Polyamines Differentially Modulate the Transcription of Growth-associated Genes in Human Colon Carcinoma Cells*

(Received for publication, January 17, 1989)

Paul Celano‡, Stephen B. Baylin, and Robert A. Casero, Jr.

From The Johns Hopkins Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21231

Increases in the transcription of genes important to cell growth frequently occur in concert with increases in intracellular polyamines after a mitogenic stimulus. Previously, we have shown that depletion of intracellular polyamines by 2-difluoromethylornithine in COLO 320 cells resulted in a significant decrease in c-myc mRNA steady state levels. We demonstrate here that polyamines have a predominant role in the regulation of transcription of c-myc, c-fos, and histone 2A, while they appear to have both a transcriptional and post-transcriptional role in expression of c-myb, ornithine decarboxylase, and β-actin mRNA levels. We further analyzed the effect of spermidine in overall and specific RNA transcription in isolated nuclei from COLO 320 cells. In nuclei from control cells, the addition of spermidine resulted in a 3-4 increase in overall [32P]UTP incorporation and a 4-8-fold increase in c-myc, c-fos, and histone 2A transcription. In contrast, ornithine decarboxylase and c-myb gene transcription were unaffected. Furthermore, in nuclei from 2-difluoromethylornithine-treated COLO 320 cells, spermidine addition resulted in less than a 2-fold increase in RNA transcription and had no effect on any specific gene analyzed. These results indicate that polyamines may have an important role in the transcription of specific growth related genes, especially c-myc and c-fos, and this role may be one mechanism by which polyamines modulate cell growth.

The regulation of transcription of c-myc (1-6), c-fos (7-10), and other growth-associated genes is dependent upon positive and negative transacting factors as well as numerous intracellular mediators. Increases in the transcription of the genes frequently occurs in concert with a rapid increase in ornithine decarboxylase activity and the biosynthesis of the polyamines, spermidine and spermine (11-13). These polycationic alkylamines are absolutely required for cell proliferation and have been well characterized as to their ability to affect DNA and chromatin conformation (14-16). However, the roles of the polyamines in specific biochemical processes believed to be important to cell growth, such as the modulation of the expression of the c-myc and c-fos protooncogenes, are unknown (17).

Our previous studies have shown that polyamine depletion of human colon carcinoma cell line COLO 320 with 2-difluoromethylornithine (DFMO), a suicide inhibitor of ornithine decarboxylase (18), results in a significant decrease in c-myc mRNA steady state levels (19). In contrast, steady state levels of ODC and β-actin mRNA levels actually increase under these conditions. Cytoplasmic half-life studies of c-myc mRNA suggested that the effect of polyamine depletion on c-myc mRNA levels were modulated at the level of transcription. In the current study, we have examined directly the role of polyamines in the transcription of specific genes such as c-myc, c-fos, and other growth-related genes. These studies, performed in isolated nuclei, demonstrate that addition of spermidine is essential for optimal performance of in vitro transcription studies and that the polyamines may have a specific role in the transcription of the c-myc and c-fos protooncogenes.

MATERIALS AND METHODS

Chemicals—DFMO was a gift from Merrell-Dow Research Institute (Cincinnati, OH). All other compounds were reagent grade or better.

Cell Culture Conditions—The human colon carcinoma cell line COLO 320 HSR (20) was obtained from American Tissue Type Culture (Rockville, MD). These cells were propagated continuously in RPMI 1640 media and 9% fetal bovine serum (GIBCO). Time course studies were initiated at cell concentrations of 1 × 10^6 cells/ml in 75-cm² flasks. Separate experiments were performed three times. Cell viability was determined by trypan blue exclusion. Cells were harvested at selected time points as indicated under "Results" for biochemical analysis.

Polyamine Analysis—Total cellular polyamine concentrations were determined by reverse-phase chromatography from precolumn dsn-sylated perchloric acid cell extracts as described by Kabra et al. (21).

