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Review

Inducing differentiation of transformed cells with hybrid polar compounds: A cell cycle-dependent process

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ABSTRACT **Transformed cells do not necessarily lose their capacity to differentiate. Various agents can induce many types of neoplastic cells to terminal differentiation. Among such inducers, a particularly potent group consists of hybrid polar compounds; hexamethylene bisacetamide (HMBA) is the prototype of this group. With virus-transformed murine erythroleukemia cells as a model, HMBA was shown to cause these cells to arrest in G_1 phase and express globin genes. This review focuses on HMBA-induced modulation of factors regulating G_1-to-S phase progression, including a decrease in the G_1 cyclin-dependent kinase cdk4, associated with inhibition of phosphorylation of the retinoblastoma protein pRB and possibly other related proteins that, in turn, sequester factors required for initiation of DNA synthesis; this provides a possible mechanism for HMBA-induced terminal cell division. Evidence that hybrid polar compounds have therapeutic potential for cancer treatment will also be reviewed.**

In normal development of cell lineages, precursor cells exist that have the capacity either for self-renewal or for differentiation and cessation of cell division (1–3). Neoplastic transformation of precursor cells leads to disordered cell proliferation and failure of cells to differentiate normally. There is abundant evidence that transformation does not necessarily destroy the capacity of transformed cells to express their differentiated phenotype, including terminal cell division (1–6). Conceptually, agents that could restore toward normal the capacity to generate differentiated progeny might be effective in treating or in preventing the progression of cancers (5).

Induced terminal differentiation of transformed cells involves both the cessation of DNA synthesis and the expression of genes for the differentiated phenotype. Induced differentiation of transformed cells has been reported with a number of different agents and a variety of transformed cell lines (4). Perhaps the most extensively studied model has been the use of hybrid polar compounds, of which hexamethylene bisacetamide (HMBA) is the prototype (6), to induce differentiation in murine erythroleukemia cells (7). This review focuses on the events triggered by HMBA that lead to arrest of murine erythroleukemia cells in G_1 phase and cessation of DNA synthesis. HMBA initiates accelerated transcription of the globin genes, but the molecular mechanism of induction of the differentiated phenotype remains unresolved. This review also summarizes the results of clinical trials with HMBA, showing that this agent can cause differentiation of neoplastic cells in patients.

HMBA Effect on Murine Erythroleukemia Cells

Murine erythroleukemia cells are virus-transformed erythroid precursors that appear to be blocked approximately at the CFU-e (colony-forming cell for erythropoiesis) stage of lineage development. These cells neither respond to nor depend on erythropoietin for growth (2, 8, 9). These cells are a model for studying differentiation of unipotent hematopoietic precursor cells along the erythrocytic lineage (2, 4, 9). When cultured with HMBA, these murine cells express a program of differentiation that has many morphological and biochemical similarities to that seen in normal erythropoietin-dependent erythropoiesis (Table 1), (2, 6, 10).

HMBA modulates the membrane surface potential of these transformed erythroid precursor cells (10). This event may activate a signaling pathway (9). No evidence has been obtained for a membrane receptor for HMBA or related compounds on these cells (11). Within minutes of initiating a culture of murine erythroleukemia cells with HMBA, there is a transient increase and then fall in diacylglycerol content (12), followed by an isozyme-specific translocation of protein kinases, protein kinase C (PKC) δ and PKC ε, from the cytosol to the membrane (13). These findings suggested that PKC-modulated signaling pathway may be involved in HMBA-induced murine erythroleukemia cell differentiation. The downstream steps in this pathway including the protein target of PKC-mediated phosphorylation are unknown. No direct evidence supports this hypothesis, but depletion of these cells of PKC activity by exposure to phorbol 12-myristate 13-acetate does render them resistant to HMBA-induced differentiation (14).

