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Prognostic significance of "short-term" effects of chemotherapy on MYC and histone H3 mRNA levels in acute leukemia patients

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ABSTRACT **We have found that administration of chemotherapy alters expression of growth-regulated genes in leukemia blast cells. To determine if such changes might be correlated with therapeutic outcome, we studied steady-state mRNA levels of MYC and histone H3 in the leukemic blasts of patients just prior to and 24 hr after the administration of the first doses of antileukemic drug therapy. Among nine patients with acute myelogenous leukemia, mRNA levels of MYC and histone H3 were reduced in five patients, and hematologic remission was achieved in three of these individuals. No remission was obtained in the four patients without reduction in MYC and histone H3 mRNA. Among acute lymphocytic leukemia patients, the mRNA levels of MYC and/or histone H3 were reduced by the therapy in seven of nine patients. A complete hematologic remission was obtained in five of them, and a partial remission was obtained in the other two. No remission was obtained in the patients in which MYC and H3 mRNA levels were unaffected by the therapy. These studies are of interest because they suggest that a decrease in the mRNA levels of MYC and histone H3 24 hr after a single dose of antineoplastic drugs may predict which patients will achieve complete remission; lack of reduction in these mRNAs correlates with failure to achieve remission. In addition, these studies also provide further proof of the heterogeneity of altered growth regulation among human leukemias.**

To elucidate potential mechanism(s) for the impaired regulation of leukemic blast-cell proliferation, we recently measured steady-state mRNA levels expressed by the growth-regulated genes encoding MYC and histone H3 in normal human myeloid precursor cells and in the blast cells of patients with acute leukemia. We then correlated steady-state mRNA levels of these genes with the percentage of cells that were actively traversing the cell cycle (1–3). The results of these studies revealed that when one utilizes mRNA levels of histone H3 as an indicator of the growth fraction (4, 5), the ratio of MYC to histone H3 is constant in normal myeloid precursors and in the majority of patients with acute leukemia (1–3). However, our studies also revealed the existence of a cohort of patients with an abnormally high MYC/H3 mRNA ratio. These results extended previously published findings by Rothberg *et al.* (6) and provided additional proof that in some leukemic patients expression of growth-regulated genes, and in particular *MYC*, might be altered.

Since the number of functional cycling cells often constitutes a minority of the leukemic cell population (7), a possible consequence of the observations noted above is that some fraction of noncycling leukemic cells might reside in a true G_0 state, whereas in other fractions heterogeneous degrees of growth arrest outside G_0 might occur. Because altered expression of *MYC* is the molecular feature most commonly associated with this putative growth arrest of leukemic cells, we hypothesized that deregulation of this gene could play a key role in determining the impairment of the proliferative control associated with the leukemic phenotype.

To test this hypothesis and to better understand the molecular basis of this putative growth arrest in leukemic cells, we ascertained whether changes in the mRNA levels expressed by genes for MYC and histone H3, as compared with levels expressed by the control genes for β-actin and β_2-microglobulin, might occur within hours after treatment of leukemic patients with chemotherapy. We reasoned that chemotherapy might induce early changes in the expression of growth-regulated genes in cycling leukemic cells, while this expression would remain unaffected in noncycling leukemic cells. Therefore, these studies were designed to determine whether early changes (or lack of changes) in the mRNA levels expressed by growth-regulated genes after initiation of chemotherapy could provide useful information on the molecular phenotype associated with induction (or lack of induction) of hematological remission in leukemic patients. It also was hoped that they would render more evident the extent of growth arrest in the leukemic blast-cell population.

MATERIALS AND METHODS

Patients. Patients with high peripheral leukocyte counts with at least 50% blast cells were selected. Use of peripheral blood allowed repeated sampling without repeated marrow aspiration. Heparinized peripheral blood leukocytes from leukemic patients were obtained by venipuncture before therapy and 24 hr after they had received their first dose of simultaneously administered antineoplastic drugs. French–American–British classification (8) of the leukemic cell populations was established by morphologic examination and cytochemical reactions (H_5IO_6/Schiff reagent, Sudan black, myeloperoxidase, chloroacetate esterase, and nonspecific esterase). Cells were further characterized by surface phenotype (common ALL antigen, Leu-1 antigen, HLA-DR, and a variety of anti-myeloid monoclonal antibodies), terminal deoxynucleotidyltransferase enzyme activity, and karyotype analysis. In patients with acute lymphocytic leukemia (ALL), the rearrangement of the Ig locus was also analyzed. Permission for the use of human specimens was granted by the Committee for Protection of Human Subjects of the Children's Hospital of Philadelphia, Temple University, and the Department of Hematology of Modena University.

