Regulatory elements that modulate expression of human *c-myc*

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Regulation of transcription from the proto-oncogene *c-myc* apparently plays an important part in cellular proliferation and the genesis of diverse tumors. Here, we report that the abundance of transcripts from the two principal promoters for human *c-myc* (P_1 and P_2) is governed by a composite of positive and negative regulators, located within a 2.3-kb domain upstream of the gene. In actively proliferating cells, the action of the positive elements is apparently dominant over that of the single negative regulator that we have identified. Nuclear proteins bind specifically to nucleotide sequences within the negative regulator and at least one of the positive regulators. The cooperative and counteracting actions of the regulatory elements described here presumably contribute to the plasticity of transcription from *c-myc* and may be affected by the tumorigenic damage that sometimes afflicts *c-myc*.

[**Key Words:** *c-myc*; proto-oncogene; transcription; negative regulation; positive regulation; protein factor]

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The proto-oncogene *c-myc* has been implicated in the control of normal cellular proliferation and in the genesis of diverse tumors (for review, see Cole 1986; Cory 1986). Expression of *c-myc* is regulated in part by changing the rate of transcription from the gene, a mechanism that contributes to both increased expression of *c-myc* during the response of cells to growth factors (Greenberg and Ziff 1984; Greenberg et al. 1985; Dean et al. 1986; Levine et al. 1986) and decreased expression during differentiation of cells to a nonproliferative state (Grosso and Pitot 1985; Bentley and Groudine 1986b; Endo and Nadal-Ginard 1986; Ramsay et al. 1986; Yarden and Kimchi 1986). Mutations of various sorts, including chromosomal translocations and insertion of retroviral DNA, apparently eliminate or override the regulation of *c-myc* and thus contribute to tumorigenesis (for review, see Cory 1986).

The topography of *c-myc* is well conserved among mammals (see Fig. 1). The gene possesses three exons, the second and third of which encode the major product of *c-myc*—a 65-kD nuclear protein whose function remains controversial (Hann and Eisenmann 1984; Ramsay et al. 1984; Cole 1986). Transcription from human *c-myc* begins at three sites, governed by separate promoters known as P_0 , P_1 , and P_2 (Battey et al. 1983; Bentley and Groudine 1986a). The initiation site for P_1 lies 161 bp upstream of that for P_2 (Battey et al. 1983). P_2 is generally the most active promoter for *c-myc* and provides the bulk of *c-myc* RNA. The RNAs initiated by P_1 and P_2 both give rise to the 65-kD *c-myc* protein. P_0 lies approximately 700 bp upstream of P_1 , accounts for no more than 5–10% of the RNA transcribed from *c-myc*

in all known settings, and may serve to express a second reading frame of *c-myc* that is located in the first exon of the human gene (Gazin et al. 1986).

Transcription from P_1 and P_2 may be regulated in at least two ways. First, activity of both promoters can be increased or decreased in parallel, in response to various physiological signals (Kelly et al. 1983; Campisi et al. 1984; Bentley and Groudine 1986b). Second, the relative activity of the two promoters may change during differentiation and in response to genetic damage found in some tumor cells (Stewart et al. 1984; Taub et al. 1984; Denny et al. 1985; Yang et al. 1985; Ruppert et al. 1986). Little is known of how these changes are effected. Here, we report that the abundance of RNAs initiated from P_1 and P_2 is governed by a composite of positive and negative regulators, located within a 2.3-kbp domain upstream of the promoters. We show that mammalian nuclei contain factors that bind to specific nucleotide sequences in the single negative element that we have identified and in at least one of the several positive elements. Our results dramatize and illuminate the combinatorial mechanisms by which transcription from *c-myc* is controlled.

Results

Preliminary identification of nucleotide sequences that regulate transcription from c-myc

As a first step toward the identification of DNA sequences that regulate the expression of human *c-myc*, we sought regions upstream of the gene that could

Figure 1. The topography of human *c-myc* and hybrid genes with bacterial CAT. The uppermost diagram illustrates the topography of human *c-myc*, drawn to scale, with exons shown as boxes, coding regions solid, and untranslated regions empty. Arrowheads indicate the sites for initiation of transcription controlled by the promoters P_1 and P_2 . In the hybrid genes, the coordinates for *c-myc* are defined relative to the site of initiation for P_1 (designated +1). Restriction sites are defined as follows: (B) *Bam*HI; (C) *Cl*AI; (H) *H*indIII; (P) *P*vuII.

control the expression of the bacterial gene chloramphenicol acetyl transferase (CAT) (Gorman et al. 1982). We constructed chimeric genes that contained CAT, the first 513 bp of *c-myc*, and various domains from upstream of *c-myc* (Fig. 1). We then tested the activity of these chimeras 45–48 hr after transfection, into mouse and human cell lines (Table 1). Maximal activity was obtained with a chimera extending 2329 bp upstream of *c-myc* (numbering from the site of initiation from P_1). Constructions with either 1257 bp or only 101 bp of upstream sequence gave two-thirds of the maximal activity. In contrast, the first 353 bp of upstream sequence reduced activity by approximately 10-fold. Similar results were obtained with diverse types of both mouse and human cells (Table 1).

Quantification of transcripts initiated by P_1 and P_2 of human *c-myc*

We next quantified the individual RNAs transcribed from the chimeric genes described above. For this purpose, we established polyclonal populations of stably transfected mouse cells that contain and express the various chimeras. RNAs transcribed from the chimeras were detected by hybridization with a radioactive probe that spanned the initiation sites for both P_1 and P_2 (Fig. 2A).

