The effect of bromodeoxyuridine and dibutyryl cyclic AMP on the induction of placental alkaline phosphatase in human choriocarcinoma cells

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The effect of 5-bromo-2'-deoxyuridine (BrdUrd) and dibutyryl cyclic AMP (Bt₂cAMP) on the expression of the placental isoenzyme of human alkaline phosphatase was examined in BeWo choriocarcinoma cells. By using a combination of specific immunoprecipitation and polyacrylamide-gel electrophoresis of cells labelled either metabolically with [³⁵S]methionine or cell-surface-labelled with ¹²⁵I, both BrdUrd (5 µg/ml) and 1 mM-Bt₂cAMP were shown to result in the enhanced accumulation of a specific protein. This protein has immunochemical identity and co-electrophoreses with placental alkaline phosphatase in two-dimensional gels. These results clearly demonstrate that the induction of placental alkaline phosphatase activity in choriocarcinoma cells treated with these agents is a consequence of the accumulation of specific enzyme protein rather than of altered catalytic activity.

The placental form of alkaline phosphatase (EC 3.1.3.1) is a structurally unique protein whose normal expression is limited to the placenta (McKenna *et al.*, 1979; Badger & Sussman, 1976). It is, however, observed with greater than random frequency in the serum of patients with both trophoblastic and non-trophoblastic tumours (Fishman *et al.*, 1968), and it has been clearly demonstrated that the tumour isoenzyme is structurally identical with placental alkaline phosphatase (Greene & Sussman, 1973; Wada *et al.*, 1979a). The molecular events governing the re-expression of this placental gene may be related to the process of neoplastic development.

It is well documented that alkaline phosphatase activity can be induced 10–20-fold in cultured cells in response to a wide variety of perturbants (Ghosh *et al.*, 1972; Singer & Herz, 1978; Goz & Walker, 1978; Hamilton *et al.*, 1979a). However, the molecular mechanisms through which this induction occurs remain unclear. Work from our laboratory has demonstrated that of the two alkaline phosphatase isoenzymes expressed in BeWo choriocarcinoma cells, only placental alkaline phosphatase

is inducible with either BrdUrd or Bt₂cAMP (Hamilton *et al.*, 1979a). By using a radioimmunoassay for that enzyme, it was also observed that placental-alkaline-phosphatase-related antigen increased in a parallel fashion with enzyme activity, suggesting that the enhanced activity results from the enhanced accumulation of enzyme protein. Others have reported that the induction of placental alkaline phosphatase in HeLa cells treated with cortisol, BrdUrd or iododeoxyuridine is a consequence of altered kinetic properties of the enzyme and not of new enzyme synthesis (Ghosh *et al.*, 1972; Goz & Walker, 1978; Morrow *et al.*, 1979). In order to evaluate this question further, we have studied choriocarcinoma cells treated with BrdUrd or Bt₂cAMP by using two independent radiolabelling techniques. The results clearly demonstrate that a protein showing immunochemical and physical identity with placental alkaline phosphatase accumulates at a rate significantly greater than a group of other unaffected placental cell-surface proteins.

Materials and methods

Cell culture

BeWo cells were grown as previously described (Hamilton *et al.*, 1979a). BrdUrd and Bt₂cAMP were added to final concentrations of 5 µg/ml and 1 mM, respectively, from concentrated stock solutions in sterile distilled water. Medium was changed

Abbreviations used: BrdUrd, 5-bromo-2'-deoxyuridine; Bt₂cAMP, dibutyryl cyclic AMP; SDS, sodium dodecyl sulphate.

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daily for both control and experimental cultures. Cultures were routinely plated at 5×10^6 – 1×10^7 cells/T-75 flask and were exposed to drugs for a 96 h period. During growth with BrdUrd, care was taken to minimize the exposure of treated cultures to sources of u.v. light.

Metabolic labelling with [^{35}S]methionine

T-75 cultures growing in the presence or absence of either BrdUrd or Bt₂cAMP were labelled on days 3 and 4 (48 h period) by treatment with [^{35}S]methionine (2 $\mu\text{Ci}/\text{ml}$; 600 Ci/mmol; New England Nuclear Corp., Boston, MA, U.S.A.) in complete medium. After the labelling period, cultures were washed three times in Earle's balanced salt solution (Gibco, Grand Island, NY, U.S.A.) and then scraped with a rubber policeman into 1 ml of TNM buffer (20 mM-Tris/HCl, pH 7.4, 150 mM-NaCl, 2 mM-MgCl₂) and extracted with 1% Triton X-100 for 10 min at 0°C. The detergent extracts were then centrifuged at 140 000 *g* for 60 min. The supernatant was used for immunoprecipitation as described below. Total incorporated radioisotope was determined by trichloroacetic acid precipitation, filtration on Whatman (Maidstone, Kent, U.K.) GF/C filters, and liquid-scintillation counting in toluene with Omnifluor (New England Nuclear).