HMBA Causes Changes in G_1-to-S Phase Progression

A decision as to whether a cell continues to divide or to withdraw from the cell cycle and differentiate occurs during G_1 phase (see ref. 15). We asked whether inducer-mediated terminal differentiation was a cell cycle-related phenomenon (16). Murine erythroleukemia cells cultured with HMBA have a prolongation in the first G_1 phase, resulting in an increase in cell cycle time from ≈12 hr (for cells in culture without HMBA) to ≈16 hr (17, 18). This prolongation in G_1 phase required exposure to HMBA during the preceding G_1 and early-S phases (18). It is during the initial prolonged G_1 phase that commitment of a portion of the cells (generally ≈15%) to terminal cell division and a marked increase in globin gene transcription are first detected (17, 19). Commitment is defined as the irreversible acquisition of the capacity to cease dividing and to express the characteristics of the differentiated state (e.g., in these cells, active transcription of globin genes) (19). Evidence from studies with other hematopoietic cells indicates that cytokines that regulate cell proliferation and differentiation also act primarily during G_1 phase (20, 21). It may be speculated that in the presence of inducers, as chromatin structure is altered with the onset of DNA replication, changes are also induced in transcription factor complexes that repress transcription of certain genes (e.g., those for proteins required for DNA synthesis) and activate transcription of other genes (e.g., globin genes).

Regulators of G_1-to-S Phase Progression Are Targets of HMBA

Prolongation of the first G_1 phase appears to occur in virtually 100% of the murine erythroleukemia cells in culture with

Abbreviations: PKC, protein kinase C; cdk, cyclin-dependent kinase; pRB, retinoblastoma protein; HMBA, hexamethylene bisacetamide.

Table 1. Characteristics of HMBA-induced differentiation of murine erythroleukemia cells

Morphological changes
Chromatin condensation
Disappearance of nucleoli
Decrease in the nuclear/cytoplasmic ratio
Early metabolic changes
Decrease in membrane fluidity
Alteration of membrane potential
Alteration at Na^+/K^+ transport
Increase of Ca^{2+} uptake
PKC activity translocated from cytosol to membrane
Transient increase, then decrease in diacylglycerol
Cessation of c-*myb* and c-*myc* gene transcription
Decrease in c-*myb*, c-*myc*, and p53 protein levels
Increase in c-fos mRNA level
Later metabolic changes
Cessation of DNA synthesis
Increase in globin gene transcription
Increase in globin synthesis
Sequential increase in enzyme activities involved in *de novo* heme synthesis: δ-aminolevulinic acid synthetase, aminolevulinic acid dehydrogenase; uroporphyrinogen-I synthetase; ferrochelatase
Accumulation of hemoglobin

For detailed references see refs. 2, 4, 9, and text, which discuss additional characteristics of HMBA-induced differentiation of these cells. This list of HMBA-induced changes does not include all reported studies.

HMBA. Nevertheless, as indicated above, during this initial prolonged G_1 phase, commitment to terminal differentiation is detected in only ≈15% of the cells, and it requires an additional five to six cell divisions to recruit >95% of cells to differentiate (19). Further, HMBA and related compounds are neither species nor cell-type specific as inducers of differentiation (5, 22–25). These observations pose at least two questions. (*i*) Why does it require several cell cycles in the presence of the inducer to achieve terminal differentiation of essentially all cells? (*ii*) Why are hybrid polar compounds relatively nonspecific with respect to the type of transformed cells sensitive to the inducer?

Cyclins and Cyclin-Dependent Kinases (cdks)

In considering these questions, we turned to examine the effects of HMBA on factors regulating cell cycle progression—in particular, the G_1-to-S phase transition because many of these factors are common to all eukaryotic cells. Thus, cdks, activated by association with cyclins, are involved as positive regulators of cell cycle progression (20). Combinations of cyclins and cdks interact at G_1 control points and regulate the G_1-to-S phase transition (20, 26–28), whereas retinoblastoma protein (pRB) and the related protein p107 may act to suppress cell cycle progression through G_1 phase (21). Different cell types display different patterns of expression of the G_1-type D cyclins and cdks (27, 29–31). Phosphorylation of pRB and possibly p107 is accompanied by loss of the activity of these proteins in blocking the G_1-to-S phase transition (32). It may be hypothesized that critical level of a factor (or factors) suppressing G_1-to-S phase progression must be achieved for a cell to be committed to cessation of DNA synthesis.