The results confirmed impressions gained previously from the measurement of CAT activity and demonstrated that P_1 and P_2 can be regulated independently. Maximal expression from both P_1 and P_2 was obtained under the influence of the 2329-bp upstream domain (Fig. 2B, lane 5). RNA initiated from P_2 was approximately fivefold in excess of that from P_1 . The endogenous *c-myc* RNA of mouse cells displayed a similar P_2/P_1 ratio (data not shown), as anticipated from pre-

vious reports (Kelly et al. 1983; Yang et al. 1985). Abundant expression was also obtained with 1257 bp of upstream sequence, but the amounts of RNA initiated from both promoters were reduced somewhat (Fig. 2B, lane 4). Shortening the upstream region to 353 bp reduced the activities of P_1 and P_2 by 10-fold, when compared to the maximum obtained with 2329 bp of upstream sequence, but the ratios between the two activities remained the same (Fig. 2B, lane 3; data not shown). Abundant expression from P_2 returned in the presence of only 101 bp of upstream sequence, but there was now no detectable activity of P_1 (Fig. 2B, lane 2). We conclude that a portion of the sequence required for P_1 activity lies upstream of nucleotide –101. A repressor element for P_2 resides somewhere between residues –101 and

Table 1. Transcriptional regulation by nucleotide sequences upstream of human *c-myc*

Hybrid gene	Relative CAT activity ^a		
	Ltk ⁻ cells	C39 cells	COLO320 cells
1	1.5 ± 0.3	1.3 ± 0.12	1.2 ± 0.15
2	1.0 ± 0.2	0.9 ± 0.15	NT ^b
3	0.12 ± 0.05	0.11 ± 0.02	0.25 ± 0.05
4	1.0	1.0	1.0

The promoters P_1 and P_2 of human *c-myc* and various upstream domains were used to drive transcription from the bacterial CAT gene. The hybrid genes are illustrated in Fig. 1 and defined by the numbers given there. Introduction of the DNAs into cells and assay of CAT activity are described in Materials and methods.

^a The activity obtained with –101/+513 was defined arbitrarily as 1.0. The data represent the results in mean values ± SD of four independent experiments.

^b (NT) Not tested.

Figure 2. Quantification of RNAs transcribed from hybrids between human *c-myc* and bacterial CAT. Polyclonal lines of Ltk⁻ cells bearing the DNAs described in Fig. 1 were established, using coselection with hygromycin B. Transcripts derived from P₁ and P₂ were then detected by hybridization with an RNA fragment synthesized with SP6 polymerase and subsequent hydrolysis with RNase A and T1. Details for all the procedures are given in Materials and methods. (A) Analytical strategy. The diagram illustrates the RNA fragment used in the assay and the length of the portions protected by transcripts initiated by P₁ and P₂. The *Sma*I restriction site is located at position -101; the *Pvu*II site at position +510. (B) Electrophoretic fractionation of RNA fragments. RNA fragments representing initiation by P₁ and P₂ are labeled with arrowheads. The electrophoresis was calibrated with end-labeled fragments of DNA, obtained by cleaving pBR322 with *Hinf*I (lane 6). The remaining lanes contain the analytical products obtained with RNAs from COLO320 HSR cells (lane 1) and polyclonal lines of Ltk⁻ cells stably transcribing the hybrid genes defined in the legend to Fig. 1 (lanes 2–5).

–353. The same element also acts on P₁ (see below).

We used the same approach to test the effect of the upstream domains on expression of the complete human *c-myc* gene (Fig. 3). The results were as before, except that at maximal activity, the excess of RNA initiated from P₂ was threefold rather than fivefold (Fig. 3, lane 3).

Other investigators have reported that a region upstream of mouse *c-myc* and analogous to the region

–353/–1257 in our studies inhibited the activity of the SV40 enhancer–early promoter (Remmers et al. 1986). In our experience, however, the SV40 enhancer–early promoter was active when attached to the domain upstream of human *c-myc* at residue –2329, augmenting the RNA initiated from P₁ and P₂ by a factor of approximately 10 (Fig. 3A, construction 4; Fig. 3B, lane 4). Although the SV40 DNA included both an enhancer and the early promoter of the virus, transcripts from the chimeric gene nevertheless began at the initiation sites for P₁ and P₂ (Fig. 3B, lane 4). As a further test of the –353/–1257 region, we used a chimeric gene in which the promoter for the herpesvirus thymidine kinase (HSV *tk*) gene drives transcription from CAT (Fig. 4A). The *c-myc* upstream region between nucleotides –350 and –1257 stimulated the activity of the *tk* promoter by about 10-fold (Fig. 4B).

Further localization of the negative regulator for P₁ and P₂ and a positive regulator for P₁

Having demonstrated that a negative regulator of *c-myc* expression resides within 353 bp upstream of the gene, we sought a better localization of the element by further manipulations of the upstream domain (Fig. 5A). The results showed that both terminal and internal deletions that removed the sequence between residues –293 and –353 eliminated repression of P₁ and P₂ (Fig. 5B, lanes 2–4, 6; Fig. 5C, lanes 2, 3). As before, restriction of the upstream sequences to the first 101 nucleotides permitted strong expression from P₂ but no detectable expression from P₁ (Fig. 5B, C, lane 1).

The data also permitted a more detailed mapping of the element required for basal activity of P₁, placing it between residues –101 and –293 (Fig. 5B, lanes 1, 2, 4, 5; Fig. 5C, lanes 1, 2). The same domain had a weak but reproducible stimulatory effect of approximately two-fold on the activity of P₂ (Fig. 5B, lanes 1, 2, 4, 5; Fig. 5C, lanes 1, 2).

Positive regulatory elements that override the repressor of *c-myc*

The data presented above demonstrated that the negative regulator of *c-myc* was overridden by the presence of nucleotide sequences from farther upstream. On further inspection, this positive effect could be ascribed to at least two domains whose effects were additive—one located downstream and the other upstream of residue –1257 (Fig. 2B, lanes 4, 5). The repressor can also be overridden by the transcriptional enhancer from the proviral DNA of murine sarcoma virus (unpubl.).

A modest impact of the repressor element was apparent, even in the presence of the positive regulatory elements. An internal deletion of the domain that contains the negatively acting element (residues –101 to –353) caused a reproducible threefold increase in the abundance of RNA initiated from P₂ (Fig. 6, lanes 1, 2). The same deletion removed an element that can sustain activity of P₁ (see above). The element appear not to be essential to the activity of P₁, however, because the pres-

Figure 3. Quantification of RNAs transcribed from human *c-myc* under the control of various upstream domains. (A) Diagram of the DNAs introduced into cells. P₁ and P₂ are as before; exons of human *c-myc* are designated as Ex1, Ex2, and Ex3; the black boxes indicate nucleotide sequences upstream of human *c-myc*; and the small arrow gives the orientation of the SV40 early promoter. (B) Electrophoretic fractionation of RNA fragments. Polyclonal lines of Ltk⁻ cells bearing the versions of human *c-myc* diagrammed in A were established and analyzed as for Fig. 2. Lanes are numbered according to the diagram in A. An RNA probe for transcripts from the hygromycin-resistance gene served as an internal standard (tk/Hm-REF).

