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Differential Expression of the Histone H1° Gene in U937 and HL-60 Leukemia Cell Lines

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Abstract The expression of the human H1° histone gene and of a main type H1 gene was analyzed in two human leukemia cell lines. The main type, replication dependent H1 gene expression reflected the state of proliferation of both cell lines. No H1° mRNA was detected in the promyelocytic HL-60 line, whereas the monocytic U937 cells showed low steady-state levels of H1° mRNA. Stimulation of HL-60 with several known inducers of differentiation failed to induce any accumulation of H1° mRNA. Treatment of U937 with phorbol ester or butyrate, on the other hand, led to an increase of the H1° mRNA concentration. © 1992 Wiley-Liss, Inc.

Key words: histone H1°, histone H1, phorbol ester, butyrate, dimethyl sulfoxide, HL-60 cell line, U937 cell line

The H1° histone is a characteristic component of the chromatin of terminally differentiated mammalian cells [Panyim and Chalkley, 1969; Smith et al., 1984]. Initially this H1 subtype was considered a characteristic component of nondividing cells [Smith et al., 1984; Zlatanova, 1980]. In the meantime, however, studies of H1° protein [Chabanas et al., 1985] and mRNA levels [Alonso et al., 1988; Bouterfa et al., 1990] showed that the induction of H1° gene expression can precede the onset of the inhibition of DNA replication. An *extreme* example of an H1° gene expression independent from an inhibition of DNA replication is the constitutively high synthesis of H1° mRNA and protein in the hepatoma cell line HepG2 [Hochhuth and Doenecke, 1990; Gabrielli et al., 1985].

The correlation between the occurrence of H1° and the state of differentiation of a given cell type [Gjerset et al., 1982] suggested that the regulation of the H1° gene forms part of a tissue specific differentiation programme. Several leukemia cell lines, such as the promyelocytic HL-60 cell [Koeffler et al., 1980; Miller and Koeffler, 1986; Collins et al., 1977; Collins, 1987] or the monocytic U937 line [Sundström and Nilsson, 1976; Radzun et al., 1983; Hass et al., 1989; Alvarez et al., 1989; Olsson et al., 1982; Hattori et al., 1983] can be induced to undergo certain steps of differentiation.

Since treatment with inducers of differentiation is frequently associated with a decreased proliferation rate [D'Anna et al., 1982; Hall et al., 1985; Kress et al., 1986], we have chosen HL-60 and U937 cells for a comparative analysis of human H1 gene regulation under varied growth conditions.

The spectrum of mammalian H1 histones comprises at least five main type species [Lennox and Cohen, 1983] and, in addition, the testicular subtype H1t [Meistrich et al., 1985] and the replacement subtype H1° [Smith et al., 1984]. To date, five human main type H1 genes [Carozzi et al., 1984; Eick et al., 1989; Albig et al., 1991], the human H1t gene [Drabent et al., 1991] and the human gene coding for H1° [Doenecke and Tönjes, 1986] have been described.

The regulation of expression of the H1° gene differs from other H1 genes in several respects. First, the synthesis of H1° does not depend on the S-phase of the cell cycle [Zlatanova and Swetly, 1980]. Secondly, the mRNA coding for H1° carries a poly(A) tail and it shows a long non coding 3′ flanking region [Kress et al., 1986]. Thirdly, in contrast to all other mammalian H1 genes, the H1° gene is not neighboured by core histone genes [Doenecke and Tönjes, 1986].

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We have analyzed steady-state levels of H1° mRNA in HL-60 and U937 cell lines prior to and after addition of butyrate, DMSO, or TPA. For comparison with H1°, we have used a main type human H1 probe to monitor the level of cell cycle dependent histone gene expression. In addition to TPA and DMSO, sodium butyrate was included in the protocol, since this compound induces H1° gene expression in several systems [D'Anna et al., 1982; Hall et al., 1985; Kress et al., 1986] prior to a decrease in the proliferation rate of the respective cells.