Nucleic Acid Isolation. Total cellular RNA was purified from leukemic cells as described by Frazier *et al.* (9). Briefly, the cells were homogenized in a Waring blender in the extraction buffer (75 mM NaCl/20 mM EDTA/10 mM

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Abbreviations: ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia.

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Tris·HCl, pH 8.0/0.2% sodium dodecyl sulfate) and mixed 1:1 (vol/vol) with buffer-saturated phenol. The aqueous phase was recovered by centrifugation and reextracted with an equal volume of phenol/chloroform/isoamyl alcohol, 25:24:1 (vol/vol) and once again with chloroform/isoamyl alcohol, 24:1 (vol/vol). The nucleic acids were precipitated with ethanol, and DNA was removed by treatment with DNase I and precipitated with 3 M sodium acetate (pH 5.5). The integrity and amount of RNA samples were monitored by ethidium bromide staining of agarose-formaldehyde gels. DNA extraction was performed essentially as described by Gross-Bellard *et al.* (10).

Blotting and Hybridization Procedures. Total cellular RNA was denatured with 6.6% formaldehyde and 50% formamide and then size-fractionated on a 1.2% agarose gel containing 6.6% formaldehyde. Blotting of RNA to nitrocellulose was done as described by Thomas (11). Nick translation of the plasmid DNA at high specific activity was performed essentially as described by Rigby *et al.* (12). Radiolabeling of DNA inserts was performed as described by Feinberg and Vogelstein (13). Prehybridization, hybridization, and post-hybridization washes were done essentially as described by Wahl *et al.* (14). DNA samples after digestion with restriction enzymes were run on 0.8% agarose gels and transferred to nitrocellulose filters as in the standard Southern blot procedure (15). Hybridization was done as described by Wahl *et al.* (14). Filters were exposed to Kodak x-ray film using intensifying screens at −70°C. Densitometric scanning of the filters was performed with the aid of a Zeineh soft-laser densitometer (Biomed Instruments, Fullerton, CA). The accuracy and the linearity of the densitometer readings were tested by analyzing the x-ray films of the same RNA blots developed after different times of exposure.

Selection of Growth-Regulated Sequences. The growth-regulated genes encoding MYC and histone H3 were used for these studies. MYC mRNA levels increase during the $G_0 \rightarrow G_1$ transition and peak in early-mid G_1, after mitogen stimulation of quiescent cells (16, 17). Histone H3 gene expression is limited to the S phase of the cell cycle (18).

Plasmids. Plasmids carrying the gene probes used in these studies have been described in detail elsewhere. Plasmids were pMC415 and pMC413 [carrying the 5′ and 3′ ends of the *MYC* probe (gifts of G. Franchini, National Institutes of Health)] by Dalla Favera *et al.* (19), pFO422 carrying a histone H3 gene (gift of G. Stein, University of Massachusetts Medical School, Worcester, MA), a human β-actin cDNA (20) (gift of E. Mercer, Temple University Medical School, Philadelphia), and a human β_2-microglobulin cDNA (gift of K. Soprano, Temple University Medical School, Philadelphia) by Suggs *et al.* (21).

RESULTS

Clinical Parameters. Nine patients with acute myeloid leukemia (AML) and nine patients with ALL were studied (Table 1). One patient in each group was in the blast phase of chronic granulocytic leukemia. It may be noticed that the number of blast cells in the peripheral blood of study patients varied from 50% to 100%, but this variation would not be expected to significantly affect our data on the mRNA levels of growth-regulated genes because the RNA yield from mature leukocytes is much lower than from blast cells (22). Table 2 lists the response to antineoplastic treatment for each patient at 24 hr and at the end of induction therapy. Hematological remission was defined as the presence of <5% of blast cells in the bone marrow at the end of treatment.