Cell-surface labelling by lactoperoxidase-catalysed iodination

Cell-surface iodination was performed on T-75 cultures grown for 96 h in the presence or absence of drugs. The method for lactoperoxidase-catalysed iodination was as described previously (Wada *et al.*, 1979b; Hamilton *et al.*, 1979b). Briefly, contents of one T-75 flask were labelled with 1 ml of phosphate-buffered saline (150 mM-NaCl/10 mM-sodium phosphate, pH 7.4) containing 100 μg of lactoperoxidase (Sigma Chemical Co., St. Louis, MO, U.S.A.), 100–200 μCi of carrier-free Na¹²⁵I (New England Nuclear) and two 10 μl batches of 250 μM -H₂O₂ added at 5 min intervals. After a 10 min labelling period, 50 μl of 0.5 M-NaCl was added and the excess free iodine was washed away with phosphate-buffered saline. Labelled cells were then harvested, extracted and centrifuged as described for [^{35}S]methionine labelling.

Preparation of placental membrane vesicles

Human placentas were obtained within 20 min of delivery, and the brush-border membrane fraction was isolated by the method of Smith *et al.* (1977). Vesicles were labelled with ¹²⁵I by using lactoperoxidase as described above.

Immunoprecipitation and gel electrophoresis

Triton X-100 extracts from ³⁵S- or ¹²⁵I-labelled cells were immunoprecipitated with a goat anti-

(human placental brush-border protein) antiserum, which has been thoroughly described elsewhere (Wada *et al.*, 1979b; Hamilton *et al.*, 1979b). In some instances, extracts were precipitated by using goat antiserum raised against homogeneous placental alkaline phosphatase as described by Sussman *et al.* (1968). Extracts were first pretreated with 10 μl of non-immune goat serum and 100 μl of a 10% (w/v) suspension of heat-killed formalin-fixed *Staphylococcus aureus* (Cowen strain I) (Kessler, 1975). Pretreated samples were then specifically precipitated with 10–20 μl of anti-(human placental brush-border protein) antiserum or anti-(placental alkaline phosphatase) serum at 0°C, followed by adsorption to *S. aureus* as described above. The immune precipitates were washed thoroughly in NET buffer (150 mM-NaCl, 5 mM-EDTA, 50 mM-Tris/HCl, pH 7.4, 0.5% Triton X-100) containing ovalbumin (1 mg/ml; Sigma) and 5 mM-NaI or 1 mM-methionine. The immune complexes were eluted in 4% SDS/0.25 M-Tris/HCl (pH 6.8)/10% (v/v) glycerol/5% (v/v) β -mercaptoethanol and boiled for 2 min before analysis by 8%-polyacrylamide-gel electrophoresis as described by Laemmli (1970). For two-dimensional electrophoretic analysis, eluted samples were diluted 10-fold with 5 mM-sodium phosphate (pH 8.0)/2% Triton X-100/8 M-urea and concentrated to 100 μl in an Amicon (Lexington, MA, U.S.A.) A25 mini-concentrator. These samples were then analysed by two-dimensional polyacrylamide-gel electrophoresis as described by O'Farrell (1975) and modified by Wada *et al.* (1977).

Gels were fixed in 7% (v/v) acetic acid/50% (v/v) methanol overnight. [^{35}S]Methionine-containing gels were impregnated with dimethyl sulphoxide/2,5-diphenyloxazole for autofluorography (Laskey & Mills, 1975). Gels were dried under vacuum and exposed by using pre-flashed Kodak (Rochester, NY, U.S.A.) RP X-Omat X-ray film at –76°C for 1–4 days. Exposure was enhanced by Dupont (Wilmington, DE, U.S.A.) 'Lightening Plus' intensifying screens. For quantification of radioautographs, films were scanned at 550 nm in a Transidyne (Ann Arbor, MI, U.S.A.) scanning densitometer.