Figure 4. A positive regulator of human *c-myc* acts on another gene. (A) Diagram of DNAs introduced into cells. Two plasmids were used to establish polyclonal lines of Ltk⁻ cells (as for previous figures). In PTE2, bacterial CAT was under the control of the promoter for the herpesvirus thymidine kinase gene (TKp). In PTE210, the region -350/-1257 upstream of human *c-myc* was added as shown. (B) Quantification of transcripts from the *tk* promoter. RNAs from the cell lines were analyzed as for previous figures. The probe was a 318-bp fragment that included a 5' portion of the CAT gene and encompassed the entire promoter (Nir et al. 1986). Bands in the gel represent transcripts initiated at a cryptic promoter in pBR322 (RT for "read-through") and promoter itself. (REF) An internal standard as described for previous figures. The electrophoresis was calibrated with DNA fragments, as in Fig. 2.

Figure 5. Mapping control elements with deletions. (A) Diagram of the hybrid genes introduced into cells. Polyclonal lines of Ltk⁻ cells were established with each of the DNAs and analyzed as for Figs. 2 and 3. (B) Electrophoretic analysis. Lanes are lettered to correspond to the diagrams in A. (C) Electrophoretic analysis with an internal standard. An RNA probe for transcripts from the hygromycin-resistance gene was included as an internal standard (REF).

ence of the upstream positive regulators compensated at least partially for the absence of the P₁ element (Fig. 6, lane 2; cf. Fig. 5B, lane 5). P₂ was now 15-fold more active than P₁, in contrast to the fivefold difference observed when the 2329 bp of upstream sequence was intact (cf. Fig. 6, lanes 1 and 2).

One or more factors bind to the repressor domain upstream of c-myc

Having identified and mapped the approximate location of a negative regulatory element for *c-myc*, we sought evidence for cellular factors that bind to the element. We used a procedure in which binding of protein to a

Figure 6. Deletion of the negative regulator for human *c-myc*. (A) Diagram of hybrid DNAs introduced into cells. Polyclonal lines of Ltk⁻ cells bearing the illustrated DNAs were established and analyzed as described for previous figures. (B) Quantification of transcription from P₁ and P₂. The lanes are numbered to correspond to the diagrams in A. An internal standard was included as in Figs. 3 and 4 (REF). Exposures of the autoradiogram for 4 hr (a) and 12 hr (b) are illustrated.

DNA fragment blocks the progress of hydrolysis by an exonuclease and thus gives rise to smaller fragments whose lengths define the approximate location of the binding site for protein (Wu 1985; Quinn et al. 1987). A reference point in the measurement is achieved by including the *lac* operator of *Escherichia coli* in the DNA and occupying that site with *lac* repressor protein (Quinn et al. 1987).

We tested binding to a 252-bp DNA fragment that includes the negative regulatory element for *c-myc* (Fig. 7A). Extracts of HeLa cell nuclei contain a factor or factors that blocked hydrolysis of this fragment by phage λ exonuclease in the vicinity of residue -350. A similar location for the blockade was obtained with the test fragment in either orientation relative to the *lac*-binding site (Fig. 7B, left). In one instance, exonuclease hydrolyzed only a short distance into the fragment; in the other, virtually the entire cloned fragment was hydrolyzed. But in either orientation, the blockade of hydrolysis gave a single product whose length defined the approximate position where cellular protein might be binding. The blockade was entirely dependent upon the presence of cellular proteins, because omission of the nuclear extracts permitted hydrolysis to proceed completely to the position of the *lac* operator (Fig. 7B, right); the blockade was absolute, indicating that the binding site was probably fully occupied (Fig. 7B, right); and pro-

tein is involved, because the specific binding is sensitive to proteinase K (data not shown).

Binding of factor(s) to the negative regulatory element is sequence specific

Having defined a site for the binding of a cellular factor, or factors, we examined the specificity of this binding by testing the ability of various DNA fragments to compete with binding to the negative regulatory region. Binding was detected by using the exonuclease assay described above. Lanes 1–4 in Figure 8B illustrate that the assay was working as expected, with hydrolysis by exonuclease blocked at the appropriate site only when nuclear extracts from HeLa cells were present. We compared the ability of two DNA fragments to compete for the factor that was binding to the labeled DNA: a 57-bp fragment that apparently contains the binding site (fragment "a" in Fig. 8) and an adjacent fragment that showed no previous evidence of binding (fragment "b" in Fig. 8). Fragment a gave complete competition with the negative element, whereas fragment b gave no detectable competition (Fig. 8B). Fragments of λ phage and plasmid pUC19 DNAs were also tested for competition, with no effect (data not shown). We conclude that the binding of nuclear protein to the negative regulatory element is specific for a circumscribed nucleotide sequence.

Figure 7. Binding site for a nuclear factor within a negative regulator for human *c-myc*. (A) DNA used to detect binding. The region -101/-353 upstream of human *c-myc* was cloned in both orientations into pUC19, adjacent to the *lac* operator domain. (B) Detection of binding by hydrolysis with exonuclease. The *Bst*NI fragment bearing the human *c-myc* insert (A) was used to detect binding of a factor from extracts of HeLa cell nuclei. The diagrams outline the topography of the *Bst*NI fragment and allow interpretation of the autoradiograms. The heavy arrows indicate the insertions of -101/-353 and the direction of transcription from *c-myc*. The oval designates the location of *lac* repressor; the stippled rectangle, the location of nuclear factor(s). Both the repressor and the factor blocked hydrolysis by λ exonuclease, giving rise to the indicated fragments (open triangles, repressor blockade; closed arrowheads, nuclear factor blockade). The left-hand gel illustrates detection of binding; the right-hand gel illustrates a dose-response relationship between amount of nuclear extract and the extent of blockade at the site of factor binding.

Figure 8. Nuclear factors bind specifically to at least two regulators of human *c-myc*. (A) DNA used in the assays. Fragment a contained the negative regulator of *c-myc*; fragment b, the positive element required for basal activity of P_1 . (B) Specificity of binding. Assays were performed as in Fig. 7, except that phage T7 exonuclease was used rather than λ exonuclease. The arrow and the bracket indicate the position of the protected fragments. (C) Enlargement of the upper portion of B. (D) Nucleotide sequence through the region of DNA containing the five binding sites highlighted in C.

Identification of binding sites for protein that may lie within a regulatory element for P_1

The assay illustrated in Fig. 8, B and C, revealed five secondary binding sites spaced at intervals of about 10 bp near the right-hand end of the test fragment (the *Sma*I site of fragment b in Fig. 8A; evidence of binding marked by bracket in Fig. 8B). Binding was not apparent when nuclear extract was omitted from the assay and was specific because it could be competed only with a DNA fragment (b in Fig. 8A) that encompassed the binding sites (Fig. 8B, lanes 5, 6; data not shown). The five binding sites defined by these data lie within the region that exerts a positive effect on expression from P_1 and may therefore represent components of the positive regulator. These sites became apparent only when phage T7 exonuclease was substituted for phage λ exonuclease in the assay. We attribute this difference to the fact that the action of T7 enzyme is distributive, whereas the λ enzyme is processive (see Discussion).