Expression of Genes Encoding MYC and Histone H3 in the Same AML Patients Before and After a Short Course of Chemotherapy. A major focus of our study was to determine if there were early differential effects of chemotherapy on

Table 1. Patients and therapy

Patient			Therapy	
No.	Age, yr	FAB type	Drugs	Dose, mg
AML				
1	64	M4	araC	150
			Thioguanine	80
			Daunomycin	40
			Methylprednisolone	80
2	17	M3	Daunomycin	40
			Methylprednisolone	60
3	55	CML*	Epirubicin	50
			araC	150
			Methylprednisolone	80
4	13	M4	M-AMSA	100
			Cyclocytidine	1000
			Prednisolone	80
5	52	M5	Vincristine	2
			Epirubicin	50
			araC	150
			Thioguanine	160
6	12	M4	araC	100
			Daunomycin	45
			Prednisolone	60
			Vincristine	2
7	28	M3	Daunomycin	40
			Methylprednisolone	60
			araC	100
			Vincristine	2
8	40	M2	Daunomycin	40
			Prednisolone	80
			araC	100
			Vincristine	2
9	48	M2	Daunomycin	40
			Methylprednisolone	80
			araC	150
			Vincristine	2
ALL				
1	15	L1	Vincristine	2
			Epirubin	60
			Methylprednisolone	60
2	9	L1	Vincristine	2
			Daunomycin	40
			Prednisolone	100
3	50	CML†	Vincristine	2
			Daunomycin	40
			Prednisolone	100
4	40	L1	Daunomycin	40
			araC	150
			Methylprednisolone	80
5	62	L1	Vincristine	2
			Daunomycin	40
			Prednisolone	100
6	16	L1	Idarubicin	15
7	40	L1	Vincristine	2
			Daunomycin	40
			Prednisolone	100
8	36	ALL Ph+	Vincristine	2
			Doxorubicin	80
			Methylprednisolone	80
9	12	L1	Vincristine	2
			Daunomycin	40
			Methylprednisolone	80

M-AMSA, *m*-amsacrine; araC, cytosine arabinonucleoside; FAB type, French–American–British type; CML, chronic myelogenous leukemia; ALL Ph+, ALL Philadelphia-positive.
*Myeloid blast crisis.
†Lymphoid blast crisis.

Table 2. Patients and response to therapy

Patient	Pretreatment WBC	Pretreatment % blast	24 hr post-treatment WBC	24 hr post-treatment % blast	Remission
AML					
1	61,000	80	55,000	82	–
2	25,000	50	19,000	50	–
3	69,000	64	61,000	77	–
4	89,000	95	108,000	95	–
5	78,000	77	81,000	85	+
6	120,600	60	43,000	30	+
7	48,000	72	45,000	76	–
8	35,000	65	32,000	60	+
9	29,000	78	31,000	80	–
ALL					
1	225,000	97	35,600	99	+
2	155,000	91	105,000	100	+
3	95,000	89	59,000	90	+
4	35,000	78	32,000	75	Partial*
5	85,000	94	80,000	90	+
6	117,000	89	53,000	95	–
7	32,000	69	28,000	68	Partial*
8	67,000	69	41,000	60	–
9	165,000	90	85,000	82	+

WBC, leukocytes.
*10% blast cells were still present in the bone marrow at the end of treatment.

cell-cycle- versus non-cell-cycle-related genes. This was accomplished by utilizing RNA blot-hybridization analysis to determine total mRNA levels expressed by the growth-regulated genes encoding MYC and histone H3 and comparing the results to levels of β-actin and β_2-microglobulin mRNAs. β-actin gene expression is abundant and, although growth factor-regulated (23, 24), is not dependent on the cell cycle because its mRNA level is the same in quiescent and proliferating cells (25). β_2-microglobulin is also abundant and equally expressed in all cells regardless of the position in the cell cycle (16). Therefore, β-actin and β_2-microglobulin are useful markers for our purposes. Fig. 1 is a composite blot of total RNA from nine patients with AML. Total RNA was isolated immediately before therapy and 24 hr after administering the first course of antineoplastic drugs. The blotted RNA was first hybridized to *MYC* and then to histone H3 genomic probes after removing the residual hybridization. Fig. 1 shows that in the patients with myeloid leukemia, the therapy affected the mRNA levels expressed by *MYC* differently: for instance, in patients 1, 3, 4, 7, and 9, the mRNA levels of MYC were essentially the same before and after