Alkaline phosphatase activity was measured as described by Bessey *et al.* (1946), and protein was determined by the method of Lowry *et al.* (1951).

Results

In order to quantify the relative amount of alkaline phosphatase protein accumulated in cells treated with BrdUrd or Bt₂cAMP, we have radio-labelled control and experimental cultures by two independent techniques, either metabolically with [^{35}S]methionine or enzymically by using cell-surface-specific lactoperoxidase-catalysed iodination. In

addition, we have analysed the radiolabelled extracts by polyacrylamide-gel electrophoresis after immunoprecipitation with antisera directed either specifically against placental alkaline phosphatase or against a set of placental brush-border membrane glycoproteins including placental alkaline phosphatase. The first antiserum provides an immunochemical identification, and the second allows quantification of the amount of incorporated radiolabel relative to a set of similar membrane proteins which appear to be unaffected by the treatments involved.

Fig. 1(a) is a schematic representation of a two-dimensional electrophoretogram of human placental brush-border glycoproteins which react with anti-(placental brush-border) serum; 13 individual subunits have been resolved and described

Fig. 1. Electrophoretic analysis of placental brush-border membrane

(a) Schematic representation of a two-dimensional electrophoretogram of placental brush-border membrane glycoprotein subunits which react with anti-(placental brush border) serum. Isoelectric focusing (IEF) was done in the horizontal dimension with the cathode and anode positions as indicated. This was followed by SDS/polyacrylamide-gel electrophoresis done in the vertical dimension running from top to bottom as described in the Materials and methods section. Filled spots represent subunits common to all human cells (6b, 8 and 9 are not on lymphoid tissue), and open spots represent subunits which exhibit differential tissue distribution. (b) Radioautogram of SDS/polyacrylamide-gel-electrophoretic analysis of ^{125}I -labelled placental membrane glycoproteins immunoprecipitated with anti-(human placental brush-border protein) antiserum. Placental membrane vesicles were prepared and labelled with ^{125}I as described in the Materials and methods section. Approx. 200 000 c.p.m. were immunoprecipitated with $10\mu\text{l}$ of anti-(human placental brush-border protein) antiserum and the immunoprecipitate was solubilized and analysed on an 8%-polyacrylamide gel. The bands are labelled to correspond roughly to individual subunits in the two-dimensional 'map' of Fig. 1(a).

(Wada *et al.*, 1977, 1979b); Hamilton *et al.*, 1979b). Fig. 1(b) shows the same subunits immunoprecipitated and analysed by one-dimensional SDS/polyacrylamide-gel electrophoresis. Eight major bands can be seen and their probable correspondence to the various numbered subunits is indicated. The subunit molecular weights (M_r) and isoelectric points (pI) for each component are detailed in Table 1. The tissue distribution of each antigen is indicated in Fig. 1(a). Filled spots are components ubiquitously expressed on all human cells examined to date, including adult liver, kidney (Wada *et al.*, 1979b) and a variety of phenotypically distinct cultured cells (Hamilton *et al.*, 1979b). Open spots represent those subunits that are differentially expressed among the various tissues and cell types examined. Subunit 23 has been previously identified as the placental isoenzyme of alkaline phosphatase on the basis of co-electrophoresis in two-dimensional gels (Wada *et al.*, 1977). Eleven (2, 7, 7a, 6b, 8, 9, 15a, 15b, 23, 21, 24) of these components have been identified in BeWo choriocarcinoma cells (Hamilton *et al.*, 1979a,b).

Fig. 2 shows gels of BeWo cells treated by BrdUrd or Bt₂cAMP which have been labelled by lactoperoxidase-catalysed cell-surface iodination. Lane 1 is from a control culture and lanes 2 and 3 are from Bt₂cAMP- and BrdUrd-treated cells respectively, all immunoprecipitated with anti-(human placental brush-border protein) antiserum. The enhancement of a band at M_r ~64 000 is clearly

Table 1. Co-ordinates of placental brush-border glycoprotein subunits in two-dimensional electrophoretograms pH was measured by using a surface electrode, and a curve of pH versus relative mobility in the isoelectric-focusing dimension was used to estimate apparent isoelectric point (pI). Molecular weights (M_r) of each subunit were estimated with β -galactosidase, human transferrin, human placental alkaline phosphatase and ovalbumin as markers.