MATERIALS AND METHODS

All chemicals used were analytical grade reagents. Media and fetal calf serum were from Gibco/BRL (Gaithersburg, MD); chemicals for electrophoresis were from Serva (Heidelberg, Germany); denaturing agents were from Fluka (Buchs, Switzerland). TPA and DMSO were from Sigma (St. Louis, MO). All other chemicals were from Merck (Darmstadt, Germany). Membrane filters were from Schleicher and Schuell (Dassel, Germany). Radioactively labelled compounds and the random prime labeling kit were obtained from Amersham (Braunschweig, Germany).

Cell Culture

HL-60 and U937 cells were grown at 37°C (5% CO_2) in RPMI 1640 medium supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, 2 mM pyruvate, 100 μg of streptomycine, and 100 units of penicillin per ml.

Depending on the different induction protocols, the medium was supplemented with either 5 mM sodium byturate, 32 nM 12-O-tetradecanoylphorbol-13-acetate (TPA), or 1,25% (v/v) DMSO (dimethylsulfoxide) for the different periods of time indicated in Figures 1 and 2.

The time course chosen for the analysis of the relation between H1° gene expression and cell proliferation was based on previously published data. Butyrate acts within few hours on H1° levels in several mammalian systems [D'Anna et al., 1982; Chabanas et al., 1985; Kress et al., 1986). In U937, the TPA-induced changes in morphology and cell surface marker patterns remain constant after 72 h of treatment [Hass et al., 1989]. Similarly, HL-60 treatment with TPA induces a macrophage like phenotype which remains unchanged beyond 72 h of TPA treatment [Rovera et al., 1979]. Finally, DMSO treatment of HL-60 cells induces a myeloid differentiation which starts within hours and reaches maximum differentiation after 5 days [Collins et al., 1978]. DMSO addition to U937 cells yields several effects within few hours, the clonogenicity of U937 cells decreases very rapidly and is barely detectable after 48 hours [Nakamura et al., 1990]. These different induction kinetics were the basis for the time course of the RNA analysis presented in Figure 2.

The cells were seeded at a density around 2×10^5/ml in parallel cell culture flasks (control versus treated cells). In the cases of DMSO or butyrate treatment, aliquots were taken from the treated and control cell suspensions after the time intervals indicated in Figures 1 and 2. Since TPA addition induces an adherent phenotype in both HL-60 and U937 cells, individual flasks for each time point to be investigated were seeded and TPA treated. Before counting and trypan blue exclusion determination (see below), the cells were detached by trypsin/EDTA treatment (0.05% [w/v] trypsin; 0.02% [w/v] EDTA, in PBS buffer: 140 mM NaCl; 2.5 mM KCl; 8.1 mM Na_2HPO_4; 1.5 mM KH_2PO_4).

Viability of cells was monitored by trypan blue exclusion. Ten microliters of cell suspension was mixed with 10 μl trypan blue (0.2%, w/v) solution in PBS (see above). The percentage of viable cells in untreated cultures was 95–97%. In the case of adherent growth due to exposure to TPA, trypan blue exclusion was determined after trypsin/EDTA treatment.

Granulocytes were isolated according to Markert et al. [1984] and RNA was extracted as described below.

Since the viability of control cells did not decrease during the respective periods of investigation and the proliferation rate remained unchanged (see Fig. 1), neither the control nor treated cells were refed during the experiments described in Figures 1 and 2 in order to avoid artifacts due to handling of cells.