Locating the binding site in the negative regulator by footprinting with DNase I

We defined the DNA-binding site for the negative regulator in greater detail by footprinting with DNase I (Fig. 9). The results showed that binding involved a sequence of 15–20 nucleotides. Both strands of the DNA were protected against DNase I by the bound factor (Fig. 9A, B). Figure 9C illustrates the nucleotide sequence of the protected region, which lies between residues 319 and 342. The site includes an AT-rich core that is flanked and overlapped by an inverted repeat of 8 nucleotides. The same binding site was occupied when exposed to extracts of nuclei from a mouse cell line ID13 (data not shown). The sequence defined by footprinting proved to be sufficient for binding: A synthetic double-stranded oligonucleotide comprising residues 318–342 bound nuclear factor in both competition assays and in the assay for binding described by Carthew et al. (1985) (data not shown).

Discussion

Regulators of expression of human *c-myc*

Transcription from human *c-myc* is driven by three promoters designated P_0 , P_1 , and P_2 (Fig. 10A). P_1 and P_2 each control a single site for initiation of transcription. They contain elements commonly found in transcriptional promoters of mammalian cells, including the TATA motif and a GC-rich sequence that may bind the transcriptional factor SP1 (Dyner and Tjian 1985; Nishikura 1986). These components are located within 60 nucleotides upstream of the initiation sites for the two promoters. Here we have shown that the nucleotide sequences between residues –293 and +513 are sufficient for strong activity of P_1 and P_2 , and we have defined several elements that modulate the activities of the two promoters (Fig. 10A): a region that can alone sustain activity of P_1 and that stimulates the activity of P_2 by approximately twofold (–101/–293); two domains that exert strong positive effects on both P_1 and P_2 (–353/–1257 and –1257/–2329); and a negative regulator that apparently acts on expression from both promoters (–293/–353).

Dalla-Favera and his colleagues have also suggested the existence of a negative regulator for human *c-myc* with a location similar to that described here (Pelicci et al. 1986). In contrast, Chung et al. (1986) and Lipp et al. (1987) reached ostensibly different results through the use of competition assays: They failed to detect the negative regulatory element described here and instead reported negative regulators of human *c-myc* farther upstream, in the first exon of *c-myc* and in the first intron. It is difficult at present to assess the significance of these discrepancies because of differences in how the regulatory domains of *c-myc* were subdivided in the several studies and in how regulatory activity was assessed.

All of the available studies employed measurements of steady-state levels of gene expression. It therefore remains uncertain whether the reported effects all reflect

Figure 9. DNase footprint of the binding site in the negative regulatory element. Binding reactions were performed as for Figs. 9 and 10, using DNA fragments labeled on their 3' termini. Fragments encompassing the negative regulatory element were cleaved from the plasmid illustrated in Fig. 7A in a manner that permitted exclusive labeling of either the coding or noncoding strand of DNA. Protein DNA complexes were formed, recovered, and subjected to footprinting with DNase I (details are in Materials and methods). (A) Footprint on the noncoding strand. Markers for electrophoresis were obtained by cleaving the DNA used in the binding with either *PvuII* (cleaves at -350) or *RsaI* (cleaves at -293) (lanes labeled accordingly). (B) Footprint from the coding strand. The electrophoresis was calibrated by chemically cleaving the DNA used in binding at dC residues (Maxam and Gilbert 1980) (lane marked C). Brackets mark the sequence protected from DNase I, which is given vertically and aligned with corresponding bands in the gel. The small open circle marks protection attributable to the binding of *lac* repressor to the DNA fragment. (C) Nucleotide sequence of the binding site defined by DNase I footprinting. Black dots define the outer boundaries of the binding site. Nucleotides contributing to dyad symmetry are underlined. Arrows designate the polarity of the symmetry. The rectangle encloses an AT-rich sequence that was fully protected in all assays (see text).

changes in the initiation of transcription or whether other mechanisms may be involved as well, such as the attenuation of transcription near the 3' end of the first exon of *c-myc* described recently (Bentley and Groudine 1986b; N. Hay, unpubl.) We also acknowledge that the effects reported here could conceivably be mediated at steps subsequent to transcription. This possibility would require unprecedented mechanisms, however, because all of the regulatory elements that we report lie well upstream of *c-myc* proper, beyond even the conventional promoter region for the gene.

Nucleotide sequences required for the activity of P_1

The region -101/-293 contains an element that is required for P_1 activity and that may influence the relative contributions of P_1 and P_2 by modest stimulation of expression from P_2 . It is possible that these two effects are dictated by different elements within the domain; mapping at higher resolution will be required to explore this possibility. There is presently no credible explanation for why two promoters are devoted to expression of the same *c-myc* protein, no hint as to why P_2 is characteristically more active than P_1 , and only provisional indica-

tions that the relative activities of the two promoters may vary from one physiological context to another (Stewart et al. 1984; Ruppert et al. 1986).

We do not believe that the positive effector for P_1 represents a portion of the promoter proper. It is located too far upstream of *c-myc* and contains none of the characteristic nucleotide sequences, such as a CAAT box or a binding site for SP1 factor.

The most striking feature of the domain $-101/-293$ is a series of five related sequences located between residues -101 and -153 . The first of these is CCTCCCC; the remaining four follow the motif C/TCC/TTCCCCA (see Fig. 8D). Our data raise the possibility that nuclear extracts contain a factor or factors that bind specifically within these sequences, although confirmation of the specific binding sites and precise mapping requires further exploration by footprinting and other techniques. There are apparently five clustered binding sites, spaced at intervals of approximately 10 nucleotides. Binding to the sites was detected as impedance of hydrolysis by T7 exonuclease. Binding could not be detected with λ exonuclease, perhaps because this enzyme is processive and might displace factors whose affinity for the DNA is below a certain level (Radding 1971). Motifs similar to the binding sequences described here are found in approximately the same location upstream of mouse *c-myc* (Corcoran et al. 1985), adjacent to an enhancer in the BK virus genome (Rosenthal et al. 1983), and in putative control regions upstream of the genes encoding chicken and mouse α_2 I collagen (McKeon et al. 1984) and the receptor for epidermal growth factor (Ishii et al. 1985). The sequences also represent the location of a hypersensitive site for cleavage

by DNase I (site III₁; see Siebenlist et al. 1984; Fig. 10A). We conclude that the domain $-101/-293$ upstream of human *c-myc* probably binds a factor (or factors) that may be essential for expression from P_1 .