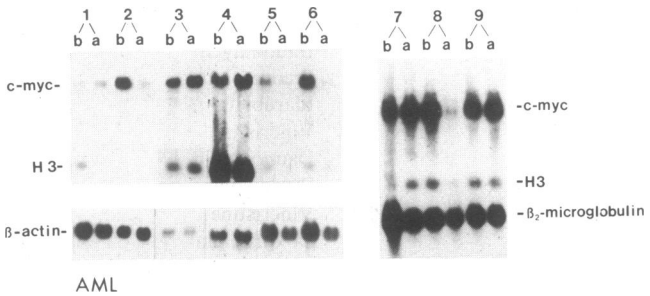

FIG. 1. Levels of MYC, H3, β-actin, and β_2-microglobulin mRNAs in AML patients before therapy (lanes b) and 24 hr after therapy (lanes a). Total RNA was isolated, and 15-μg samples were electrophoresed in each lane of a 1.2% agarose/formaldehyde gel and subsequently transferred to nitrocellulose as described by Thomas (10). Hybridization was carried out first with *MYC* insert, then with a H3 genomic insert, and finally with a β-actin or β_2-microglobulin cDNA insert.

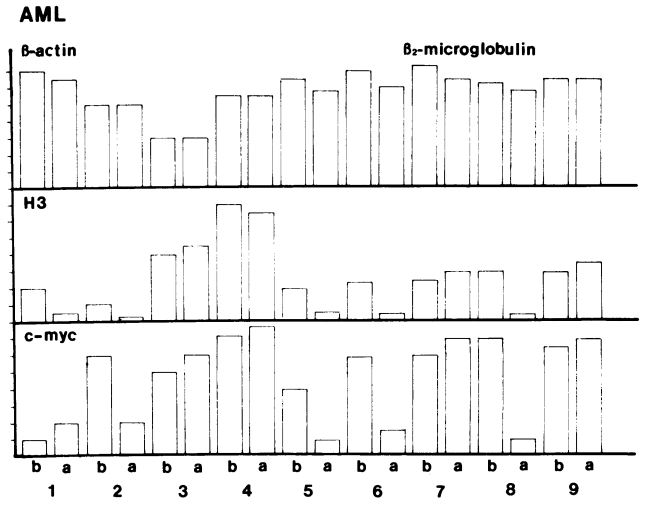

FIG. 2. Densitometric readings of levels of c-myc, H3, β-actin, and β_2-microglobulin mRNAs before (lanes b) and after (lanes a) therapy in AML patients.

therapy, whereas in patients 2, 5, 6, and 8, there was a significant decrease in MYC mRNA. Fig. 1 also shows that the mRNA level of histone H3 was reduced by the therapy in five patients (1, 2, 5, 6, and 8). In no patient was the mRNA level of β-actin or β_2-microglobulin significantly modified by the therapy. The mRNA levels of MYC, histone H3, β-actin, and β_2-microglobulin were also measured before and after therapy by densitometric readings of the Northern blots. Such analysis is shown in Fig. 2.

Expression of Genes Encoding MYC and Histone H3 in ALL Patients Before and After a Short Course of Chemotherapy. The levels of MYC and histone H3 mRNAs in nine patients with ALL before and after therapy are shown in Fig. 3. It is apparent that the expression of the histone H3 gene was dramatically decreased in patients 1, 2, 5, 7, and 9 24 hr after the first administration of antineoplastic drugs in comparison with the untreated patients. The level of histone H3 mRNA was also moderately decreased in patient 3, while it was essentially unchanged or even increased in patients 4, 6, and 8. The expression of *MYC* was drastically reduced by the therapy in patients 1, 2, 5, and 9, and it was slightly reduced in patient 4. The MYC mRNA level was unchanged after therapy in patients 3, 7, and 8, while it increased in patient 6. The filter of Fig. 3 was hybridized to a β-actin or β_2-microglobulin cDNAs and, as shown in the bottom of Fig. 3, the chemotherapy did not induce significant changes in the mRNA levels expressed by these genes. The levels of MYC, histone H3, β-actin, and β_2-microglobulin mRNAs were also measured before and after therapy by densitometric readings of the Northern blots. Such analysis is shown in Fig. 4.