In murine erythroleukemia cells cultured without inducer, the protein levels of cyclins D3, E, and A fluctuate during the cell cycle, whereas the level of cyclin D2 changes little, if at all (33). Cyclin D1 protein is not detected in these cells. Cyclin D3 increases as cells progress through G_1 phase to a maximum level in S phase. Cyclin E peaks at the transition from G_1-to-S phase. Cyclin A increases somewhat later than cyclin E, peaking in G_2/M phase. The level of cdk4 fluctuates in a pattern similar to that of cyclin D3, peaking in S phase. cdk2 kinase activity is low in G_1 phase and increases during S phase, whereas the level of cdk2 protein is constant during the cell cycle. These patterns of change in cyclins and cdks in murine erythroleukemia cells are similar to those seen in growing HeLa and MANCA cells (a human B cell line) (34–36).

HMBA-induced differentiation of murine erythroleukemia cells is associated with striking changes in these patterns of expression of certain cyclins and cdks (Fig. 1). During the transiently prolonged G_1 phase the level of cyclin A and the activity of cdk2 remain low for the duration of G_1 phase. The levels of cyclin A protein and cdk2 kinase activity increase as cells enter S phase. HMBA does not alter the pattern of fluctuation of cyclin E compared with that in cells cultured without inducer (33).

The most striking HMBA-induced changes in the pattern of expression of cyclins and cdks are a 20-fold decrease in cdk4 protein, a 75% decrease in cdk4-dependent kinase activity, and a 3-fold increase in cyclin D3 protein level (33), beginning during the initial prolonged G_1 phase.

The striking decrease in cdk4 protein reflects a marked decrease in its stability. The rate of synthesis of the cdk4 protein and the level of mRNA are unaffected by HMBA. The increase in cyclin D3 protein level in conjunction with a fall in cdk4 protein results in a fraction of the cyclin D3 not bound to the kinase, and it is found in complex with hypophosphorylated pRB. The transcription factor E2F is also found in complex with cyclin D3 in HMBA-cultured cells (33).

To evaluate the significance of the HMBA-induced decrease in cdk4, murine erythroleukemia cells transfected with cdk4 cDNA and with cdk2 cDNA were isolated. Overexpression of cdk4 protein inhibits HMBA-induced differentiation of these cells (33). Overexpression of cdk2 protein does not have this effect. These results suggest that selective suppression of cdk4 is critical during induced terminal differentiation.

pRB and Related Proteins

pRB and the related proteins p107 and p130 are implicated in the control of cell cycle progression (21, 32, 37). The ability of pRB to regulate cell progression through G_1 phase is controlled by phosphorylation and dephosphorylation of the protein. It is thought that hypophosphorylated pRB is the active form in suppressing progression through G_1-to-S phase (21, 32). Transforming growth factor β_1 inhibits cell proliferation, causing cell cycle arrest in G_1 phase. This effect of transforming growth factor β correlates with an accumulation of hypophosphorylated pRB (37) and is associated with inhibition of cdk4 synthesis (38) and inhibition of the G_1 cdks, such as cyclin E-cdk2 (39). Similar changes in pRB phosphorylation may be involved in regulating expression of differentiation (32).

pRB is hypophosphorylated in early G_1 phase and is progressively phosphorylated during mid-to-late G_1 phase, possibly by cyclin D-cdk4 kinase (21, 32). DNA tumor virus oncoproteins—such as simian virus 40 large tumor antigen, the adenovirus E1A, and the papilloma virus E7—complex with pRB only in its underphosphorylated forms (32, 40–42). Complex formation inactivates the growth suppressor function of pRB, in part by blocking a binding domain on pRB for other cellular proteins such as E2F. As a result, transcription of E2F-regulated genes is suppressed (43). Genes with E2F sites include DNA polymerase α, dihy-