Our description of the domain required for activity of P_1 contrasts with the work of Nishikura, who reported that the first 60 bp upstream of human *c-myc* was sufficient for strong and accurate transcription from P_1 when human *c-myc* was injected into oocytes of *Xenopus laevis* (Nishikura 1986). We cannot presently explain this discrepancy, although it is possible that the factors that regulate *c-myc* in *Xenopus* differ from those in mammalian cells.

Positively acting regulators of P_1 and P_2

The regions $-353/-257$ and $-1257/-2329$ act additively to stimulate expression from P_1 and P_2 and can facilitate the activity of P_1 in the absence of an element ($-293/-101$) that is otherwise required for activity of the promoter. Both domains contain a hypersensitive site for hydrolysis by DNase I (Siebenlist et al. 1984; see Fig. 10A), and both contain sequences that may be binding sites for NF1 (Siebenlist et al. 1984)—a protein that is reported to be a positively acting transcriptional factor in mammalian cells (Shaul et al. 1986; Jones et al. 1987). Overlapping the putative NF1-binding site at residue -700 is the sequence GTGGAAGG, which closely approximates the consensus sequence found in numerous transcriptional enhancers (Goodbourn et al. 1985). In combination, these observations suggest that the region $-353/-1257$ may be a conventional transcriptional enhancer. We have shown that this domain

Figure 10. Elements that control transcription from human *c-myc*. (A) Location of regulatory elements. The negative, positive, and P_1 elements were defined by the present work. Other details have been taken from the pertinent literature. Stippling denotes the maximal extent of the P_1 element. Vertical bars designate the locations of binding sites identified by exonuclease assays (P_1 and negative elements) or DNase I footprinting (negative element). Roman numerals mark the location of sites that are hypersensitive to DNase I in one circumstance or another (see text). Two regions where NF1 might bind are marked by diagonal cross-hatching. (B) Similarities between the nucleotide sequences in the negative regulatory elements for human *c-myc* and β -interferon. Nucleotides contributing to dyad symmetry are underlined. (*) Identities between the two sequences.

can stimulate transcription from the promoter for the herpesvirus TK gene, but we have yet to test the effects of the element when its orientation with respect to a promoter has been reversed or when it has been placed downstream of a promoter. Similarly, it is possible that the region $-1257/-2329$ is a conventional enhancer, but we have yet to explore this possibility in detail.

A negative regulator for human c-myc

We have found a domain ($-293/-353$) that can inhibit expression from P_1 and P_2 in actively proliferating cells when the promoters are subject only to elements downstream of the negative regulator. A 15- to 20-bp sequence within this domain binds a nuclear factor (or factors) from human cells. The binding sequence features an AT-rich core, flanked and overlapped by inverted repeats of eight residues. A similar sequence is present in the same location upstream of mouse *c-myc*, and a nuclear factor (or factors) from mouse cells binds to the human sequence. We do not know how many factors might be involved in the binding or what the nature of these might be. We hypothesize that the binding is involved in the inhibition of transcription from *c-myc*.

The role of negative regulators of transcription in mammalian cells is just coming into view. One prominent example is an element that apparently represses transcription from the gene for β -interferon in the absence of an inducing agent (Goodbourn et al. 1985). There are several provocative similarities between this element and the potential negative regulator of *c-myc* described here. First, both negative elements lie downstream of positive or enhancer elements (although the negative and positive elements for human β -interferon are more closely approximated than are those for *c-myc*). Second, the positive elements are dominant over the negative ones in the induced state (we regard the activity of *c-myc* in proliferating cells as the induced state). Third, both negative elements can be overridden by viral enhancers. Fourth, the nucleotide sequences that bind factor within the two negative elements are roughly the same length, display dyad symmetry, and resemble one another in other regards (Fig. 10B). Our findings therefore raise the possibility that at least some of the negative regulators of transcription in mammalian cells may take a common form and that the specificity of regulation lies in another component, or components, of the regulatory machinery.

A composite of regulators for c-myc

Transcription from *c-myc* can be modulated both positively and negatively in response to different physiological signals (Greenberg and Ziff 1984; Greenberg et al. 1985; Dean et al. 1986; Endo and Nadal-Ginard 1986; Levine et al. 1986; Ramsay et al. 1986). Here we have reported the identification of several regulatory elements that may contribute to this plasticity. Some of the elements appear to be additive with one another, but others are counteracting. For example, the positive regulators located upstream of -353 appear to override the

effect of the negatively acting element, although the override may be incomplete because removal of the negative element causes a further increase (about three-fold) in the activity of P_2 . Similarly, the effect of the negative element can be overridden by a transcriptional enhancer from the provirus of murine sarcoma virus. This finding conforms to the view that integration of retroviral enhancers in the vicinity of *c-myc* can initiate tumorigenesis in lymphoid cells by activating the gene (for review, see Cory 1986).

A number of eukaryotic genes whose activities respond to physiological signals are under the control of juxtaposed transcriptional enhancers and repressor elements. In each instance, the negative regulators are effective only in physiological contexts where the enhancers are not active (Chambon et al. 1984; Gonzalez et al. 1985; Goodbourn et al. 1985). There may be settings in which the positive regulators of *c-myc* are silent and the repressor element is therefore in command. For example, we have yet to test the activity of the various regulatory elements in cells that have been differentiated or otherwise arrested within the cell cycle. We propose that the domain between -353 and -2329 contains two or more regulators that serve to activate *c-myc* when its expression is required. This view is supported by the fact that a hypersensitive site for DNase I located at residue -750 (site II₂; see Fig. 10) is prominent when *c-myc* is actively transcribed but diminishes or disappears when the gene is inactive (Siebenlist et al. 1984; Dyson et al. 1985).

Our findings sustain the view that the expression of *c-myc* is controlled by a composite of regulatory elements, provide an initial dissection of where several of those elements might be located, and suggest the existence of nuclear factors that bind to at least two of the elements—one, a positive effector, and the other, a negative regulator. We hope now to determine how these elements participate in the response of *c-myc* to various physiological signals and how they are affected by the damage that afflicts *c-myc* in several types of human tumors.