Nuclear Run on Transcription Assays—Nuclei were prepared by techniques utilized in our previously published studies (22-24). Briefly, COLO 320 cells suspended in buffer A (20 mM Tris-HCl, pH 7.4, 10 mM NaCl, and 3 mM MgCl₂) and 0.1% Nonidet P-40, v/v, and homogenized in a sterile Dounce homogenizer. Nuclei were pelleted at 1000 × g and washed once in buffer A. The nuclear pellet was resuspended in 40% glycerol, 50 mM Tris-HCl, pH 8.3, 5 mM MgCl₂, and 0.1 mM EDTA at 1 µg of DNA/ml and frozen in liquid nitrogen and stored at −70 °C. Nuclei isolated as described were reproducibly intact and free of cellular debris as assessed by phase contrast microscopy. Nuclear transcription activity was determined by measurement of [α-32P]UTP incorporation in RNA transcripts elongated in vitro as described by McNight and Palmer (24). Nuclear transcription assays were carried out in a transcription buffer composed of 35% glycerol, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl₂, 80 mM KCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 4 mM ATP, GTP, CTP, and 200 µCi of [α-32P]UTP (Amersham Corp. 3000 Ci/nmol) at 26 °C for 10 min. In indicated experiments, spermidine (0.25-2.0 mM final concentration) was added to reaction mixtures. RNA was extracted by a modification of the acid guanidium phenol method (25) followed by two ethanol precipitations. Total RNA synthesis was determined using an Ambion A. 32P-labeled RNA probe. This work was supported by National Institutes of Health Grants CA 6973-26, CA 37606, and CA 47492 and the Clayton Fund and the McGrorty Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of the Clinician-Scientist Award, The Johns Hopkins University School of Medicine. To whom reprint requests should be addressed: The Johns Hopkins Oncology Center Laboratories, 424 N. Bond St., Baltimore, MD 21231.

* This work was supported by National Institutes of Health Grants CA 6973-26, CA 37606, and CA 47492 and the Clayton Fund and McGrorty Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† The abbreviations used are: DFMO, 2-difluoromethylornithine; ODC, ornithine decarboxylase; H2A, histone 2A; SSC, saline-sodium citrate.
by measurement of trichloroacetic acid precipitable [$\alpha$-$^{32}$P]UTP/mg of nuclear protein.

Immobilization of DNA Plasmids and Hybridization—DNA from PUC18 control plasmid and plasmids containing human c-myc exon 1 (HindIII-XbaI fragment) and exon 3 (ClaI-EcoRI), human ornithine decarboxylase (Dr. O. Janne, The Population Council, New York, NY), $\beta$-actin (Dr. D. Cleveland, The Johns Hopkins University), histone 2A (Dr. W. Marzluff, University of Florida, Gainesville, FL), c-fos (Neol-XhoI), and c-myb (26) were prepared as follows: DNA was linearized by digestion with HindFI, boiled and blotted (2 $\mu$g of DNA/blot) onto nylon membrane (Gene Screen, Du Pont). These membranes were dried at 80 °C for 4 h. $\alpha$-$^{32}$P-Labeled nuclear RNA (10$^6$ - 20 $\times$ 10$^6$ cpm) isolated from nuclear transcription experiments were used in 3 ml of hybridization buffer (19) and hybridized to the filters for 24 h at 42 °C. Filters were washed in 2 X SSC, 1% sodium dodecyl sulfate at 65 °C for 1 h and then in 0.1 X SSC, 0.1% sodium dodecyl sulfate at room temperature for 1 h. Filters were exposed to Kodak XAR-2 film at −70 °C. Quantitative results were obtained by densitometric scanning and are expressed with reference to the signal for $\beta$-actin.

Northern Blot Analysis—Cytoplasmic RNA from cells used for nuclear run on experiments was isolated and electrophoresed, and Northern hybridization were performed according to previously published methods (19). Filters were serially hybridized to nick-translated [$\alpha$-$^{32}$P]dCTP-labeled DNA probes corresponding to those listed above.

