancer-promoter cassette, was reproducibly stimulated by serum (Fig. 8 and 9). Hence, expression of transfected human *myc* is independent of the transcriptional activity of the SV40 enhancer.

To test whether the serum stimulation of human c-*myc* genes was due to differential stabilization of mRNA, RNase protection analyses were performed on total cellular RNA isolated from serum-starved and -stimulated cultures in the presence of actinomycin D (which rapidly inhibits elongation of RNA polymerase II-directed transcription [51]) (Fig. 7). When actinomycin D was added at time zero (concurrently with serum), induction of the c-*myc* gene was effectively blocked, indicating that ongoing transcription is necessary for serum-responsive expression. c-*myc* transcripts, however, were degraded at similar rates regardless of whether the cells were serum stimulated (Fig. 7, compare lanes 8 to 10 with lanes 3 to 5). If actinomycin D was added 45 min after serum addition (to allow for the induction of a putative stabilizing factor), the levels of c-*myc* RNA were induced in stimulated cells, but again the rate of degradation was the same in both starved and stimulated cultures (Fig. 7; compare lanes 15 to 18 with lanes 19 to 22). These observations effectively rule out any straightforward mechanism of serum-dependent stabilization of c-*myc* transcripts.

Nuclear run-on assays were used to determine the extent of transcriptional attenuation (4, 5) of the transfected human genes. Human *myc* genes were strongly attenuated in FR3T3 cells (Fig. 9C). Both cell lines hfa8 and hf1 exhibited much greater transcriptional activity on exon 1 than on downstream intron 1 or exon 2 sequences. This profile of polymerase density is characteristic of c-*myc* attenuation (4, 5). The transcriptional activity detected by the human *myc* probes was specific, since no *myc* hybridization was detected in run-on assays performed on nuclei isolated from line gpt3 transfected with the pSV2.*gpt* vector alone (Fig. 9B). There was evidence that relief of attenuation contributed to exogenous *myc* gene induction. Treatment of starved cells with serum resulted in enhanced transcriptional activity on intron 1 and exon 2 sequences in the absence of increased exon 1 transcriptional activity (Fig. 9C). In cell line hfa8, serum treatment clearly stimulated transcription of antisense RNA in every region of the *myc* gene analyzed. The significance of this observation is presently unknown. A definitive assessment of the degree to which attenuation relief accounts for serum stimulation of c-*myc* mRNA levels awaits an analysis of the serum response of mutant *myc* gene constructs defective in attenuation.

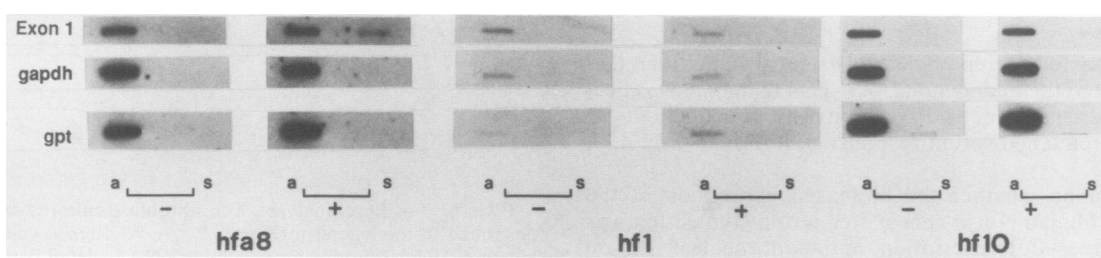

FIG. 8. Nuclear run-on transcription in serum-starved (−) and serum-stimulated (+) transfected fibroblast cultures. Isolation of nuclei and run-on transcription were performed essentially as described previously (28). Labeled products hybridized to single-stranded M13 c-*myc* exon 1 probes (see Fig. 9A). Clone s detects products of sense transcription; clone a detects antisense transcripts. Rat gapdh and bacterial gpt probes are derived from double-stranded plasmid DNA.