Materials and methods

Cells and transfection

Mouse Ltk⁻ cells and C39, a human fibroblastic cell line immortalized by SV40 (a gift from D. Neufeld and H. Ozer), were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum. COLO320 HSR cells (Quinn et al. 1979) were grown in RPMI, supplemented with 10% fetal bovine serum.

Transfections of cells were performed using calcium phosphate precipitates (Graham and Van der Eb 1973). For transient transfection, 1×10^6 cells were transfected with 5 μ g of vector DNA. After 12 hr of exposure to precipitate, cells were washed with phosphate-buffered saline (PBS) and refed with complete medium. Cells were harvested for CAT assay, 44–48 hr after transfection. For stable transfection, 1×10^6 Ltk⁻ cells were cotransfected with 5 μ g of vector DNA and 0.5 μ g of pHMR272 DNA. After 12 hr of exposure to precipitate, cells were washed with PBS and refed with complete medium. Forty-eight hours later, cells were harvested by trypsinization and reseeded into

selective medium containing 200 μ g/ml hygromycin B. After 3–4 weeks in selective medium, about 1000 hygromycin-resistant clones were pooled and maintained as cell lines.

Plasmid constructions

Human *c-myc* sequences were subcloned from an *EcoRI* fragment of human *c-myc* in pML (a gift of A. Levinson). The plasmid pBRCAT (Walker et al. 1983) was used to construct *c-myc*–CAT hybrids. Sequences between the *PvuII* site at the 3' end of the human *c-myc* first exon and various lengths of the 5'-flanking region were cloned into the *ClaI* site of pBRCAT. The restriction sites *SmaI*, *ScaI*, *PvuII*, *NarI*, *ClaI*, and *HindIII* in the 5' flanking region were used to create deletion mutants that possess 101, 293, 353, 416, 1257, and 2329 bp upstream of the transcription start site. The sites *PvuII*, *ClaI*, and *HindIII* were left intact in the constructions.

The plasmid PTE2 (a gift from M. Walker) is the same as plasmid PTE1 (Edlund et al. 1985) but with pUC18 background instead of pBR322. PTE210 was constructed by cloning the *PvuII*–*ClaI* fragment between nucleotides –350 and –1257 of human *c-myc* into the *NruI* site of PTE2 (15 bp upstream of residue –109 of the HSV TK promoter) and the *AccI* site in the polylinker of PTE2.

The plasmids pSP64 and pSP65 (Promega Biotec) were used to generate complementary-strand RNA probes. To generate the human *c-myc* probe, the fragment *SmaI* (–101)–*PvuII* (+510) of human *c-myc* was used (see Fig. 2A). To generate the RNA probe for hygromycin B fused to HSV TK promoter in pHMR272 (Bernard et al. 1985), a fragment, *PstI*–*EcoRI*, that spans the TK promoter and the 5' portion of hygromycin B was used.

Plasmids were constructed and prepared by standard procedures (Maniatis et al. 1982). Full details of each construction are available upon request.

CAT assays

Preparation of cell extract, CAT assays, and thin-layer chromatography were performed according to Gorman et al. (1982). Assays contained 100–150 μ g of extract protein. After thin-layer chromatography, the acetylated and nonacetylated forms of chloramphenicol were cut out and counted. CAT activity was calculated as percentage of chloramphenicol converted to acetylated forms.

RNA analysis

Total cellular RNA was isolated by the guanidinium thiocyanate procedure, followed by centrifugation through CsCl (Ullrich et al. 1977). RNA was analyzed by the RNase protection procedure, as described by Zinn et al. (1983). Samples (15 μ g of total RNA plus RNA probe) were denatured at 85°C for 10 min, followed by hybridization at 56°C for 12–14 hr. RNase treatment was with 10 μ g/ml RNase A and 500 U/ml T1 (Sigma) for 1 hr at 33°C, followed by addition of 10 μ g/ml proteinase K for 10 min at 37°C. RNase-resistant fragments were analyzed by running on 5% polyacrylamide sequencing gel.

Preparation of extracts

Nuclear extracts were prepared according to the procedure of Dignam et al. (1983), with the following modifications: Buffer C contained 20% glycerol and 0.45 M NaCl, and buffer D contained 20% glycerol and 80 mM KCl. All buffers contained 20 mM HEPES (pH 7.5). Extracts contained 40 mg/ml protein.

Immunobead complexes

Immunobead complexes were prepared as described by Levens and Howley (1985).

Exonuclease reactions

Exonuclease footprint experiments (Wu 1985) employed either of a pair of 458-bp *BstNI* restriction fragments that contained the –101 to –353 segment of *c-myc* cloned into the *HindIII* site of pUC19 in either orientation (as specified in the figure legends) and adjacent *lac* operon sequences. These fragments were labeled with α -³²P-labeled deoxyribonucleotides at both 3' ends by DNA polymerase I, large fragment. Prebinding reactions immobilized 1–5 ng of probe, and in some experiments 50 ng of unlabeled, carrier pUC8 DNA, on Immunobeads via a *lac* repressor– β -galactosidase fusion protein. Binding reactions were performed in a total of 15 μ l of buffer D plus extract (5 μ l, unless noted otherwise), the indicated additions, and 7.5–10 μ g of poly[d(I–C)]. Reactions were incubated for 30 min at 20°C; the beads were recovered centrifugally (10,000 g, 30 sec) and resuspended in 15 μ l of 10 mM Tris–Cl (pH 8.0), 4 mM MgCl₂, 0.2 mM dithiothreitol (DTT), and 5% glycerol. Three to four units of λ exonuclease (Bethesda Research Laboratories) or 10–15 U of T7, gene 6 exonuclease (United States Biochemicals) were added; reactions were incubated for 3 min at 30°C and stopped with the addition of an equal volume of 10 mM Tris–Cl (pH 7.5), 20 mM EDTA, and 0.5% SDS. The reactions were then extracted with phenol, and the nucleic acid was recovered from the aqueous phase by ethanol precipitation, analyzed by electrophoresis (6% acrylamide, 8.3 M urea), and visualized with autoradiography.

DNase I analysis

DNase I footprint analysis (Galas and Schmitz 1978) of the noncoding strand employed a probe generated by labeling the 458-bp *BstNI* fragment as above, cleaving with *SalI*, and immobilizing the desired *lac* operator containing product on Immunobeads. The probe for analysis of the coding strand was generated by cleaving at, and 3'-labeling, the *BssII* site, followed by hydrolysis with *BstNI* and immobilization as above. Binding reactions were performed as described for exonuclease assays, except that 10–30 μ l of extract was included. The beads with bound factors were recovered centrifugally and resuspended in buffer D plus 7.5 mM MgCl₂ and 0.1 mM CaCl₂. DNase I (0.2–10 μ g/ml, final concentration) was added; reactions were incubated for 45 sec at 30°C, stopped, and analyzed by polyacrylamide gel electrophoresis. In some experiments, base-specific, partial chemical degradation (Maxam and Gilbert 1980) of the probe was performed to provide a reference.