RESULTS

Effect of Polyamine Depletion on Gene Expression—We initially examined the effects of polyamine depletion in COLO 320 cells on the steady state mRNA levels of c-myc, c-fos, and c-myb protooncogenes as well as on the ODC, histone 2A (H2A), and $\beta$-actin genes. As in our previous study (19), depletion of polyamines by DFMO in COLO 320 cells had a differential effect on the overall expression of the genes under study. DFMO treatment resulted in a 90% inhibition in cell growth, a reduction of putrescine and spermidine to undetectable levels by 72 h, and a 50% decrease in spermine levels (Table I). No morphologic changes suggestive of differentiation were noted. Northern blot analysis of cytoplasmic RNA obtained from COLO 320 cells used in nuclear run on experiments demonstrated that, compared with control cells, polyamine depletion of COLO 320 cells was associated with a greater than 90% reduction in c-myc, c-fos, and H2A mRNA steady state levels by 96 h (Fig. 1). These changes did not appear to be the result of a generalized reduction in gene expression since c-myb mRNA levels were unaffected, and ODC and $\beta$-actin mRNA levels steadily increased during polyamine depletion.

Effect of Spermidine in Overall Transcription in Isolated Nuclei from COLO 320 Cells—We next examined the effect of polyamine concentrations on overall transcription rates in nuclei isolated from COLO 320 cells by a technique standardly employed to study transcription in vitro (22-24). These studies first determined that the polyamine content in nuclei isolated for the nuclear run on assay were significantly less than total cellular polyamine concentrations. Most importantly, spermidine was undetectable in nuclear pellets from both control and DFMO-treated COLO 320 cells (Table I).

Prior to the readdition of spermidine, the overall transcription rate as measured by [$\alpha$-$^{32}$P]UTP incorporation into cell RNA, was identical in nuclei from control COLO cells and DFMO-treated cells for 4 and 8 days and was somewhat but not significantly lower for DFMO-treated cells after 12 days ($p<0.20$) (Fig. 2). However, addition of spermidine to nuclei isolated from control cells resulted in a greater than 3-fold increase in total RNA synthesis (Fig. 3). In contrast, similar addition of spermidine to nuclei from DFMO-treated cells resulted in a less than 2-fold increase in overall transcription (control versus DFMO nuclei at spermidine concentration of 0.5 mM $p<0.05$) (Fig. 3).

The effects of spermidine addition on overall transcription rates in isolated nuclei could not be accounted for by replacement of positive changes. Increasing MgCl$_2$ concentrations by 0.5 to 2.0 mM over the standard conditions of 5 mM MgCl$_2$ in the assay system revealed no increase in [$\alpha$-$^{32}$P]UTP incorporation (data not shown).

Effect of the Polyamines on the Transcription of Growth-related Genes in COLO 320 Cells—When the transcription rates of individual genes were examined before and after spermidine addition to isolated nuclei, important selective differences between control and DFMO-treated cells were observed. Treatment of COLO 320 cells with DFMO results in a decrease in the relative transcription rates of the c-myc, c-fos, H2A, ODC, and c-myb genes (Fig. 4A). The decrease in transcription for c-myc, c-fos, and H2A parallels the decrease in their respective cytoplasmic mRNA levels. In contrast,
Polyamines Differentially Modulate Growth Gene Transcription

FIG. 2. Total RNA synthesis from nuclei preparations from 5 mM DFMO-treated COLO 320 cells. Nuclei from control and DFMO-treated COLO 320 cells were isolated and incubated in the presence of \([\alpha-^32P]UTP\) in the nuclear run on assay as described under “Materials and Methods.” RNA was isolated and total trichloroacetic acid precipitable \([\alpha-^32P]UTP\) was quantitated by scintillation counting. Results represent the mean ± S.E. of three separate experiments.

FIG. 3. Effect of the addition of spermidine on total RNA synthesis in nuclei from 5 mM DFMO-treated COLO 320 cells (day 8). Spermidine was added to the nuclear run on assay at the final concentration indicated and assays were performed and RNA was analyzed as described under “Materials and Methods.” Results represent the mean ± S.E. of three experiments.

although c-myb transcription decreases by day 4, its cytoplasmic mRNA level remains relatively unaltered. Furthermore, despite a decrease in ODC transcription in polyamine-depleted COLO 320 cells, cytoplasmic ODC mRNA levels actually increase. Finally, although the transcription rate of \(\beta\)-actin is unaffected, its mRNA levels increase (Fig. 1).