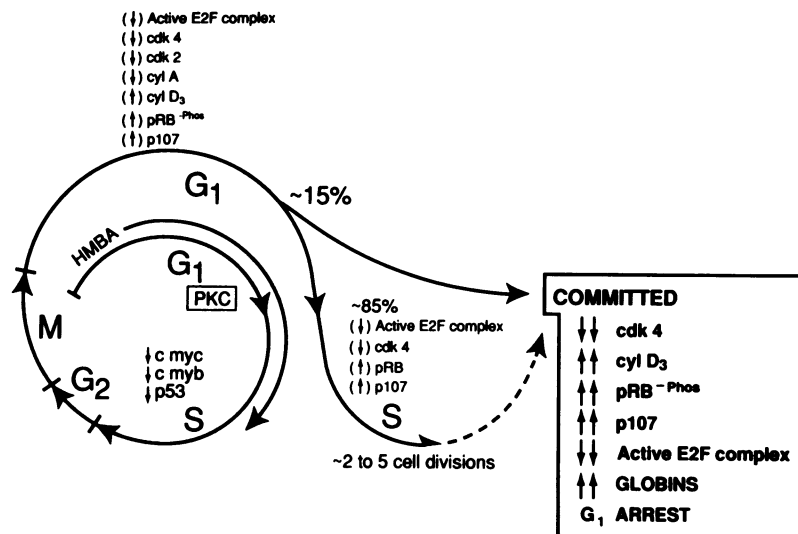

FIG. 1. Schematic representation of HMBA-induced changes in murine erythroleukemia cell cycle progression, cyclins (cyl), cdks, pRB, p107, and globin gene expression. (↑), increased; (↓), decreased. Phos, phosphorylated.

drofolate reductase, thymidylate synthetase, c-*myc*, and c-*myb*, all of them encoding proteins involved in cell cycle regulation and differentiation (43). The phosphorylation of pRB during mid-to-late G_1 phase is accompanied by release of transcription factor E2F from the E2F/pRB complex and, presumably, consequent activation of transcription of these E2F-regulated genes. The HMBA-induced initial G_1 phase prolongation is associated with an increase in the relative amount of hypophosphorylated pRB (44, 45). The variant cell line (DS19/VCR/C) has accelerated kinetics of HMBA-induced differentiation, owing to commitment of a greater proportion of cells to differentiation during the first G_1 phase than with the variant DS19 cells. DS19/VCR/C cells have a more marked increase in the relative amount of hypophosphorylated pRB during the initial prolonged G_1 phase than do DS19 cells. With progressive recruitment of DS19 cells into the differentiation program, the total amount of pRB and p107 increases, and these increases are associated with an increased proportion of cells arrested in G_1 phase. These studies suggest that pRB and p107 participate in determining HMBA-induced terminal cell division of murine erythroleukemia cells. One hypothesis that may explain the requirement for several cell cycles in the presence of HMBA to achieve terminal differentiation of >95% of these cells is that during the initial G_1 phase only a fraction of cells have sufficient levels of hypophosphorylated pRB or related proteins to permanently arrest cells in G_1 phase. The remaining cells enter S phase as the level of phosphorylated pRB increases, but the relative amount of hypophosphorylated pRB in a fraction of the cells remains higher than in cells cultured without inducer. With subsequent passage to G_1 phase, a further proportion of cells achieves levels of hypophosphorylated pRB sufficient to cause G_1 phase arrest. With each subsequent cell cycle, cells are recruited to arrest in G_1 phase by this mechanism.

E2F Proteins

As indicated above, E2F proteins can complex with cell cycle-regulatory proteins, including pRB and p107, as well as cyclins A and E and the cdks. The complexing of E2F with these proteins inactivates its promotor function and may cause it to act as a repressor of gene function (32, 43). In uninduced murine erythroleukemia cells, E2F–DNA complexes are present in at least two forms, based on electrophoretic mobility (unpublished observations). The E2F complex with the faster electrophoretic mobility appears to be free E2F complexed to DNA. By 8 hr of culture with HMBA, this form of E2F complex is no longer detected. The remaining complexes, which persist during culture with inducer, contain transcription factor E2F associated with p107, cyclin A, and cdk2. These findings suggest a role for p107 in regulating the transcriptional activity of E2F during HMBA-induced murine erythroleukemia cell terminal differentiation.