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References

- Batney, J., C. Moulding, R. Taub, W. Murphy, T. Stewart, H. Potter, G. Lenoir, and P. Leder. 1983. The human *c-myc* oncogene: Structural consequences of translocation into the IgH locus in Burkitt lymphoma. *Cell* **34**: 779–787.
- Bentley, D.L. and M. Groudine. 1986. A block to elongation is largely responsible for decreased transcription of *c-myc* in differentiated HL60 cells. *Nature* **321**: 702–706.
- Bentley, D.L. and M. Groudine. 1986. Novel promoter upstream of the human *c-myc* gene and regulation of *c-myc* expression in B-cell lymphomas. *Mol. Cell. Biol.* **6**: 3481–3489.
- Bernard, H.U., G. Krammer, and W.G. Rowekamp. 1985. Construction of a fusion gene that confers resistance against hygromycin B to mammalian cells in culture. *Exp. Cell Res.* **158**: 237–243.
- Campisi, J., H.E. Geay, A.B. Pardee, M. Dean, and G.E. Sonenshein. 1984. Cell-cycle control of *c-myc* but not *c-ras* expression is lost following chemical transformation. *Cell* **36**: 241–247.
- Carthew, R.W., L.A. Chodosh, and P.A. Sharp. 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. *Cell* **43**: 439–448.
- Chambon, P., M.P. Gaub, J.P. Lepennec, A. Dierich, and D. Astinotti. 1984. Steroid hormones relieve repression of the ovalbumin gene promoter in chick oviduct tubular gland cells. In *Endocrinology* (ed. F. Labrie and L. Proulx). Excerpta Medica, Amsterdam.
- Chung, J., E. Sinn, R.R. Reed, and P. Leder. 1986. Trans-acting elements modulate expression of the human *c-myc* gene in Burkitt lymphoma cells. *Proc. Natl. Acad. Sci.* **83**: 7918–7922.
- Cole, M.D. 1986. The *myc* oncogene: Its role in transformation and differentiation. *Annu. Rev. Genet.* **20**: 361–384.
- Corcoran, L.M., S. Cory, and J.M. Adams. 1985. Transposition of the immunoglobulin heavy chain enhancer to the *myc* oncogene in a murine plasmacytoma. *Cell* **40**: 71–79.
- Cory, S. 1986. Activation of cellular oncogenes in hemopoietic cells by chromosome translocations. *Adv. Cancer Res.* **47**: 189–234.
- Dean, M., R.A. Levine, W. Ran, M.S. Kindy, G.E. Sonenshein, and J. Campisi. 1986. Regulation of *c-myc* transcription and mRNA abundance by serum growth factors and cell contact. *J. Biol. Chem.* **261**: 9161–9166.
- Denny, C.T., G.F. Hollis, I.T. Magrath, and I.R. Kirsch. 1985. Burkitt lymphoma cell line carrying a variant translocation creates new DNA at the breakpoint and violates the hierarchy of immunoglobulin gene rearrangement. *Mol. Cell. Biol.* **5**: 3199–3207.
- Dhar, R., W.L. McClements, L.W. Enquist, and G.F. Van de Woude. 1980. Nucleotide sequences of integrated Moloney sarcoma provirus long terminal repeats and their host and viral junctions. *Proc. Natl. Acad. Sci.* **77**: 3937–3941.
- Dignam, J.D., P. Martin, B.S. Shastri, and R.G. Roeder. 1983. Eukaryotic gene transcription with purified components. *Methods Enzymol.* **101**: 582–598.
- Dynan, W.L. and R. Tjian. 1985. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. *Nature* **316**: 774–777.
- Dyson, P.J., T.D. Littlewood, A. Forster, and T.H. Rabbitts. 1985. Chromatin structure of transcriptionally active and inactive human *c-myc* alleles. *EMBO J.* **4**: 2885–2891.
- Edlund, T., M.D. Walker, P.J. Barr, and W.J. Rutter. 1985. Cell-specific expression of the rat insulin gene: Evidence for role of two distinct 5'-flanking elements. *Science* **230**: 912–916.
- Endo, T. and B. Nadal-Genard. 1986. Transcriptional and post-transcriptional control of *c-myc* during myogenesis: Its mRNA remains inducible in differentiated cells and does not suppress the differentiated phenotype. *Mol. Cell. Biol.* **6**: 1412–1421.
- Galas, D. and A. Schmitz. 1978. DNase footprinting: A simple method for detection of protein-DNA binding specificity. *Nucleic Acids Res.* **5**: 3157–3170.
- Gazin, C., M. Rigolet, J.P. Briand, M.H.V. Van Regenmortel, and F. Galibert. 1986. Immunochemical detection of proteins related to the human *c-myc* exon 1. *EMBO J.* **5**: 2241–2250.
- Gonzalez, F.J. and D.W. Nebert. 1985. Autoregulation plus positive and negative control regions associated with transcriptional activation of the mouse P1-450 gene. *Nucleic Acids Res.* **13**: 7269–7288.
- Goodbourn, S., H. Burstein, and T. Maniatis. 1986. The human β -interferon gene enhancer is under negative control. *Cell* **45**: 601–610.
- Gorman, C.M., L.F. Moffat, and B.H. Howard. 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol. Cell. Biol.* **2**: 1044–1051.
- Graham, F.L. and A.J. Van der Eb. 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* **52**: 456–467.
- Greenberg, M.E. and E.B. Ziff. 1984. Stimulation of 3T3 cells induces transcription of the *c-fos* protooncogene. *Nature* **311**: 433–438.
- Greenberg, M.E., L.A. Greene, and E.B. Ziff. 1985. Nerve growth factor and epidermal growth factor induce rapid transient changes in proto-oncogene transcription in PC12 cells. *J. Biol. Chem.* **260**: 14101–14111.
- Grosso, L.E. and H.C. Pitot. 1985. Transcriptional regulation of *c-myc* during chemically induced differentiation. *Cancer Res.* **45**: 847–850.
- Hann, S.R. and R.N. Eisenman. 1984. Proteins encoded by the human *c-myc* oncogene: Differential expression in neoplastic cells. *Mol. Cell. Biol.* **4**: 2486–2497.
- Ishii, S., Y.-H. Xu, R.H. Stratton, B.A. Roe, G.T. Merlino, and I. Pastan. 1985. Characterization and sequence of the promoter region of the human epidermal growth factor receptor gene. *Proc. Natl. Acad. Sci.* **82**: 4920–4925.
- Jones, K.A., J.T. Kadonaga, P.J. Rosenfeld, T.J. Kelly, and R. Tjian. 1987. A cellular DNA-binding protein that activates eukaryotic transcription and DNA replication. *Cell* **48**: 79–89.
- Kelly, K., B.H. Cochran, C.D. Stiles, and P. Leder. 1983. Cell-specific regulation of the *c-myc* gene by lymphocyte mitogens and platelet-derived growth factor. *Cell* **35**: 603–610.
- Levens, D. and P.M. Howley. 1985. Novel method for identifying sequence-specific DNA-binding proteins. *Mol. Cell. Biol.* **5**: 2307–2315.
- Levine, R.A., J.E. McCormack, A. Buckler, and G.E. Sonenshein. 1986. Transcriptional and posttranscriptional control of *c-myc* gene expression in WEHI 231 cells. *Mol. Cell. Biol.* **6**: 4112–4116.
- Lipp, M., R. Schilling, S. Wiest, G. Laux, and G.W. Bornkamm. 1987. Target sequences for *cis*-acting regulation within the dual promoter of the human *cmyc* gene. *Mol. Cell. Biol.* **7**: 1393–1400.
- Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Maxam, A.M. and W. Gilbert. 1980. Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**: 499–560.
- McKeon, C., A. Schmidt, and B. DeCrombrughe. 1984. A se-