RNA Analysis

Total cellular RNA from control and treated cells was prepared by the guanidinium isothiocyanate/cesium chloride method [Chirgwin et al., 1979] and centrifuged in a Beckman SW60 rotor for 16 h at 180,000 *g* (15°C). The RNA was precipitated with ethanol, washed several times with 80% ethanol, dried, and dissolved in water. Then 10 μg samples of RNA were denatured in glyoxal and dimethyl sulfoxide [McMaster and Carmichael, 1977], transferred onto nylon membranes, hybridized with labeled DNA probes,

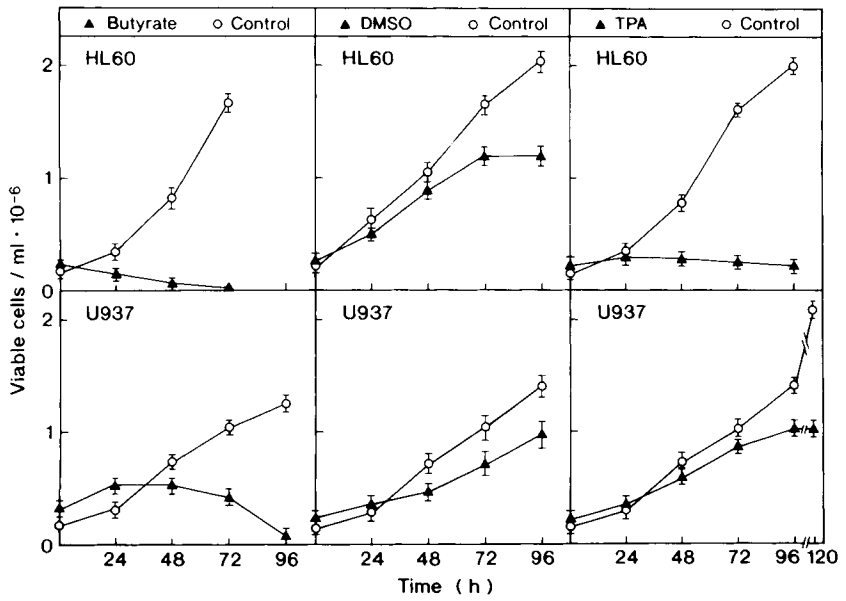

Fig. 1. Effects of sodium butyrate, dimethyl sulfoxide (DMSO) and 12-O-tetradecanoylphorbol 13-acetate (TPA) on growth kinetics of HL-60 and U937 cells. The final concentrations of compounds were 5 mM butyrate, 1.25% (v/v) DMSO, or 32 nM TPA, respectively. The ordinate indicates the number of viable cells/ml (defined as cells excluding trypan blue, see Materials and Methods) and the abscissa refers to the time interval after addition of the respective substance (untreated cells: open circles). Each experiment was done at least three times.

and analyzed by autoradiography as described [Kress et al., 1986]. The endogenous ribosomal RNA could be used as a marker, since human H1° mRNA has a size of 2.3 kb [Kress et al., 1986] and an electrophoretic mobility intermediate between rRNA bands, whereas main group H1 mRNA measures about 0.75 kb [Eick et al., 1989; Albig et al., 1991]. In addition, ethidium bromide stained rRNA bands served as an internal control for identical RNA concentrations per electrophoresis slot. DNA probes were derived from the human H1° gene [Doenecke and Tönjes, 1986] and the human main type gene H1.2 [Eick et al., 1989]. The DNA probes were labeled by nick translation [Rigby et al., 1977] or by the random prime labeling procedure [Feinberg and Vogelstein, 1983].

RESULTS

Treatment of leukemic cell lines with phorbol ester (TPA), DMSO, or several other substances is associated with a loss of proliferative capacity and an induction of cellular differentiation [Koeffler, 1983]. Figure 1 shows the growth kinetics of HL-60 and U937 cells in the absence or presence of either TPA, DMSO, or sodium butyrate. In HL-60, TPA as well as butyrate caused a rapid decline of the rate of cell proliferation, whereas addition of DMSO resulted just in a gradually decreasing rate of proliferation, and no further cell division occurred after 72 h of DMSO treatment.

As previously described [Hochhuth et al., 1990], TPA leads to a growth inhibition of U937. Similarly, inhibitory effects were elicited by treatment of U937 cells with butyrate and DMSO, but just the administration of butyrate caused a complete stop of cell proliferation within 24 h.

