ARTICLES

Cell Cycle Arrest by Prostaglandin A_1 at the G_1/S Phase Interface With Up-Regulation of Oncogenes in S-49 cyc⁻ Cells

Millie Hughes-Fulford

Research Service, Veterans Administration Medical Center, and Department of Medicine, University of California Medical Center, San Francisco, California 94121

Abstract   Our previous studies have implied that prostaglandins inhibit cell growth independent of cAMP. Recent reports, however, have suggested that prostaglandin arrest of the cell cycle may be mediated through protein kinase A. In this report, in order to eliminate the role of c-AMP in prostaglandin mediated cell cycle arrest, we use the-49 lymphoma variant (cyc⁻) cells that lack adenylate cyclase activity. We demonstrate that dimethyl prostaglandin A_1 (dmPGA$_1$) inhibits DNA synthesis and cell growth in cyc⁻ cells. DNA synthesis is inhibited 42% by dmPGA$_1$ (50 μM) despite the fact that this cell line lacks cellular components needed for cAMP generation. The ability to decrease DNA synthesis depends upon the specific prostaglandin structure with the most effective form possessing the α,β unsaturated ketone ring. Dimethyl PGA$_1$ is most effective in inhibiting DNA synthesis in cyc⁻ cells, with prostaglandins PGE$_1$ and PGB$_1$ being less potent inhibitors of DNA synthesis. DmPGE$_2$ caused a significant stimulation of DNA synthesis. S-49 cyc⁻ variant cells exposed to (30–50 μm) dmPGA$_1$, arrested in the G$_1$ phase of the cell cycle within 24 h. This growth arrest was reversed when the prostaglandin was removed from the cultured cells; growth resumed within hours showing that this treatment is not toxic. The S-49 cyc⁻ cells were chosen not only for their lack of adenylate cyclase activity, but also because their cell cycle has been extensively studied and time requirements for G$_1$, S, G$_2$, and M phases are known. Within hours after prostaglandin removal the cells resume active DNA synthesis, and cell number doubles within 15 h suggesting rapid entry into S-phase DNA synthesis from the G$_1$ cell cycle block. The S-49 cyc⁻ cells are known to have a G$_1$/S boundary through M phase transition time of 14.8 h, making the location of the prostaglandin cell cycle arrest at or very near the G$_1$/S interface. The oncogenes, c-fos and c-myc which are normally expressed during G$_1$ in proliferating cells have a 2–3 fold enhanced expression in prostaglandin G$_1$ arrested cells. These data using the S-49 variants demonstrate that dmPGA$_1$ inhibits DNA synthesis and arrests the cell cycle independent of cAMP-mediated effects. The prostaglandin arrested cells maintain the gene expression of a G$_1$ synchronous cell which suggests a unique molecular mechanism for prostaglandin action in arresting cell growth. These properties indicate that this compound may be an effective tool to study molecular mechanisms of regulation of the cell cycle.   © 1994 Wiley-Liss, Inc.*

Key words: DNA synthesis, cAMP, cell growth inhibition, lymphoma, PGA$_1$

Adenosine 3'5' cyclic monophosphate (cAMP) is thought to play a central role in the regulation of cell growth [Ramwell and Shaw, 1970; Peery et al., 1971; Pastan et al., 1971; Millis et al., 1972]. The growth of many types of cultured cells is arrested by adding exogenous dibutyryl adenosine 3'5' cyclic monophosphate (dbcAMP) [Millis et al., 1972; Ottan et al., 1976; Johnson and Pastan, 1971]. In 1971, a report by Pastan

et al. demonstrated that growth of L-929 cells was inhibited by prostaglandin E$_1$ (PGE$_1$). Like epinephrine and glucagon, certain prostaglandins are able to trigger adenylate cyclase activity in the cell membrane and stimulate a rise in cAMP; it was therefore logical to suggest that the block in cell growth caused by PGE$_1$ was solely due to the increased intracellular concentration of cAMP. However, we have published data supporting the hypothesis that the prostaglandins may inhibit cell growth of BHK-21 cells independent of increases in cAMP levels [Wiley (Hughes-Fulford) et al., 1983].