Phase I/II Clinical Trials with HMBA

After investigations on the effects of HMBA on cells in culture, preclinical animal pharmacokinetic and toxicology studies, and phase I clinical trials (45, 46), a phase II clinical trial was conducted using HMBA to treat patients with myelodysplastic syndrome or acute myelogenous leukemia (47). Of 28 patients who received at least two or more 10-day courses of therapy, a complete remission was observed in three patients (1.3- to 16-mo duration), and a partial remission was seen in six patients (1- to 7-mo duration). Further, these clinical studies provided direct evidence that HMBA can induce differentiation of transformed precursor cells in patients. In four separate courses, a patient with myelodysplastic syndrome with monosomy 7 karyotype in bone marrow blast cells achieved peripheral blood granulocyte counts that approached normal levels. These morphologically mature granulocytes carried the chromosomal marker of the malignant clone, monosomy 7.

HMBA caused severe thrombocytopenia, which limits its administration and its efficacy. Recently, compounds have been synthesized that are effective inducers of transformed cells in culture at concentrations as low as 1/500th of that optimal for HMBA (Table 2). These agents induce expression of differentiation in several types of transformed cells in culture, including murine erythroleukemia cells (20), human promyelocytic leukemia HL-60 cells (21), human breast cancer (22), human colon carcinoma (21), human melanoma (23), and others (5). Compounds that are more potent as cytodifferentiation agents than HMBA and without serious toxic side effects have to be developed if hybrid polar compounds are to provide effective drugs for inducing differentiation of transformed cells, as either therapeutic or chemopreventive agents.

Conclusion

Considerable progress has been made toward elucidating the pathway of induction of terminal differentiation of transformed cells by hybrid polar compounds such as HMBA. HMBA alters factors controlling G_1-to-S phase transition, leading to G_1 arrest and inhibition of DNA synthesis. Among the inducer-mediated changes, suppression of cdk4, which may be required for phosphorylation of pRB and perhaps p107, is critical in the pathway of terminal differentiation. Hypophosphorylated pRB, as well as p107, can complex with transcription factor E2F, which, in turn, may alter E2F-dependent gene transcription. Much less has been defined with respect to the relationship of the inducer-mediated changes in cyclins, cdks, regulatory proteins, and transcription factors and the expression of differentiation-specific genes. The hybrid polar compounds are potent inducers of differentiation of a wide variety of transformed cells, and HMBA can induce differentiation of neoplastic cells in patients. Taken together, our present knowledge suggests that

Table 2. Hybrid polar compounds active as inducers of differentiation of transformed cells

Compound	Optimal concentration, μM	Transformed cells* MEL	HL-60	HT-29
1. $CH_3\text{-}\overset{O}{S}\text{-}CH_3$	280,000	+	+	+
2. $CH_3\text{-}\overset{O}{C}\text{-}\overset{H}{N}\text{-}(CH_2)_6\text{-}\overset{H}{N}\text{-}\overset{O}{C}\text{-}CH_3$	5,000	+	+	+
3. $(CH_3)_2\text{-}N\text{-}\overset{O}{C}\text{-}(CH_2)_6\text{-}\overset{O}{C}\text{-}N\text{-}(CH_3)_2$	5,000	+	+	+
4. $CH_3\text{-}N\text{-}\overset{O}{C}\text{-}(CH_2)_6\text{-}\overset{O}{C}\text{-}N\text{-}CH_3$	5,000	+	+	+
5. $(CH_3)_2\text{-}N\text{-}\overset{O}{C}\text{-}(CH_2)_5\text{-}\overset{CH_3CH_2O\text{-}C\text{=}O}{\underset{O\text{=}C\text{-}OCH_2CH_3}{C}}\text{-}(CH_2)_5\text{-}\overset{O}{C}\text{-}N\text{-}(CH_3)_2$	200–600	+	+	+
6. $HO\text{-}\underset{H}{N}\text{-}\overset{O}{C}\text{-}(CH_2)_6\text{-}\overset{O}{C}\text{-}\underset{H}{N}\text{-}OH$	10–60	+	+	+
7. $HO\text{-}\underset{H}{N}\text{-}\overset{O}{C}\text{-}\underset{H}{C}\text{=}\overset{H}{C}\text{-}C_6H_4\text{-}\overset{O}{C}\text{-}NH\text{-}OH$	10–60	+	+	+