These effects on the proliferation of HL-60 as well as U937 cells were paralleled by changing levels of mRNA hybridizing with a human H1 histone gene probe (Fig. 2). This main type H1 histone probe should describe the H1 histone turnover related to the rate of DNA synthesis as described for other main type human H1 species [Baumbach et al., 1984]. The probe used here (termed H1.2 in Eick et al. [1989]) detects H1 gene transcripts in the size range of 0.75 kb, whereas the non-cross-hybridizing H1° probe would detect a 2.3 kb mRNA [Kress et al., 1986].

Treatment of HL-60 with either DMSO, butyrate, or TPA resulted in a decrease of the H1.2 histone mRNA level. The delayed effect of DMSO on the H1.2 expression agreed with the minor effect of this compound on the HL-60 proliferation rate as indicated in Figure 1. On the other hand, the addition of butyrate to HL-60 cells caused an early inhibition of cell growth and no

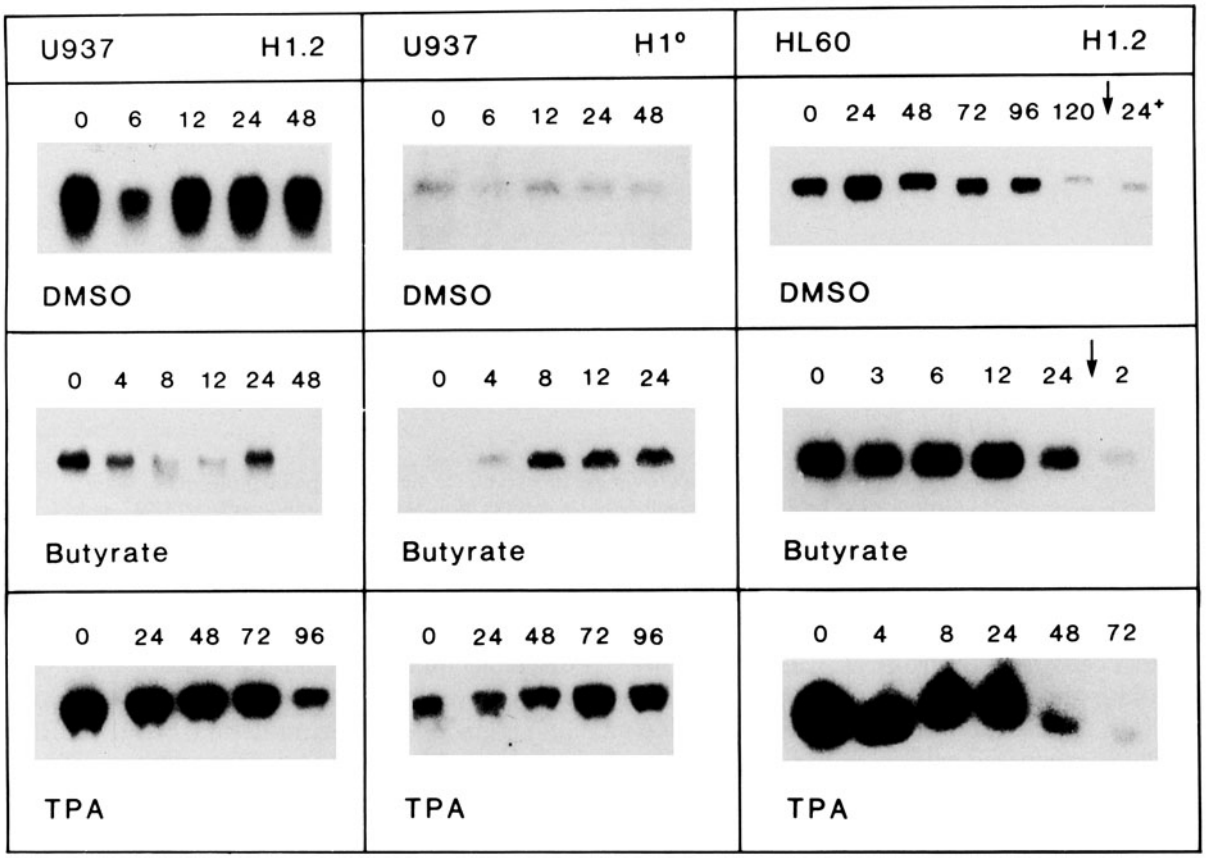

Fig. 2. Steady-state levels of mRNA coding for histone H1° [Kress et al., 1986] or H1.2 [Eick et al., 1989] in HL-60 and U937 cells after treatment with DMSO, butyrate or TPA (as in Fig. 1). The U937 data from TPA treated cells were taken from Hochhuth et al. [1990]. RNA was extracted from cells after different periods of exposure to the respective substance: hours of treatment are indicated above the electrophoretic slots. Vertical arrows indicate washing of cells and resuspension in medium without butyrate or DMSO. Identical amounts of RNA (10 μg) were applied per electrophoresis slot, blotted, and hybridized with ^{32}P-labeled probes. Since hybridization of HL-60 RNA with H1° probes was absolutely negative, these series are omitted from the figure. The time periods presented here were chosen on the basis of the differential induction kinetics as previously determined (references in Materials and Methods section). Each experiment was performed at least three times; each panel shows a representative pattern of RNA data.