There have been reports of inhibition of cell growth by prostaglandins [Honn et al., 1981;

Thomas et al., 1974; Santoro and Jaffe, 1979; Choi et al., 1992], with some supporting evidence that this effort is solely mediated by cAMP. In order to disassociate the effect of the prostaglandins from that of induction of cAMP synthesis in malignant tissues, we have carried out experiments in an S-49 lymphoma cell (cyc⁻) variant that was first isolated by Bourne et al. [1975]. Protein membranes from the cyc⁻ variant fail to respond to five effectors—adrenergic amines, PGE_1, cholera toxin, guanine nucleotides, and fluoride ion—that normally stimulate adenylate cyclase to increase cAMP in the wild type S-49 cells. The cyc⁻ phenotype possesses the catalytic unit of adenylate cyclase, but lacks the α subunit of guanine nucleotide binding regulator protein ($G_{s\alpha}$) which is needed for coupling the hormone receptor and adenylate cyclase components for transmitting the external hormonal signal to activate adenylate cyclase [Johnson et al., 1980].

In this study, we used the cyc⁻ S-49 variants as tools to explore the mechanism of prostaglandin action on cell growth inhibition. We found $dmPGA_1$ causes a late G_1 cell cycle arrest with enhanced expression of c-fos and c-myc oncogenes. We show here that $dmPGA_1$ inhibits DNA synthesis, cell growth, and cell cycle of the S-49 cyc⁻ lymphoma cell independent of cAMP mediated pathways.

METHODS
Materials

[³H]-methyl dTdR (20 Ci/nmol) and cAMP radioimmunoassay kits were obtained from New England Nuclear (Boston, MA). Prostaglandin E_1, PGE_2, PGB_1, 16, 16 dimethyl PGA_1 ($dmPGA_1$), 16, 16 $dmPGE_2$ and $PGF_{2\alpha}$ were purchased from Sigma Chemical Co. (St. Louis, MO) and/or Cayman Chemical (Ann Arbor, MI). Chromomycin A_3 was purchased from Calbiochem (San Diego, CA). Dulbecco's Modified media (DME) with Earle's salts was purchased from the University of California, San Francisco, Cell Culture Facility. The media was supplemented with 10% v/v heat inactivated horse serum (HyClone 100298), and 110 mg/l of sodium pyruvate.

Cell Culture

The S-49 wild type and cyc⁻ variant cells were obtained from the University of California, San Francisco, Cell Culture Facility. The cells were grown in suspension while maintained in a humidified incubator (5% CO_2) at 37°C in 250 ml flasks containing from 20–40 ml of DME supplemented with 10% heat inactivated horse serum. All experiments were carried out in either triplicate or duplicate with individual assays. Prostaglandin treatments (20–50 μM) contained traces of ethanol, therefore all control cells were treated with the same concentration of ethanol as the experimental cells. The final concentration of ethanol never exceeded 0.05%.

Measurement of DNA Synthesis

This method was previously described [Wiley (Hughes-Fulford) et al., 1983]. At the specified time points, cells were incubated with [³H]-methyl thymidine (2 μCi/ml) for 15 min at 37°C. After the incubation, media was suctioned into a radioactive trap bottle. Cells were washed three times with 6% TCA (5°C) followed by 3 more times with equal volumes of 95% ETOH (5°C). Cells were allowed to air dry for 5 min before solubilizing in lysis buffer (0.5 M NaOH, 0.5 M NaCl, 1% n-lauryl sarcosine, 4 m MEDTA, and 0.01 M Na pyrophosphate). After 30 min, 200 μl were analyzed for protein by the method of Lowry et al (1951) and a second 200 μL sample was counted in liquid scintillation fluid (Beckman "Ready-Solv," Fullerton, CA) for isotope analysis in a Beckman LS-330 counter. Samples were assayed for protein using the method of Lowry et al. [1951] as modified by Brown et al. [1973]. The majority of the experiments were carried out 2–3 times in triplicate. All statistics were calculated using student's t-test and data is reported in S.E.M. ± S.D.

Measurement of cAMP Content

Cyclic AMP levels were measured according to the modified method of Rapoport [1970] on the same cells in which the DNA synthesis was determined. Briefly, after centrifugation, the tritiated thymidine solution was rapidly aspirated and 1.0 ml of cold 5% TCA was added. After 30 min at 0–4°C, the TCA solution containing cAMP from the disrupted cells was collected, frozen, and later extracted and assayed for cAMP by radioimmunoassay. All data is reported in S.E.M. ± S.D. using Student's t-test.