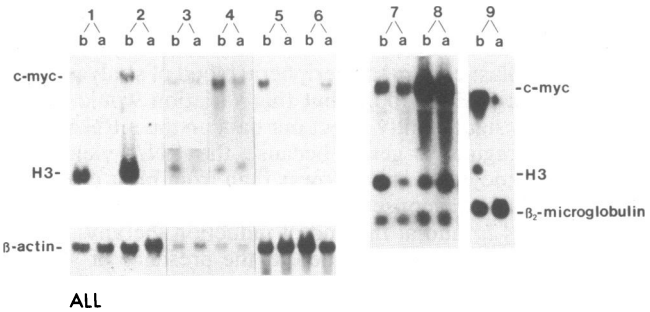

FIG. 3. Levels of MYC, H3, β-actin, and β_2-microglobulin mRNAs in ALL patients before therapy (lanes b) and 24 hr after therapy (lanes a). The conditions are as in Fig. 1.

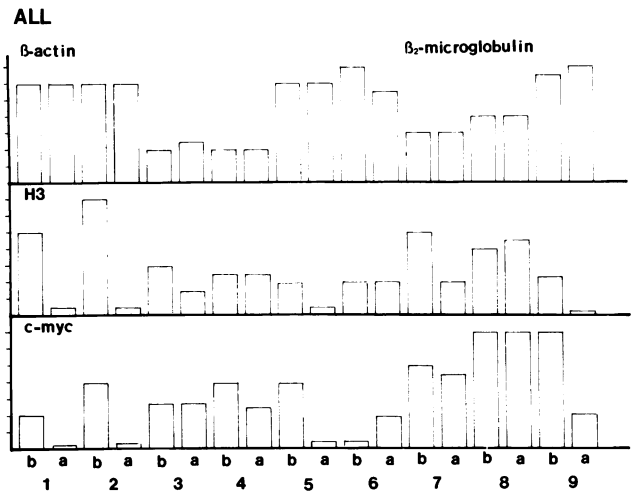

FIG. 4. Densitometric readings of levels of MYC, H3, β-actin, and β_2-microglobulin mRNAs before and after therapy in ALL patients.

Finally, it should also be mentioned that we could not detect major rearrangements or amplification of the *MYC* locus by Southern blot analysis of DNA from the leukemic patients studied (data not shown).

DISCUSSION

In these studies, we have compared the expression of two growth-regulated genes, those encoding MYC and histone H3, with the expression of non-cycle-dependent genes such as those encoding β-actin and β_2-microglobulin in patients with acute leukemia before and 24 hr after treatment with chemotherapy. Our purpose was to investigate the effects of antineoplastic drugs on the expression of growth-regulated genes before the marked cytoreduction that is usually associated with a prolonged treatment. In fact, Table 2 shows that, except in the first patient with ALL, the number of leukocytes and the percentage of blast cells were not significantly altered by the single dose of antineoplastic drugs that the patients had received. The absence of significant modifications on the percentage of blast cells indicates that the variations on the mRNA levels expressed by growth-regulated genes after therapy are due to an early effect of the antineoplastic drugs used on the metabolic pathways necessary for the cell-cycle progression of leukemic cells. Our studies also show that the first dose of chemotherapy induces early changes in the mRNA levels expressed by the growth-regulated genes encoding MYC and H3 in ≈65% of leukemic patients, while it does not affect the mRNA levels of β-actin and β_2-microglobulin in any patient.