+, Compound induces terminal differentiation of the transformed cells.
*MEL are murine erythroleukemia cells, HL-60 are human promyelocytic leukemia cells, and HT-29 are human colon cancer cells.

these agents may be effective in treating cancers.

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1. Pierce, G. B. & Wallace, C. (1971) *Cancer Res.* **31**, 127–134.
2. Marks, P. A. & Rifkind, R. A. (1978) *Annu. Rev. Biochem.* **47**, 419–448.
3. Sachs, L. (1987) *Cancer Res.* **47**, 1981–1986.
4. Michaeli, J., Marks, P.A. & Rifkind, R. A. (1992) in *Cancer Chemotherapy and Biological Response Modifiers: Annual 13*, eds. Pinedo, H. M. P., Longo, D. & Chabner, B. A. (Elsevier, New York), pp. 288–307.
5. Marks, P. A. & Rifkind, R. A. (1984) *Cancer* **54**, 2766–2769.
6. Reuben, R. C., Wife, R. L., Breslow, R., Rifkind, R. A. & Marks, P. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 862–866.
7. Friend, C., Scher, W., Holland, J. & Sato, T. (1971) *Proc. Natl. Acad. Sci. USA* **68**, 378–382.
8. Clarke, B. J., Axelrod, A. A., Shreeve, M. M. & McLead, D. L. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3556–3560.
9. Tsiftsoglou, A. S. & Wong, W. (1985) *Anticancer Res.* **5**, 81–100.
10. Arcangeli, A., Carla, M., del Bene, M. R., Becchetti, A., Wanke, E. & Olivotto, M. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 5858–5862.
11. Reuben, R. C., Rifkind, R. A. & Marks, P. A. (1980) *Biochim. Biophys. Acta* **606**, 325–346.
12. Michaeli, J., Busquets, X., Orlow, I., Younes, A., Colomer, D., Marks, P. A., Rifkind, R. A. & Kolesnick, R. N. (1992) *J. Biol. Chem.* **267**, 23463–23466.
13. Leng, L., Yu, F., Dong, L. Busquets, X., Osada, S.-i., Richon, V. M., Marks, P. A. & Rifkind, R. A. (1993) *Cancer Res.* **53**, 5554–5558.
14. Melloni, E., Pontremoli, S., Michetti, M., Sacco, O., Cakiroglu, A. G., Jackson, J. F., Rifkind, R. A. & Marks, P. A. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 5282–5286.
15. Pardee, A. B. (1989) *Science* **246**, 603–608.
16. Levy, J., Terada, M., Rifkind, R. A. & Marks, P. A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 28–32.
17. Gambari, R., Marks, P. A. & Rifkind, R. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4511–4515.
18. Gambari, R., Terada, M., Bank, A., Rifkind, R. A. & Marks, P. A. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3801–3804.
19. Fibach, E., Reuben, R. C., Rifkind, R. A. & Marks, P. A. (1977) *Cancer Res.* **37**, 440–444.
20. Sherr, C. J. (1993) *Cell* **73**, 1059–1065.
21. Cobrinik, D., Dowdy, S. F., Hinds, P. W., Mittnacht, S. & Weinberg, R. A. (1992) *Trends Biochem. Sci.* **17**, 312–315.
22. Marks, P. A., Breslow, R., Rifkind, R. A., Ngo, L. & Singh, R. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6358–6362.