In the previous section, we demonstrated that the addition of spermidine to isolated nuclei resulted in a marked increase in overall RNA transcription, especially in nuclei from untreated COLO 320 cells. To more fully examine the effect of polyamine depletion on transcription of individual genes, identical studies utilizing the addition of physiological concentrations of spermidine were performed. No significant alteration in the transcription rate of any gene was observed in the nuclei of DFMO-treated COLO 320 cells (Fig. 5A) after the addition of spermidine. In contrast, spermidine addition to control nuclei resulted in an 8-fold increase in the transcription of the c-myc and c-fos genes and a 3-fold increase in the H2A gene (Fig. 5B). The increase in c-myc transcription in response to spermidine addition to nuclei appears to be concentration dependent with a maximum increase observed at a concentration of 0.5 mM (Fig. 5C). Similar concentration-dependent trends were observed for c-fos and H2A transcription (Fig. 5B). The changes in the transcription rates of these genes does not appear to result simply from a generalized increase in gene transcription because in nuclei from either control or DFMO-treated COLO 320 cells the transcription rates of the c-myc and ODC genes were unaffected by the addition of spermidine. Furthermore, increasing MgCl\(_2\) by 0.5 to 2.0 mM over the standard nuclear run on assay conditions of 5 mM had no effect on the transcription of any specific gene (data not shown).

Polyamines do not appear to influence the site-specific block in elongation in the c-myc protooncogene. Several workers have described that the regulation of the transcription of c-myc in several cell types is partially mediated by a block in the elongation of nascent nuclear RNA at the exon 1-intron 1 junction (5, 27). In COLO 320 cells, we observed that c-myc transcription also has such a block as evidenced by an increased relative transcription rate of exon 1 as compared with...
Polyamines Differentially Modulate Growth Gene Transcription

Fig. 5. Effect of the addition of spermidine on the rate of gene transcription in nuclei from control and DFMO-treated COLO 320 cells in the nuclear run on assay. Representative autoradiograms from control (A) and 5 mM DFMO treated for 8 days COLO 320 cells (B). Conditions for nuclear run on transcription were performed as described under "Materials and Methods." Equal amounts of [32P]UTP labeled RNA (5–10 × 10⁶ cpm) were hybridized to a nylon filter containing the indicated immobilized plasmid DNA sequences. C, quantitative analysis derived from densiometric analysis of autoradiograms from A and B. Data points represent the mean of three separate experiments with a S.E. of approximately 15%.

exon 3 (Fig. 4A). Polyamine depletion by DFMO did not appear to augment this block in RNA chain elongation because the transcription rate of both c-myc regions was equally decreased by approximately 70% (Fig. 4B). Similarly, the addition of spermidine to nuclei from control cells resulted in an equal increase in transcription of both regions of the c-myc gene (Fig. 5C).

DISCUSSION

Following a mitogenic stimulus, a nearly simultaneous increase in both polyamine biosynthesis and the transcription of genes such as the c-myc and c-fos protooncogenes have been shown to occur in a number of cell systems (1, 4, 28, 29).

The temporal association of these events suggested that polyamines may be involved in modulating the expression of growth-related genes. Our previous studies (19) and the present work support this hypothesis and suggest that variations in polyamine concentrations may play a critical role in modulating gene expression at both the transcriptional and post-transcriptional level.

In the present study, we show that c-myc and c-fos mRNA steady state levels are dramatically decreased while mRNA levels for the other genes studied are either unchanged or actually increased during polyamine depletion of COLO 320 cells. The direct measurement of the transcription rates for these genes shows that the effects of polyamine depletion on c-myc and c-fos expression are mediated by a decrease in transcription while the effects on c-myb, ODC, and β-actin expression appear to be predominantly post-transcriptional. These findings suggest classes of gene expression in response to alterations in intracellular polyamine concentration.