H1.2 mRNA could be detected at times beyond 24 h (Fig. 2).

No H1° mRNA could be detected in HL-60 cells. Neither RNA from proliferating cells nor from growth-inhibited or differentiation-induced HL-60 showed any hybridization with the H1° probe (not shown in Fig. 2, but see Fig. 3).

Addition of butyrate, TPA, DMSO, or retinoic acid failed to induce any accumulation of H1° mRNA in HL-60 cells (not shown). According to Collins et al. [1978], a 5 day treatment of HL-60 cells with DMSO induces myeloid differentiation. Five days of DMSO exposure of HL-60 cells was similarly without effect on H1° mRNA induction as TPA or butyrate treatment. Serum depletion, which is another mode of H1° induction [Pehrson and Cole, 1980; Hall and Cole, 1986] was also without any effect on the expression of the H1° gene in HL-60 cells (not shown).

U937 cells have the capacity to differentiate towards cells of the monocytic lineage [Radzun et al., 1983]. As in HL-60, TPA induces a macrophage-like phenotype in U937 cells [Nilsson et al., 1980]. As shown previously for U937 [Hochhuth et al., 1990] and for comparison again in Figure 1 (above), this phorbol ester induces a decline in the cell proliferation rate, a decrease of the level of main type H1 mRNA and an increase of the steady state level of H1° mRNA (Fig. 2).

The middle panel of Figure 2 shows that DMSO, which is an inducer of H1° in other systems [Hall et al., 1985] and causes an induction of phospholipase A_2 within 2 h of treatment