23. Breslow, R., Jursic, B., Fa Yan, Z., Friedman, E., Ngo, L., Rifkind, R. A. & Marks, P. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 5542–5546.
24. Guilbaud, N. F., Gas, N., Dupont, M. A. & Valette, A. (1990) *J. Cell. Physiol.* **145**, 162–172.
25. Huberman, E., Heckman, C. & Langenbach, R. (1979) *Cancer Res.* **39**, 2618–2624.
26. Ajchenbaum, F., Ando, K., DeCaprio, J. A. & Griffin, J. D. (1993) *J. Biol. Chem.* **268**, 4113–4119.
27. Matsushime, H., Roussel, M. F., Ashmun, R. A. & Sherr, C. J. (1991) *Cell* **65**, 701–713.
28. Won, K.-A., Xiong, Y., Beach, D. & Gilman, M. Z. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9910–9914.
29. Kiyokawa, H., Busquets, X., Powell, C. T., Ngo, L., Rifkind, R. A. & Marks, P. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2444–2447.
30. Motokura, T., Bloom, T., Kim, H. G., Juppner, H., Ruderman, J. V., Kronenberg, H. M. & Arnold, A. (1991) *Nature (London)* **350**, 512–515.
31. Motokura, T., Keyomarsi, K., Kronenberg, H. M. & Arnold, A. (1992) *J. Biol. Chem.* **267**, 20412–20415.
32. Ewen, M. E. (1994) *Cancer Metastasis Rev.* **13**, 45–66.
33. Kiyokawa, H., Richon, V. M., Rifkind, R. A. & Marks, P. A. (1994) *Mol. Cell. Biol.*, in press.
34. Dulic, V., Lees, E. & Reed, S. I. (1992) *Science* **257**, 1958–1961.
35. Koff, A., Giordano, A., Desai, D., Yamashita, K., Harper, J. W., Elledge, S., Nishimoto, T., Morgan, D. O., Franza, B. R. & Roberts, J. M. (1992) *Science* **257**, 1689–1694.
36. Pines, J. & Hunter, T. (1990) *Nature (London)* **346**, 760–763.
37. Laiho, M., DeCaprio, J. A., Ludlow, J. W., Livingston, D. M. & Massague, J. (1990) *Cell* **62**, 175–185.
38. Ewen, M. E., Sluss, H. K., Whitehouse, L. L. & Livingston, D. M. (1993) *Cell* **74**, 1009–1020.
39. Koff, A., Ohtsuki, M., Polyak, K., Roberts, J. M. & Massague, J. (1993) *Science* **260**, 536–539.
40. DeCaprio, J. A., Ludlow, J. W., Figge, J., Shew, J.-Y., Huang, C. M., Lee, W. H., Marsillo, E., Paucha, E. & Livingston, D. M. (1988) *Cell* **54**, 275–283.
41. Whyte, P., Buchkovich, K., Horowitz, J. M., Friend, S. H., Raybuck, M., Weinberg, R. A. & Harlow, E. (1988) *Nature (London)* **334**, 124–129.
42. Dyson, N., Howley, P. M., Munger, K. & Harlow, E. (1989) *Science* **243**, 934–937.
43. La Thangue, N. B. (1994) *Curr. Opin. Cell Biol.* **6**, 443–450.
44. Richon, V. M., Rifkind, R. A. & Marks, P. A. (1992) *Cell Growth Differ.* **3**, 413–420.
45. Egorin, M. J., Sigman, L. M., VanEcho, D. A., Forrest, A., Whitacre, M. Y. & Aisner, J. (1987) *Cancer Res.* **47**, 617–623.
46. Young, C. W., Fanucchi, M. P., Walsh, T. D., Baltzer, L., Yaldaei, S., Stevens, Y. W., Gordon, C., Tong, W., Rifkind, R. A. & Marks, P. A. (1988) *Cancer Res.* **48**, 7304–7309.
47. Andreeff, M., Stone, R., Michaeli, J., Young, C. W., Tong, W., Sogoloff, H., Ervin, T., Kufe, D., Rifkind, R. A. & Marks, P. A. (1992) *Blood* **80**, 2604–2609.