Cell Counts and Flow Cytometry

All cell counts were made on a ZBI Coulter Counter using Isotone II as the diluent. For flow

cytometry, cells were fixed and stained as described by Coffino et al. [1975]. Briefly, 1×10^6 cells were centrifuged and washed twice with PBS. After centrifugation, the pellet was resuspended in 1 ml of 15 mM $MgCl_2$ in 95% EtOH and stored at 4°C. For staining, the cells were centrifuged and resuspended in 2 ml chromomycin A_3 solution. This solution was filtered through 37 μm nylon mesh, capped, and measured for DNA content using a Coulter flow cytometer. Flow cytometry was carried out on three different experiments; representative cytometric graphs are shown. The flow cytometry data were graphically calculated by the method of Gray et al. [1979]. Once the total area for G_1, S, and G_2/M were graphically calculated, the values were added together and percent of total determined.

Dye Exclusion

Aliquots of the cell solutions were centrifuged in a tabletop centrifuge. The media was aspirated and replaced by an equal volume of .04% trypan blue in PBS. The solution was vortexed and allowed to sit for a minimum of 10 min. Cells were counted for dye exclusion under a microscope in a hema-cytometer.

Detection of Oncogenes c-fos and c-jun Messenger RNA

All molecular biologic techniques were obtained or adapted from standard references [Sambrook et al., 1989]. RNA was prepared from previously harvested and frozen cultures of S-49 cyc⁻ cells using RNAzol (Cinna-Biotecz, Friendswood, TX). Total RNA was transferred to nylon membranes (Hybond, Amersham, Arlington Heights, IL) by overnight blotting on slot blot. RNA was cross linked to the nylon by calibrated UV exposure [Church and Gibert, 1984] in a Stratalinker (Stratagene, La Jolla, CA). The blots were hybridized for 18–20 h to the biotinylated labeled fos or jun probes (Oncor Non-Isotopic System, Gaithersburg, MD) in a buffer containing 200 mM sodium phosphate, pH 7.2, 10 mg/ml bovine serum albumin, 35% formamide, 7% sodium dodecyl sulfate, and 1 mM NaEDTA in rotating capsules in a hybridization oven (Robbins Scientific, Sunnyvale, CA). On removal, the blots were washed at moderate stringency in $0.1 \times SSC$ [Sambrook et al., 1989] for 30 min at 52°C and subjected to autoradiography for periods of 1–4 days.

TABLE I. Effect of dmPGA₁ on S-49 Lymphoma DNA Synthesis and cAMP†

		Tritiated thymidine incorporation into DNA (cpm/μg/15 min)	cAMP (pmol/mg)
Wild type	Control	1,237 ± 42	1.7 ± 0.1
	dmPGA₁	839 ± 3*	3.9 ± 0.2**
cyc⁻	Control	1,140 ± 21	0.98 ± 0.1
	dmPGA₁	619 ± 136**	0.82 ± 0.1 (N.S.)

†The effect of prostaglandin on the wild type (W.T.) adenylate cyclase (cyc⁻) S-49 cells is shown here. The S-49 cells were in stationary suspension culture in Dulbecco's medium with 3 g/liter glucose plus 10% fetal calf serum which was heat inactivated. The S-49 cells in exponential growth were treated with 50 μM dmPGA₁ or vehicle for 2 h before measuring DNA synthesis or cAMP generation. The data is reported in ±S.E.
*$P < 0.001$.
**$P < 0.02$.

RESULTS

Effect of dmPGA₁ on DNA Synthesis in Lymphoma Cells

As shown in Table I, dmPGA₁ is a potent inhibitor of DNA synthesis in S-49 wild type and cyc⁻ lymphoma cells. After exposure to dmPGA₁ for 2 h, DNA synthesis of cyc⁻ cells was inhibited by 46%. Dimethyl PGA₁ caused a significant increase in cAMP levels in the S-49 wild type cell. In comparison, the basal cAMP level of the cyc⁻ cells were a fraction of the wild type and, as expected, there was no increase in intracellular cAMP in the prostaglandin-treated cyc⁻ cell. Therefore, the inhibition of DNA synthesis in the cyc⁻ variants cannot be attributed to a cAMP-mediated mechanism.