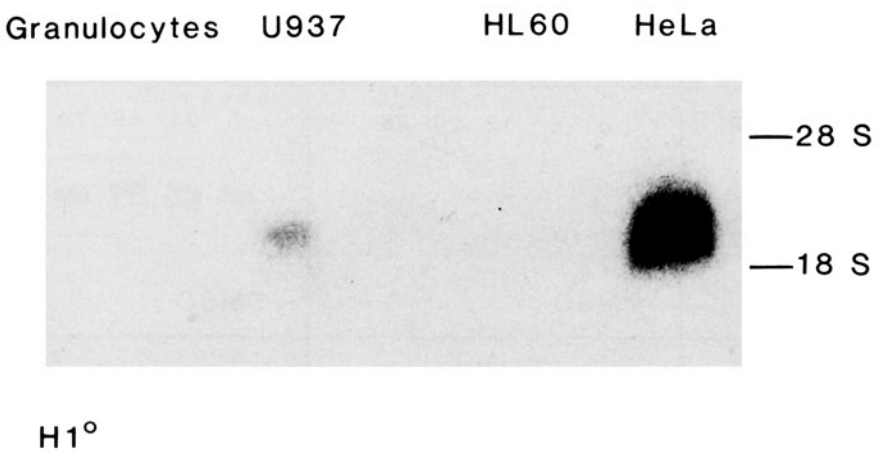

Fig. 3. Comparison of hybridization of different RNA preparations with the human H1° probe [Kress et al., 1986]. Granulocyte RNA was extracted from mature granulocytes [Markert et al., 1984], RNA from uninduced U937 and HL-60 cells was extracted and analyzed as described above. Butyrate induced HeLa cell RNA served as a positive control [Kress et al., 1986]. In each lane, 10 μg RNA were analyzed, the intensity of ethidium bromide stained rRNA bands was identical in the four slots.

of U937 cells [Nakamura et al., 1990], failed to stimulate the accumulation of H1° mRNA in U937. In fact, the low basal level of H1° mRNA rapidly disappeared within a few hours after addition of DMSO. The replication-dependent H1.2 mRNA level in DMSO-treated U937 cells rapidly reached its initial level after an early decrease. The comparison with the growth kinetics (Fig. 1, lower middle panel) showed that this maintenance of the H1.2 mRNA level agreed with the ongoing cell division.

Butyrate treatment of U937 cells, which caused an inhibition of cell proliferation within 24 hours, affected the H1.2 mRNA level with similar kinetics as in the case of the DMSO treatment. Again, the H1.2 probe detected a decreased mRNA concentration around 6 to 8 hours of treatment and an increase thereafter. With decreasing numbers of viable cells (as indicated in Fig. 1: 48 h and later) the H1.2 mRNA was no more detectable.

Sodium butyrate leads to a broad spectrum of effects in several cell types [Prasad and Sinha, 1976]. An induction of H1° protein [D'Anna et al., 1982; Hall et al., 1985] as well as mRNA [Kress et al., 1986] by butyrate has been observed in CHO, HeLa, and other cells. Figure 2 shows that butyrate induces an accumulation of H1° mRNA in U937 within a few hours, reaching a maximum at around 8 h. These kinetics are within the same range as butyrate induction of H1° mRNA accumulation in HeLa [Kress et al., 1986] or in mouse B16 cells [Chabanas et al., 1985]. At this early stage of induction, an obvious effect on the proliferation rate of U937 cells has not yet been observed (Fig. 1). Thus, the induction of the accumulation of H1° mRNA preceded the inhibitory effect on the proliferation rate. This agreed with the H1.2 hybridization data as described above.

TPA effects on the differentiation of U937 have been described by several authors [Gidlund et al., 1981; Hass et al., 1989; Hochhuth et al., 1990]. We have included data from Hochhuth et al. [1990] in Figure 2 in order to allow a comparison with effects of butyrate and DMSO, respectively, on proliferation and H1° induction within the same U937. According to Gidlund et al. [1981], 2 to 3 days of exposure of U937 cells to TPA lead to a macrophage-like phenotype of these cells. Within the same period, we have found an accumulation of H1° mRNA in TPA treated U937 cells (Hochhuth et al. [1990] and Fig. 2).

Figure 3 shows a comparison of Northern blots of U937 and HL-60 RNA with RNA extracted from mature granulocytes. RNA from butyrate treated HeLa cells served as a H1° control [Kress et al., 1986]. In rapidly growing U937 cells, a very faint hybridization band was obtained with the H1° probe, whereas neither HL-60 nor granulocytes yielded any detectable H1° mRNA band. This suggests that the promyelocytic cell line HL-60 already represents a differentiation state beyond a (as yet hypothetical) stage of repression of the H1° gene connected with the granulocyte lineage.

DISCUSSION

Changes in the state of differentiation and in the rate of proliferation of a given cell type may require alterations in the functional organization of chromatin. This may involve a removal or redistribution of H1 histones upon activation or inactivation of specific portions of the genome [Schlissel and Brown, 1984; Sharp, 1991; Crosston et al., 1991].