- quence conserved in both the chicken and mouse $\alpha 2(I)$ collagen promoter contains sites sensitive to S_1 nuclease. *J. Biol. Chem.* **259**: 6636–6640.
- Nir, U., M.D. Walker, and W.J. Rutter. 1986. Regulation of rat insulin 1 gene expression: Evidence for negative regulation in nonpancreatic cells. *Proc. Natl. Acad. Sci.* **83**: 3180–3184.
- Nishikura, K. 1986. Sequences involved in accurate and efficient transcription of human *c-myc* genes microinjected into frog oocytes. *Mol. Cell. Biol.* **6**: 4093–4098.
- Pellicci, P.-G. II, D.M. Knowles, I. Magrath, and R. Dalla-Favera. 1986. Chromosomal breakpoints and structural alterations of the *c-myc* locus differ in endemic and sporadic forms of Burkitt lymphoma. *Proc. Natl. Acad. Sci.* **83**: 2984–2988.
- Quinn, J.T., N. Holbrook, and D. Levens. 1987. Binding of a cellular protein to the gibbon ape leukemia virus enhancer. *Mol. Cell. Biol.* (in press).
- Quinn, L.A., G.E. Moore, R.T. Morgan, and L.K. Woods. 1979. Cell lines from human colon carcinoma unusual cell products, double minutes, and homogeneously staining regions. *Cancer Res.* **39**: 4914–4924.
- Radding, M.C. 1971. The purification of B protein and exonuclease made by phage λ . *Methods Enzymol.* **21**: 273–280.
- Ramsay, G., G.I. Evan, and J.M. Bishop. 1984. The protein encoded by the human proto-oncogene *c-myc*. *Proc. Natl. Acad. Sci.* **81**: 7742–7746.
- Ramsay, R.G., K. Ikeda, R.A. Rifkind, and P.A. Marks. 1986. Changes in gene expression associated with induced differentiation of erythroleukemia: Protooncogenes, globin genes, and cell division. *Proc. Natl. Acad. Sci.* **83**: 6849–6853.
- Remmers, E.F., J.-Q. Yang, and K.B. Marcu. 1986. A negative transcriptional control element located upstream of the murine *c-myc* gene. *EMBO J.* **5**: 899–905.
- Rosenthal, N., M. Kress, P. Gruss, and G. Khoury. 1983. BK viral enhancer element and a human cellular homolog. *Science* **222**: 749–755.
- Ruppert, C., D. Goldowitz, and W. Wille. 1986. Proto-oncogene *c-myc* is expressed in cerebellar neurons at different developmental stages. *EMBO J.* **5**: 1897–1903.
- Shaul, Y., R. Ben-Levy, and T. De Medina. 1986. High affinity binding site for nuclear factor I next to the hepatitis B virus S gene promoter. *EMBO J.* **5**: 1967–1971.
- Siebenlist, U., L. Hennighausen, J. Battey, and P. Leder. 1984. Chromatin structure and protein binding in the putative regulatory region of the *c-myc* gene in Burkitt lymphoma. *Cell* **37**: 381–391.
- Stewart, R.A., A.R. Bellve, and P. Leder. 1984. Transcription and promoter usage of the *myc* gene in normal somatic and spermatogenic cells. *Science* **226**: 707–710.
- Taub, R., C. Moulding, J. Battey, W. Murphy, T. Vasicek, G.M. Lenoir, and P. Leder. 1984. Activation and somatic mutation of the translocated *c-myc* gene in Burkitt's lymphoma cells. *Cell* **36**: 339–348.
- Ullrich, A., J. Shine, R. Chirgwin, R. Pictet, E. Tischer, W.J. Rutter, and H.M. Goodman. 1977. Rat insulin genes: Construction of plasmids containing the coding sequences. *Science* **196**: 1313–1319.
- Walker, M.D., T. Edlund, A.M. Boulet, and W.J. Rutter. 1983. Cell-specific expression controlled by the 5' flanking region of insulin and chymotrypsin genes. *Nature* **306**: 557–561.
- Wu, C. 1985. An exonuclease protection assay reveals heat shock element and TATA box DNA-binding proteins in crude nuclear extracts. *Nature* **317**: 84–87.
- Yang, J., S.R. Bauer, J.F. Mushinski, and K.B. Marcu. 1985. Chromosome translocations clustered 5' of the murine *c-myc* gene qualitatively affect promoter usage: Implications for the site of normal *c-myc* regulation. *EMBO J.* **4**: 1441–1449.
- Yarden, A. and A. Kimchi. 1986. Tumor necrosis factor reduces *c-myc* expression and cooperates with interferon-gamma in HeLa cells. *Science* **234**: 1419–1421.
- Zinn, K., D. DiMaio, and T. Maniatis. 1983. Identification of two distinct regulatory regions adjacent to the human β -interferon gene. *Cell* **34**: 865–879.

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