Specificity of the Prostaglandins' Effect

The specificity of the prostaglandin effect on DNA synthesis in cyc⁻ is shown in Figure 1. This figure demonstrates that in S-49 cyc⁻ cells, the response of DNA synthesis varies with the specific prostaglandins tested. The most dramatic effect was that of dimethyl PGA₁, which reduced DNA synthesis to almost one-half of control within 2 h of exposure. PGE₁ was the next most effective and PGB₁ was a less active inhibitor. Not all the prostaglandins caused inhibition of DNA synthesis; PGE₂ tended to increase DNA synthesis and dmPGE₂ and PGF₂ₐ caused a significant increase in DNA synthesis.
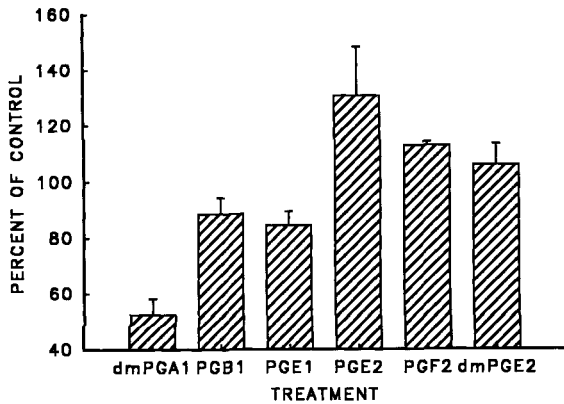
Fig. 1. The effect of prostaglandin on DNA synthesis of (cyc⁻) S-49 cells. The S-49 cyc⁻ cells were grown in stationary suspension culture in Dulbecco's medium with 3 g/liter glucose plus 10% fetal calf serum. The S-49 cells in exponential growth were treated with 50 μM dmPGA₁, PGB₁, PGE₁, PGE₂, PGF₂ₐ, dmPGE₂, or vehicle for 2 h before measuring DNA synthesis or cAMP generation. The data is reported in ±S.E.
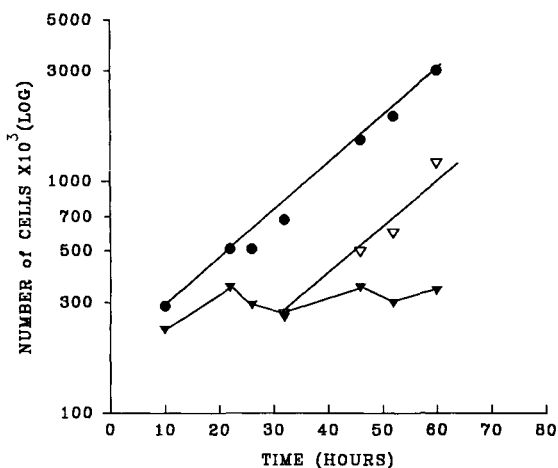

Fig. 2. Growth curve of S-49 lymphoma cyc⁻ cells. Cells were untreated and teamed with 30 μM dmPGA₁ for 60 h. Cells were grown in duplicate flasks (●—●, control; ▲—▲, PGA₁ treated, and △—△, PGA₁ removed). Exponentially growing cells were seeded at 2 × 106 cells/ml. At 26 h, the cells were resuspended in fresh culture medium or in medium containing dmPGA₁.

Effect of PGA₁ on Cell Growth

As seen in Figure 2, cell growth was inhibited over the entire 60 h of dmPGA₁ exposure. In one set of cell cultures, dmPGA₁ containing media was removed after 26 h of exposure and logarithmic growth was totally reestablished within 16 h after removal of prostaglandin. The cell cycle was analyzed by flow cytometry and plotted as cell population ordinate vs. DNA distribution; as seen in Figure 3A, the first peak represents the cell population at 1 × DNA content (G_1), the

second peak is the number of cells at the 2 × DNA content produced by cells in G_2 and M phases; the interval between the two peaks represents cells in S phase. When logarithmically growing cyc⁻ cells were analyzed by flow cytometry, approximately 52% of the cells were in G_1, 27% were in S phase, and 21% were in G_2/M (Fig. 3A). As seen in Figure 3B, when cyc⁻ cells are exposed to dmPGA₁ for 26 h, there is a striking *decrease* in the cell population in S phase and a concomitant rise in G_1 (i.e., 92% in G_1, 3% in S, and 6% in G_2/M). Conversely, when cells are exposed for 26 h and the dmPGA₁ is removed for 18 h, there is a corresponding increase in S and G_2/M, with a concomitant decrease in cells in G_1 (60% in G_1, 21% in S, and 18% in G_2/M) (Fig. 3C). Thus, 18 h after PGA₁ removal (Fig. 3C), the cells are returning to the distribution seen in the control cells (Fig. 3A).