We have chosen two leukemia cell lines to investigate the influence of established inducers of differentiation [Koeffler, 1983] on the rate of proliferation and on the steady state mRNA levels of two different H1 subtypes, i.e., H1° and the main type histone H1.2. Here, we describe effects of a phorbol ester (TPA), dimethyl sulfoxide (DMSO), and sodium butyrate on the promyelocytic HL-60 and the monocytic U937 cell lines.

The analysis of H1 histone mRNA levels in HL-60 cells growing at differential rates in the absence or presence of butyrate, DMSO, or TPA revealed two major results. First, predictably, the steady-state mRNA level of the main type H1.2 histone reflected the state of proliferation of the respective HL-60 cells. Secondly, the H1° gene is apparently inactive in this cell line, and none of the established inducers of H1° synthesis could elicit any detectable expression of this gene.

As described above for HL-60, we have also treated U937 cells with sodium butyrate, DMSO and TPA, and we have studied the changes in main type H1 (H1.2) and replacement type (H1°) gene expression in comparison with the rate of proliferation.

We have shown previously [Hochhuth et al., 1990] that treatment of U937 with TPA or antineoplastic phospholipids yields a decrease of the H1.2 gene expression and an increase of the H1° mRNA level (RNA data after TPA treatment from Hochhuth et al. included in Fig. 2). These previous TPA effects were included in this study in order to demonstrate the differential reactivity of HL-60 and U937 at the level of H1 histone subtype gene expression.

In contrast to TPA, addition of DMSO to U937 cells did not induce an accumulation of H1° mRNA despite its inhibitory effect on the proliferation rate. Few data have been published about the effect of DMSO in U937. Nakamura et al. [1990] showed that the proliferation rate of U937 decreased under the influence of DMSO. Furthermore, they found an increase of nonspecific esterase activity and the expression of a monocyte specific surface antigen. These data indicated that treatment of U937 cells with DMSO caused an induction of terminal differentiation.

Since DMSO treatment of neither HL-60 nor U937 cells was able to induce enhanced H1° expression (Fig. 2), we conclude that the expression of the H1° gene is neither a prerequisite nor a result of the process of cellular differentiation in these systems. For example, HL-60 cells differentiate to granulocytic or macrophage-like cells [Koeffler, 1983], but this process is not associated with a detectable H1° gene expression. On the other hand, U937 cells react by increasing the level of H1° mRNA when the cells are treated with TPA or sodium butyrate. This suggests that the substances studied here act at different steps of control of cellular proliferation and differentiation and that the expression of the H1° gene contributes to a discrimination between these stages.

In our previous analysis of the effects of the phospholipid analogue hexadecylphosphocholine (HePC) on U937 cells, we have shown that this lipid is an inducer of H1° mRNA accumulation and causes a reduced proliferation rate [Hochhuth et al., 1990]. The signal transduction pathway of these lipids is yet unknown, but the plasma membrane should be the prime site of interaction of these substances with the cell [Unger and Eibl, 1991]. Similarly, butyrate as a short chain fatty acid may act primarily via membrane effects. The broad spectrum of effects elicited by butyrate indicates that it acts on a rather early step of a pleiotropic signal transduction system [Prasad and Sinha, 1976]. Thus, if both HePC and butyrate influence certain steps of U937 differentiation upon interaction with the cell membrane, we must conclude that DMSO must act differently on these signal pathways, since it does not cause an increased level of H1° mRNA in U937 cells.

The mode of action of phorbol ester is partly understood. At the level of the genome, the AP1 recognition sequence mediates responsiveness to this tumor promoter. This protein binding motif (TGACTCA) [Lee et al., 1987] is, however, not found in the H1° promotor [Bouterfa and Doenecke, submitted]. Thus, other steps of the protein kinase C mediated signal transfer of TPA may be involved.

In conclusion, the differential expression of H1° in tumor cell lines in the absence or presence of exogenous inducers indicates a complex pattern of signalling pathways which differ in the involvement of H1° in modulating the chromatin structure of the differentiating cell.

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