Subunit no.	Apparent pI	$10^{-3} \times$ Apparent M_r
2	5.80	235.0
6b	5.68	170.0
7	5.88	161.0
7a	5.80	120.0
8	5.68	148.0
9	5.18	175.0
13	5.86	123.0
15a	5.82	100.0
15b	6.57	100.0
18	5.65	92.4
21	4.60	72.5
23	6.30	64.0
24	5.60	64.2

Fig. 2. Electrophoretic analysis of cell-surface- ^{125}I -labelled BeWo cells treated with BrdUrd or Bt_2cAMP
For each experiment, one T-75 flask of confluent cells was labelled with ^{125}I , extracted, immunoprecipitated and analysed as described in the Materials and methods section. Approx. 250 000 c.p.m. were used for each immunoprecipitation. Experiments using BrdUrd and Bt_2cAMP were done at different times. Lanes 1–3, immunoprecipitation with anti-(human placental brush-border protein) antiserum; lanes 4–6, immunoprecipitation with anti-(placental alkaline phosphatase) serum; lanes 1 and 4, untreated cells; lanes 2 and 5, cells treated with 1 mM- Bt_2cAMP ; lanes 3 and 6, cells treated with BrdUrd ($5\text{ }\mu\text{g/ml}$). All gels were exposed for 96 h.

evident and is most remarkable in cells treated with BrdUrd, which also show the largest enhancement of enzyme activity. Lanes 4, 5 and 6 show gels of immunoprecipitation of the same samples with antiserum specific for placental alkaline phosphatase. A single prominent band can be seen in both induced-cell samples which is barely detectable in control cells. The band appearing in the induced cells immunoprecipitated with either anti-(human placental brush-border protein) antiserum or anti-(placental alkaline phosphatase) sera co-migrates with homogeneous placental alkaline phosphatase. These data clearly demonstrate that induced cells contain an increased amount of iodinated placental alkaline phosphatase-like protein identifiable both by immunoprecipitation and by electrophoretic mobility in SDS/polyacrylamide gels.

Fig. 3 presents radioautograms of two-dimensional gels of immunoprecipitates from (a) control and (b) BrdUrd-treated cultures surface-labelled with

Fig. 3. Two-dimensional electrophoresis of BeWo cells treated with BrdUrd

For each experiment, one T-75 flask of cells was labelled with ^{125}I , extracted with Triton X-100, immunoprecipitated and analysed by two-dimensional gel electrophoresis as described in the Materials and methods section. Approx. 200 000–400 000 c.p.m. were used for each immunoprecipitation with anti-(human placental brush-border protein) antiserum, and the final applied samples contained 3000–5000 c.p.m. The spots are labelled to indicate their identity with subunits identified in Fig. 1. (a) Untreated cells; (b) cells treated with BrdUrd ($5\text{ }\mu\text{g/ml}$).

^{125}I . The appearance of a series of spots at M_r 64 000 and pI 6.2–6.4 in induced cells provides confirmation of the identity of the inducible protein identified in Fig. 2 (lanes 2, 3, 5 and 6) as placental alkaline phosphatase. It is noteworthy that spots 21 and 24 also show a relative increase in density in the two-dimensional gels of cells treated with BrdUrd. However, these subunits are apparently not related to alkaline phosphatase because they are present in cells which do not express detectable enzyme activities. Most of the other spots do not appear to have changed in density. However, quantification of

spot intensity in two-dimensional gels is an unreliable procedure.

Fig. 4 shows gels of matched sets of control and BrdUrd- or Bt₂cAMP-treated BeWo cells labelled with [³⁵S]methionine for 48 h before immunoprecipitation and gel electrophoresis. Lanes, 1, 2, 3 and 4 are of total detergent-soluble cellular proteins from cells grown in control medium (lanes 1 and 3) or medium containing BrdUrd (5 µg/ml) (lane 2) or 1 mM-Bt₂cAMP (lane 4). No apparent differences can be seen in these samples. Lanes 5, 6, 7 and 8 show gels of anti-(human placental brush-border protein) antiserum immunoprecipitates of these same samples. In lanes 5 and 6 more of the lower-

molecular-weight components can be seen than in lanes 7 and 8, owing to longer exposure time and greater radioactivity in the sample applied. Nine major bands are resolved by scanning the radioautographs at 550 nm, and these are indicated by the numbering in lane 8. Comparison of control gels (lanes 5 and 7) with those from drug-treated cultures (lanes 6 and 8) shows the defined complement of glycoproteins to be present in apparently similar quantities, except for a band of *M_r* 64 000, which is considerably enhanced. This induced band co-migrates with homogeneous placental alkaline phosphatase.