Our results and those of others indicate that polyamines can have a role in the modulation of the expression of specific genes. Several investigators have demonstrated that chronic DFMO exposure can result in increases in ODC mRNA steady state levels (30–33). Although these increases can be directly attributed to amplification of the ODC gene in some cell systems (30, 32), others have ruled out this mechanism (31). Our data suggest that post-transcriptional stabilization may be one mechanism by which ODC mRNA levels increase in response to DFMO treatment. Furthermore, polyamines have also been shown to be required for the basal and maximal cAMP-stimulated production of human chorionic gonadotropin mRNA in JEG-3 choriocarcinoma cells (34) as well as the transcriptional induction of globin mRNA in differentiating mouse erythroleukemia cells (35).

In the present study, we have focused upon the role of spermidine in overall transcription and in the transcription of specific genes. Our findings suggest that the presence of spermidine in the nuclear run on assay may be necessary for an adequate assessment of gene transcription rate. We also show that the polyamines are markedly diminished in nuclei isolated by standard procedures in preparation for the nuclear run on assay. This is not surprising because polyamines only rarely bind covalently with macromolecules (12, 13) and generally interact ionically with numerous intracellular macromolecules, most notably DNA (14–16, 36–38). Although studies addressing the true subcellular distribution of polyamines have been somewhat conflicting, studies using rapid methods of nuclei isolation have generally found intranuclear polyamines to be present in high concentration (39). Therefore, the intranuclear polyamine concentrations determined in the current study are most likely the result of dilution during the prolonged nuclei isolation process.

Our data indicate that under the usual conditions in which the nuclear run on assay is performed, spermidine can improve overall RNA transcription and can have a differential effect on specific gene transcription rates. Our findings are further supported by studies which have demonstrated that spermidine is important in a transcription system analyzing bacteriophage SP6 RNA polymerase (40, 41). Therefore, since intracellular polyamine concentration can vary dramatically in response to numerous mitogenic agents, tumor promoters, and other cell stimuli (12, 13, 28), polyamines should be considered in the assessment of the transcription of specific genes in the nuclear run on assay.

Polyamines may influence the level of transcription of specific gene by several mechanisms including changes in
DNA structure and conformation (42, 43). In cell-free systems, they have been shown to influence B to Z conformational changes in DNA (16, 42, 43) as well as chromatin and nucleosomal structural changes (15). The specific alterations achieved by the polyamines in these cell-free systems is not a simple result of altering ionic environment by the polyamines. The specific effects cannot be accomplished by addition of similar concentrations of simple divalent cations such as Mg²⁺ (15). Interestingly, additional MgCl₂ had no effect in our nuclear transcription assays. Our studies in isolated nuclei are also consistent with studies which have demonstrated spermidine as having structural specificity to overcome a block in cell proliferation following polyamine depletion by DFMO (44, 45).