Theoretically, the effects observed should reflect the multiplicity of cell-cycle targets of the chemotherapeutic agents used in this study (26). Our observation that the expression of three other growth-regulated genes (genes expressing p53, cyclin, and ornithine decarboxylase) is similarly modified by the chemotherapy provides further support for a direct effect on the expression of growth-regulated genes in cycling cells. The reduction in the levels of MYC and H3 mRNAs after therapy might be the consequence of a direct effect on the transcription of growth-regulated genes in cycling cells. This interpretation is supported by the observation that a reduction of the mRNA levels of histone H3 can be observed in some patients as early as 6 hr after therapy (data not shown). However, it is likely that the magnitude of the effect is increased by the short half-life of the MYC and H3 messages (27, 28) as compared with the much longer half-life of β-actin mRNA (29). Independent of the mechanism involved, early variations on the levels of MYC and H3 histone mRNAs, as compared with the unchanged levels of β-actin and β_2-microglobulin mRNAs, are due to a specific effect rather than being the result of a general inhibition of cellular RNA metabolism.

Among the patients with AML, the expression of genes for MYC and histone H3 was reduced by the therapy in five of nine cases (Fig. 1, patients 1, 2, 5, 6, and 8). Among the patients with ALL, a decrease of H3 or MYC RNA or both RNAs was observed in seven of nine cases (Fig. 3, patients 1, 2, 3, 4, 5, 7, and 9). However, the ratio of MYC/H3 mRNAs expressed was similar in most patients before and after therapy, although there were some exceptions. For instance in patient 1 of the AML group, the ratio MYC/H3 24 hr after therapy was higher than it was before therapy. Within the group of ALL patients, the ratio MYC/H3 was increased after therapy in patients 3, 6, and 7 but was slightly decreased in patient 4. Together, these findings support our suggestion that the protooncogene *MYC* is expressed not only by cycling leukemic cells but also by functionally out-of-cycle leukemic cells (1–3).

We also have tried to establish a correlation between the early effects of the chemotherapy on the mRNA levels expressed by growth-regulated genes and the attainment of hematologic remission when the entire course of chemotherapy was completed. Hematologic remission was obtained in three patients with AML and in five patients with ALL. A decrease in the level of histone H3 mRNA measured 24 hr after a single dose of chemotherapy was observed in five patients with AML, whereas the MYC mRNA level was decreased in four of these patients. Hematologic remission was obtained only in patients 5, 6, and 8. Levels of H3 mRNA were decreased by the therapy in six of nine patients with ALL; the expression of *MYC* was decreased in four of these six patients; five patients were in complete remission at the end of the chemotherapy. Partial remissions were obtained in patient 4 (Fig. 3), in whom only the expression of *MYC* was reduced 24 hr after therapy, and in patient 7 (Fig. 3), in whom only the expression of the H3 gene was reduced 24 hr after therapy.

Although our sample is small, the consistency of our findings suggests that (*i*) lack of a concomitant reduction of MYC and H3 mRNAs is an unfavorable prognostic factor (none of six patients with no changes or increase of MYC and H3 mRNA levels after therapy achieved remission), and (*ii*) a decrease in the mRNA levels of MYC or histone H3 24 hr after the administration of a single dose of chemotherapy might predict eventual attainment of complete hematologic remission. Whether decrease in both MYC and H3 mRNAs will be more significant as a prognostic factor than the decrease of histone H3 or MYC mRNA alone remains to be determined. In this regard, in AML patient 2 (Fig. 1), the decrease in the levels of MYC and H3 mRNAs after a short course of chemotherapy was not accompanied by the attainment of hematological remission. In contrast, a patient with ALL (Fig. 3, patient 7) obtained partial hematological remission despite the lack of early changes in the level of MYC mRNA. Another patient of the same group (Fig. 3, patient 4) obtained partial hematological remission despite the lack of early changes in the level of histone H3 mRNA. It is of interest, however, that a decrease of the histone H3 mRNA level was detected 48 hr after therapy in this patient. This result suggests that monitoring changes in MYC and H3 mRNA levels at 24 hr might not be sufficient to predict the therapeutic outcome in each case and that an additional measurement at 48 hr might be of even greater utility.

In conclusion, we have provided evidence that the analysis of the early effects of chemotherapy on the mRNA levels expressed by growth-regulated genes gives additional information on the molecular phenotype associated with the

deregulation of leukemic cell proliferation. Of equal importance, if the relationships observed are confirmed in a larger study, the observations described could form the basis of a sensitive test for predicting which patient will achieve hematologic remission after chemotherapy. By extension, in those patients who fail to respond to a first dose of chemotherapy with a decrease in either MYC or histone H3 mRNA, more aggressive treatment of their disease might well be indicated.