Table 2 gives the results of quantification of the gels shown in Fig. 4 by scanning the radioautographs at 550 nm and integrating each band. This analysis resolves a total of eight major bands, which are indicated by numbers in Fig. 4 (lane 8). The amount of each component relative to the other components in the gel was quantified by determining the ratio of individual peak size to peak sizes for two other bands from the same gel (nos. 2 and 4), which appeared to be relatively constant between the matched control and drug-treated cells. The data are given as percentages of the ratios in control cells. BrdUrd results in a minor decrease (<50%) in the relative amount of component 1 and a major in-

Fig. 4. Electrophoretic analysis of BeWo cells treated with BrdUrd and Bt₂cAMP

For each experiment, one T-75 flask of cells was labelled with [³⁵S]methionine, extracted with Triton X-100, immunoprecipitated and analysed on 8% acrylamide gels as described in the Materials and methods section and in the text. Between 1×10^6 and 4×10^6 acid-insoluble c.p.m. were used for each immunoprecipitation and 1×10^4 – 4×10^4 c.p.m. were used for crude extracts. Molecular weights determined from standards are indicated in the left-hand margin, and bands are numbered for reference to the text in the right-hand margin. Lane 1, untreated cells, crude extract; lane 2, cells treated with BrdUrd (5 µg/ml), crude extract; lane 3, untreated cells, crude extract; lane 4, cells treated with 1 mM-Bt₂cAMP, crude extract; lane 5, untreated cells, anti-(human placental brush-border protein) antiserum immunoprecipitate; lane 6, cells treated with BrdUrd (5 µg/ml), anti-(human placental brush-border protein) antiserum immunoprecipitate; lane 7, untreated cells, anti-(human placental brush-border protein) antiserum immunoprecipitate; lane 8, cells treated with 1 mM-Bt₂cAMP, anti-(human placental brush-border protein) antiserum immunoprecipitate. Lanes 1 and 2 were exposed for 24 h, and lanes 3–8 for 96 h.

Table 2. Relative amounts of placental glycoproteins in BrdUrd- or Bt₂cAMP-treated choriocarcinoma cells

Gels from Fig. 4 were scanned at 550 nm and the relative intensity of each band was estimated by integrating each peak. Bands 2 and 4 showed constant values between matched control and experimental samples. The ratios of the integrated values for all bands relative to nos. 2 and 4 were calculated and the percentage of a matched control ratio was determined. The values given are the means of both comparisons $\pm \frac{1}{2}$ the range.

Drug treatment	Gel band	Ratio (% of control)
BrdUrd	1	62 $\pm$ 3
	2	95 $\pm$ 6
	3	104 $\pm$ 8
	4	108 $\pm$ 8
	5	80 $\pm$ 5
	6	775 $\pm$ 5
	7	85 $\pm$ 7
	8	94 $\pm$ 6
Bt ₂ cAMP	1	74 $\pm$ 2
	2	113 $\pm$ 2
	3	88 $\pm$ 1
	4	100 $\pm$ 1
	5	100 $\pm$ 1
	6	256 $\pm$ 6
	7	100 $\pm$ 1
	8	49 $\pm$ 4

crease in amount of placental alkaline phosphatase (>700%). Bt₂cAMP caused a small decrease in components 1 (~40%) and 8 (~60%), and again a major increase in placental alkaline phosphatase (250%). Otherwise, all components showed nearly identical quantities in control and drug-treated cells. Alkaline phosphatase enzyme activity in control cultures was approx. 0.05 unit/mg of protein, and BrdUrd and Bt₂cAMP treatment increased this value to 0.38 and 0.29 unit/mg respectively. The relative enhancement of enzyme activity (5–10-fold) agrees within less than a factor of 2, with the determination from the densitometric data for [³⁵S]methionine-labelled proteins (Fig. 4) presented in Table 2.