In addition to chromatin structure, the proper and efficient transcription of eukaryotic genes requires the coordinated action of multiple protein factors for initiation, elongation, and termination (46–51). Since polyamine levels are highly regulated with cell proliferation, alterations in intranuclear polyamines may provide a modulatable ionic environment that could influence the binding or off binding of transcriptional regulatory factors (52). For instance, the affinity of the lac repressor to lac operator DNA in vitro changes by a factor of 20 as NaCl changes from 0.1 to 0.2 M (30). Furthermore, spermidine has been demonstrated to achieve more specific binding of a c-fos gene DNA-binding protein (10). Multivalent cations have also been shown to be critical to the site-specific binding of oligonucleotides used to repress the transcription of the human c-myc gene in vitro (54). In DFMO-treated COLO 320 cells which are severely polyamine depleted, the ionic influence of the polyamines may be simply lost from the nucleus leading to abnormalities in the interaction between transcription factors and their cognate DNA sequences. Importantly, addition of spermidine to nuclei from DFMO-treated cells does not reverse the decreased transcription of any of the genes studied. Loss of polyamines may thus irreversibly alter gene structure in gene manner or lead to loss of synthesis, in the whole cells, of critical transcription factors. Polyamines may also play a role in the regulation of elongation during gene transcription. It should be emphasized, that the transcription rates measured in the nuclear run on transcription assay performed in the current study reflects the elongation of initiated nascent RNA transcripts but has not been definitively shown to permit reinitiation for RNA polymerase II transcripts (55, 56). The regulation of gene transcription by a block in elongation has been demonstrated in the c-myc (5, 27) and c-fos protooncogenes (57). We confirm the findings of others that c-myc exhibits a transcription block at the exon 1-intron 1 junction in COLO 320 cells. However, our data show that polyamine depletion does not specifically augment the transcription block but causes a generalized decrease in c-myc transcription. This may be due to a decrease in elongation rate or a decrease in transcription initiation. Furthermore, in nuclei from control cells, exogenous spermidine enhances transcription throughout the c-myc gene suggesting that spermidine plays a role in RNA chain elongation. Although the mechanisms that control elongation in eukaryotes is poorly understood, changes in DNA secondary structure have been shown to block the elongation of nascent RNA transcripts in prokaryotes (58). Although polyamines have been demonstrated to modulate B to Z form DNA conformational changes (42, 43), they may also participate in other aspects of the elongation mechanism (59).

In summary, our current study supports a mechanistic link between spermidine and the transcription of the c-myc, c-fos, and H2A genes. The isolated nuclei system utilized in our study may, in part, imitate the typical coordinate increases in intracellular polyamine levels which accompany growth onset and the transcription of genes important to cell proliferation. Further study will be necessary to determine if the polyamines play a specific role in the formation of the chromatin structure of these genes or in the interaction or synthesis of key transcriptional regulatory factors as in the regulation of RNA chain elongation.

Acknowledgments—We are grateful to A. de Bustros, M. Mabry, and B. Nelkin for critical discussions, to C. Berchtold, S. Ervin, J. Smith, and E. Setaler for their expert technical assistance, to S. Lund and T. Hess for preparation of the manuscript, and to Albert H. Owens, Jr. for his continuing support.