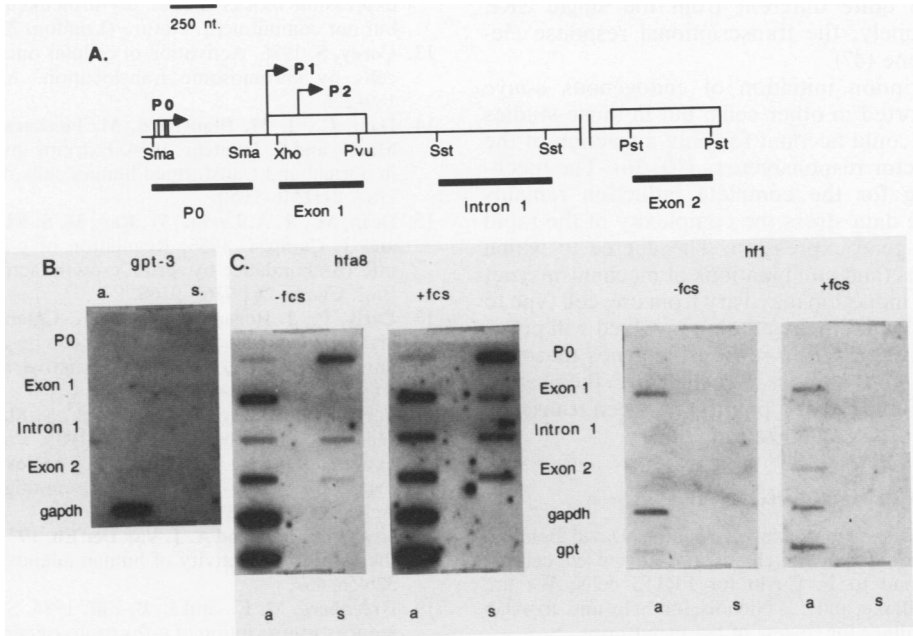

FIG. 9. Attenuated transcriptional activity of transfected human c-*myc* genes in isolated fibroblast nuclei. Labeled products of transcriptional elongation reactions were hybridized to immobilized DNA probes representing different regions of the human c-*myc* gene. Attenuation of c-*myc* transcription is manifested by a greater density of polymerase molecules on exon 1 sequences than on intron 1 or exon 2 sequences. (A) Single-stranded M13 probes corresponding to different regions of human c-*myc* (a gift of Mark Groudine) are indicated by bold lines beneath a partial map of the human c-*myc* gene. Both antisense (a) and sense (s) clones from each region were used, detecting sense and antisense transcription, respectively. Probe sizes (in nucleotides; uridine residues in sense transcripts): P0 (535; 134); exon 1 (443; 74); intron 1 (606; 135); exon 2 (414; 68). (B) Hybridization of labeled run-on transcription products from control cell line gpt3, transfected with the pSV2.*gpt* parent vector only, demonstrating species specificity of the human c-*myc* probes used in these experiments. The gapdh probe serves as a constitutive control and indicates that run-on transcription indeed occurred in this assay. (C) The attenuated transcriptional response to serum starvation (−fcs) and stimulation (+fcs) in human *myc*-transfected cell lines hfa8 and hf1. Confluent cell cultures were starved in medium containing 1% serum and stimulated for 1 h with 15% serum. Nuclei were isolated in parallel from starved and stimulated cells, and run-on transcription assays were performed simultaneously. The transcriptional activity of the SV40 promoter-enhancer-driven bacterial *gpt* gene (gpt) is also shown.

DISCUSSION

It is shown here that stably assimilated normal c-*myc* genes are serum responsive in FR3T3 rat fibroblasts. Expression was accurately initiated at both major *myc* promoters, and expression of RNA from promoter P2 in particular was induced in every clone examined by amounts comparable to that of the endogenous rat c-*myc* genes. The kinetics of induction were also entirely consistent with those reported for endogenous *myc* genes. Hence, the transfected DNA contains one or more functional serum-responsive elements (SREs).

Expression of endogenous c-*myc* genes can be induced in vertebrate cells by the addition of serum growth factors. The induction occurs at both the RNA and protein levels and is rapid, peaking between 1 and 2 h poststimulation. c-*myc* induction nevertheless represents a different class of modulation from the extremely rapid induction shown by the c-*fos* gene (19, 46). For c-*fos*, the capacity to regulate the activity of transfected genes has led to the definition of a potent SRE as part of the gene promoter (46–48). While the complete profile of c-*fos* induction is due to several mechanisms (e.g., mRNA turnover [46, 51]), the influence of this SRE on transcription initiation appears to be a major component in the rapid induction of *fos* gene expression (46). By contrast, no SRE has been identified in the *myc* gene.

The transfected *myc* sequence used in this study includes both an upstream AP2 site (23) and a sequence reported to be a binding site for a Fos-containing protein complex (20). Although the possibility cannot be excluded that low-level P1 activity is modulated by serum at the level of transcription initiation, the fact that no serum-responsive modulation of transcription initiation is detected in nuclear run-on assays suggests that protein-DNA interactions at these sites are insufficient to account for the growth factor-dependent expression in transfected FR3T3 cells. Treatment with actinomycin D reveals, however, that induction requires ongoing transcription of c-*myc* in stimulated cells. Furthermore, serum-dependent stabilization of c-*myc* mRNA cannot account for the observed induction.

It seems probable that most of the induction of c-*myc* occurs within the cell nucleus at a point between transcription initiation and the stabilization of mature mRNA. Such a point may be transcriptional attenuation, occurring at the 3′ end of c-*myc* exon 1 (5, 6). The transfected human c-*myc* genes do attenuate strongly in FR3T3 cells and appear to modulate the extent of downstream polymerase activity in response to serum (Fig. 9). In addition, *myc* gene expression may be serum responsive at the level of splicing or of nucleus-to-cytoplasm transport. It is now quite clear that splicing can be a highly regulated phenomenon (3, 16, 22, 30). The data presented here describe a system in which these possibilities may be distinguished genetically. At this point, it is clear that SREs exist within the 10.4-kbp c-*myc* fragment used to transfect the cells, and at least one such

element will prove quite different from the single SRE defined to date, namely, the transcriptional response element of the c-*fos* gene (47).

Inducible transcription initiation of endogenous c-*myc* genes has been reported in other cells, but in those studies increased initiation could account for only a fraction of the observed growth factor responsiveness (19, 36). The mechanism(s) accounting for the complete induction remains unelucidated. Those data stress the complexity of the rapid induction of c-*myc* gene expression. The degree to which different mechanisms (and combinations of mechanisms) act to effect c-*myc* gene induction may vary from one cell type to another. It is possible that the regulation involved will prove a paradigm for a class of serum-responsive genes that may include the genes *mr-1* and *mr-2*, which are themselves putatively regulated by *myc* at a point(s) between transcriptional initiation and RNA stabilization (38).

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