Discussion

BeWo cells, a human choriocarcinoma-derived cell line, express both the placental and liver isoenzymes of alkaline phosphatase (Hamilton *et al.*, 1979a), each of which is structurally distinct (Badger & Sussman, 1976). Only the placental isoenzyme is specifically induced by BrdUrd or Bt₂cAMP, demonstrating the independent regulation of separate alkaline phosphatase genes (Hamilton *et al.*, 1979a). The present paper provides evidence for internal labelling of the molecule, to show a quantitative increase in this molecule in response to these perturbants. It also describes the effect of these perturbants on the expression of placental alkaline phosphatase relative to a defined set of cell-surface glycoprotein antigens, which include placental alkaline phosphatase, and which are expressed by the BeWo cell line. This set of membrane antigens provides an internal standard against which a determination of the relative quantity of specific enzyme protein can be made. Quantitative examination of this set of placental membrane glycoproteins by SDS/polyacrylamide-gel electrophoresis in ¹²⁵I- or [³⁵S]methionine-labelled cells showed a specific 3–8-fold enhancement of the accumulation of one subunit with an apparent *M_r* of approx. 64 000 after treatment with either BrdUrd or Bt₂cAMP. We have determined this component to be the subunit of the placental isoenzyme of alkaline phosphatase on the basis of its immunochemical reactivity with specific antiserum and its electrophoretic mobility in both one- and two-dimensional polyacrylamide-gel-electrophoretic systems. The use of two technically independent methods for radioactive labelling of cellular protein indicates that the increased labelling of placental alkaline phosphatase does not result from differential labelling efficiency as a consequence of drug treatment.

The enhancement of alkaline phosphatase activity in cultured human cells by various perturbants has been previously documented both in our laboratory

and elsewhere (Ghosh *et al.*, 1972; Singer & Herz, 1978; Goz & Walker, 1978; Hamilton *et al.*, 1979a; Morrow *et al.*, 1979). Nevertheless, these earlier studies have not entirely resolved the question of whether the enhanced activity results from an increased quantity of specific enzyme protein. Ghosh *et al.* (1972) have reported that the induction of alkaline phosphatase in HeLa cells with cortisol results from a change in the kinetic properties of the enzyme, with no new enzyme synthesis required, and others have suggested that this may be the case with BrdUrd or iododeoxyuridine induction of HeLa cells (Morrow *et al.*, 1979; Goz & Walker, 1978). In contrast, Hamilton *et al.* (1979a) have shown that the increase in phosphatase activity in BrdUrd- or Bt₂cAMP-treated choriocarcinoma cells is paralleled by an increase in specific placental alkaline phosphatase antigen as detected by radioimmunoassay. This observation suggested that the induction could be entirely accounted for on the basis of increased enzyme protein. In the present paper, the enhanced incorporation of [³⁵S]methionine or ¹²⁵I into placental alkaline phosphatase subunits shown in Figs. 2, 3 and 4 demonstrates that specific enzyme protein does accumulate during the induction of placental alkaline phosphatase with BrdUrd and Bt₂cAMP in choriocarcinoma cells. The difference between the data reported for HeLa cells and those reported here for choriocarcinoma cells could result from different technical approaches to the problem, or from true differences in expression of placental genes in trophoblastic (choriocarcinoma) and in non-trophoblastic (HeLa) phenotypes.

The quantitative extent of induction of band 6 (see Fig. 4, lanes 6 and 8, and Table 2) and of alkaline phosphatase activity agree closely, but the calculated enhancement of band-6 protein consistently underestimates the measured enhancement of alkaline phosphatase activity. These differences probably result from the methodology employed in quantifying band-6 protein by using autoradiography. Because the labelling period with [³⁵S]methionine (48 h) could be long relative to the turnover time for alkaline phosphatase, it cannot be determined whether the increased accumulation of placental alkaline phosphatase protein results from altered rates of enzyme synthesis or degradation.

Although the synthesis of placental alkaline phosphatase appears to be highly specific to the placenta under normal conditions (McKenna *et al.*, 1979), its re-expression in both trophoblastic and non-trophoblastic neoplasia has been clearly documented (Fishman *et al.*, 1968; Sussman, 1978). The high degree of specificity of induction for the placental isoenzyme compared with the other trophoblast cell-surface glycoprotein antigens demonstrated in the current study, and the frequency of its induction under a variety of conditions in other cell

types, may be related to the frequent re-expression of placental alkaline phosphatase in malignancy. Furthermore, because placental alkaline phosphatase is representative of the differentiated trophoblast in the term placenta, the effect of BrdUrd and Bt_2cAMP observed in choriocarcinoma cells, which represent a less differentiated form of the cell, may represent limited biochemical differentiation of this cell type. The molecular mechanisms involved in this process require further investigation.

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