REFERENCES
1. Kelly, K., Cochran, B. H., Stiles, C. D., and Leder, P. (1983) Cell 35, 603–610
2. Lipp, M., Schilling, R., Wiest, S., Laux, G., and Bomkamp, G. W. (1987) Mol. Cell Biol. 7, 1393–1400
3. Chung, J., Sinn, E., Reed, R., and Leder, P. (1986) Proc. Natl. Acad. Sci. U.S.A. 83, 7918–7922
4. Greenberg, M. E., Hermanoski, A. L., and Ziff, E. B. (1986) Mol. Cell Biol. 6, 1056–1057
5. Salehi, Z., Taylor, J. D., and Neidel, J. E. (1988) J. Biol. Chem. 263, 1898–1903
6. Dublik, D., and Shiui, R. P. C. (1988) J. Biol. Chem. 263, 12705–12708
7. Muller, R., Bravo, R., Burchhardt, T., and Curran, T. (1984) Nature 312, 716–720
8. Prywes, R., and Roeder, R. G. (1986) Cell 47, 777–784
9. Hayes, T. E., Kitchen, A. M., and Cochran, B. H. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 1272–1275
10. Treisman, R. (1986) Cell 46, 567–574
11. Mamont, P. S., Duchen, M. E., Grove, J., and Bey, P. (1978) Biochem. Biophys. Res. Commun. 81, 58–66
12. Tabor, C. W., and Tabor, H. (1984) Annu. Rev. Biochem. 53, 749–790
13. Pegg, A. E. (1986) Biochem. J. 234, 249–262
14. Denstman, S. C., Ervin, S. J., and Casero, R. A. (1987) Biochem. Biophys. Res. Commun. 149, 194–202
15. Morgan, J. E., Blankenship, J. W., and Matthews, H. R. (1987) Biochemistry 26, 3643–3649
16. Tofillon, P. J., Oredsson, S. M., Deen, D. F., and Marton, L. J. (1982) Science 217, 1044–1046
17. Pegg, A. E. (1988) Cancer Res. 48, 759–774
18. Metcalf, W. B., Bey, P., Denzin, C., Jung, M. J., Casera, P., and Vespet, J. P. (1978) J. Am. Chem. Soc. 100, 2551–2553
19. Celano, F., Baylin, S. B., Giardiello, F. M., Nelkin, B. D., and Casero, R. A. (1988) J. Biol. Chem. 263, 5491–5494
20. Altalato, K., Schwab, M., Lin, C. C., Varmus, H. E., and Bishop, J. M. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 1707–1711
21. Kabra, P. M., Lee, H. K., Luich, W. P., and Marton, L. J. (1986) J. Chromatogr. Biomed. Appl. 380, 19–32
22. de Bustros, A., Baylin, S. B., Berger, C. L., Roos, B. A., Leong, S. S., and Nelkin, B. D. (1988) J. Biol. Chem. 263, 98–104
23. Nakagawa, T., Nelkin, B. D., Baylin, S. B., and de Bustros, A. (1988) Cancer Res. 48, 2096–2100
24. McNight, G. S., and Palmeter, R. P. (1979) J. Biol. Chem. 254, 9050–9058
25. Chomczynski, P., and Sacchi, N. (1987) Anal. Biochem. 162, 156–159
26. Gonda, T. J., Gough, N. M., Dunn, A. R., and de Glaquier, J. (1988) EMBO J. 4, 2003–2008
27. Bentley, D. L., and Groudine, M. (1986) Nature 321, 702–706
28. Katz, A., and Kahana, C. (1987) Mol. Cell Biol. 7, 2641–2643
29. Greenberg, M. E., Greenberg, L. A., and Ziff, E. B. (1985) J. Biol. Chem. 260, 14101–14110
30. Pohjanpelto, P., Hollta, E., Janne, O. A., Jnuutila, S., and Alitalo, K. (1985) J. Biol. Chem. 260, 8532–8537
31. Alhoosen-Hongisto, L., Sinervirta, R., Janne, O., and Alitalo, K. (1986) Biochem. J. 232, 605–609
32. Kahana, C., and Nathans, D. (1984) Proc. Natl. Acad. Sci. U.S.A. 81, 3645–3649
33. McConlogue, L., Dana, S. L., and Coffino, P. (1986) Mol. Cell Biol. 6, 2865–2871
Polyamines Differentially Modulate Growth Gene Transcription

Moore, J. J., Lungren, D. W., Moore, R. M., and Andersen, B. (1988) J. Biol. Chem. 263, 12765–12769

Watanabe, T., Sherman, M., Shafman, T., Iwata, T., and Kufe, D. (1986) J. Cell Physiol. 127, 480–484

Manning, G. S. (1978) Q. Rev. Biophys. 2, 179–246

Vertino, P. M., Bergeron, R. J., Cavenaugh, P. F., and Porter, C. W. (1987) Biopolymers 26, 691–703

Thomas, T. J., and Bloomfield, V. A. (1985) Biopolymers 24, 725–729

Mach, M., Egert, P., Popp, R., and Ogi1ive, A. (1982) Biochem. Biophys. Res. Commun. 104, 1327–1334

Butler, E. T., and Chamberlin, M. J. (1982) J. Biol. Chem. 257, 5772–5778

Melton, D. A., Kreig, P. A., Rebalati, M. R., Maniatis, T., Zinn, K., and Green, M. R. (1984) Nucleic Acid Res. 12, 7035–7056

Gross, D. S., and Garrard, W. T. (1988) Annu. Rev. Biochem. 57, 159–197

Elgin, S. C. R. (1988) J. Biol. Chem. 263, 19259–19262

Porter, C. W., and Bergeron, R. J. (1983) Science 219, 1083–1085

Casero, R. A., Bergeron, R. J., and Porter, C. W. (1984) J. Cell Physiol. 121, 476–482

Ptashne, M. (1988) Nature 335, 683–689