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1. Calabretta, B., Venturelli, D., Kaczmarek, L., Narni, F., Talpaz, M., Anderson, B., Beran, M. & Baserga, R. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 1495–1498.
2. Torelli, U., Selleri, L., Venturelli, D., Donelli, A., Emilia, G., Ceccherelli, G., Turchi, L. & Torelli, G. (1986) *Leuk. Res.* **10,** 1249–1254.
3. Ferrari, S., Narni, F., Mars, W., Kaczmarek, L., Venturelli, D., Anderson, B. & Calabretta, B. (1986) *Cancer Res.* **46,** 5162–5166.
4. Steel, G. G. (1977) *Growth Kinetics of Tumors* (Clarendon, Oxford).
5. Calabretta, B., Kaczmarek, L. & Baserga, R. (1988) *UCLA Symp. Mol. Cell. Biol.* **58,** 161–170.
6. Rothberg, P. G., Erisman, M. D., Diehl, R. E., Rovigatti, V. G. & Astrin, S. M. (1984) *Mol. Cell. Biol.* **4,** 1096–1103.
7. Vincent, P. C. (1983) in *Leukemia*, eds. Gunz, F. W. & Henderson, E. S. (Grune & Stratton, New York), pp. 77–98.
8. Bennett, J. M., Catovsky, D., Daniel, M. T., Flandrin, G., Galton, D. A. G., Gralnick, H. R. & Sulton, C. (1976) *Br. J. Haematol.* **33,** 451–465.
9. Frazier, M. L., Mars, W., Florine, L. D., Montagna, R. A. & Saunders, G. F. (1983) *Mol. Cell. Biochem.* **56,** 113–122.
10. Gross-Bellard, M., Oudet, P. & Chambon, P. (1973) *Eur. J. Biochem.* **36,** 32–38.
11. Thomas, P. S. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5201–5205.
12. Rigby, P. W. J., Dieckman, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
13. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **132,** 6–13.
14. Wahl, G. M., Stein, M. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3683–3687.
15. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
16. Kelly, K., Cochran, H. B., Stiles, C. D. & Leder, P. (1983) *Cell* **35,** 603–610.
17. Kaczmarek, L., Calabretta, B. & Baserga, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 5375–5379.
18. Plumb, M., Stein, J. & Stein, G. (1983) *Nucleic Acids Res.* **11,** 2391–2410.
19. Dalla Favera, R., Gelmann, R. E., Martinotti, V. S., Franchini, G., Papas, T. S., Gallo, R. & Wong-Staal, F. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6497–6501.
20. Gunning, P., Ponte, P., Okayama, H., Engel, J., Blau, H. & Kedes, L. (1983) *Mol. Cell. Biol.* **3,** 787–795.
21. Suggs, S. V., Wallace, R. B., Hirose, T., Kawashima, E. H. & Itakura, K. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6613–6617.
22. Calabretta, B., Kaczmarek, L., Mars, W., Ochoa, D., Gibson, C. W., Hirschhorn, R. R. & Baserga, R. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 4463–4467.
23. Elder, P. K., Schmidt, L. J., Ono, T. & Getz, M. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7476–7480.
24. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311,** 433–438.
25. Thompson, C. B., Challoner, P. B., Neiman, P. E. & Groudine, M. (1985) *Nature (London)* **314,** 363–366.
26. Goodman, L. S. & Gilman, A. (1980) *The Pharmacological Basis of Therapeutics* (Macmillan, New York), 6th Ed., pp. 1256–1313.
27. Dani, C., Blanchard, J. M., Piechaczyk, M., El Sabouty, S., Marty, L. & Jeanteur, P. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 7046–7050.
28. Heintz, N., Sive, H. L. & Roeder, G. R. (1983) *Mol. Cell. Biol.* **3,** 539–550.
29. Khalili, K. & Weinmann, R. (1984) *J. Mol. Biol.* **180,** 1007–1021.