Location of the Site of Cell Cycle Arrest Caused by dmPGA₁

It is known from previous work that the S-49 cells are arrested in early G_1 of the cell cycle by dBcAMP [Coffino et al., 1975; Gray et al., 1979; Sambrook et al., 1989]. In order to define the location of prostaglandin induced arrest in the G_1 phase more precisely, we studied the kinetics of DNA synthesis and cell growth in cells released from the prostaglandin arrest. S-49 cyc⁻ cells were synchronized in G_1 by incubation in the presence of dmPGA₁ for 22 h, followed by a media wash to remove any residual prostaglandin and the rapid addition of media containing dBcAMP (0.2 mM) to assure a single cell cycle after release of prostaglandin arrest. As illustrated in Figure 4, the cells exhibit approximately one cell doubling within 15 h. In data not shown, cells that are first arrested in G_1 by dBcAMP for 22 h, followed by a media rinse and immediate addition of dmPGA₁, do not progress through a cell cycle. During this procedure, we measured [³H-methyl] dThd incorporation into DNA and observed a period of high DNA synthetic activity followed by distinct decrease of DNA synthesis at approximately 12 h, a time period that is compatible with previous estimations of length of S phase duration [Gray et al., 1979].

Oncogene Expression in Growing and dmPGA₁ Arrested Cells

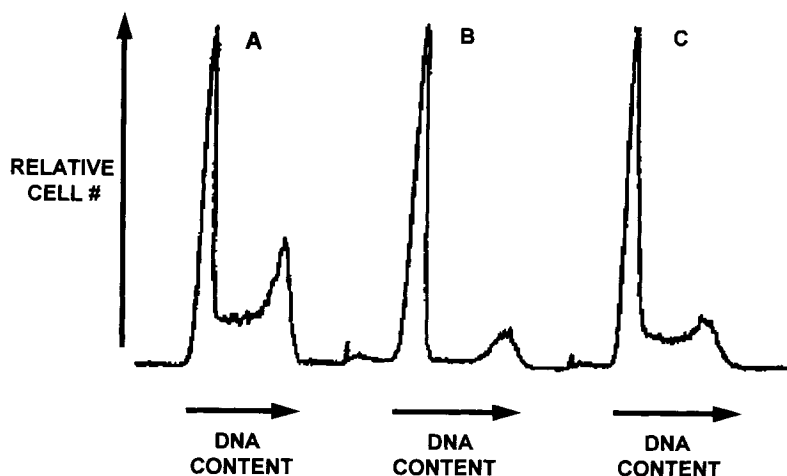S-49 cyc⁻ cells were treated with and without prostaglandin and after 24 h approximately 90%

Fig. 3. Effect of dimethyl prostaglandin A₁ on cell DNA distribution of the adenylate cyclase mutant (cyc⁻) S-49 cells. The cells represented in **A** and **B** were in exponential growth before treatment with vehicle or 50 μM dmPGA₁ for 44 h. Data in **C** represent the histogram of cells that were treated with 50 μM PGA₁ for 26 h before removal of the prostaglandin and re-

incubation in fresh media for 18 h. At 44 h all three groups were analyzed by flow cytometry to determine the frequency of distribution of DNA content per cell. Data are displayed as fluorescence per cell on the abscissa and cell number on the ordinate; both are arbitrary units.
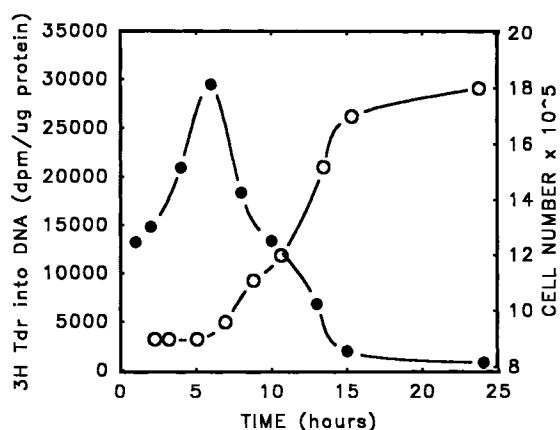

Fig. 4. Exit from the prostaglandin cell cycle arrest. The competence of the dmPGA₁ cells to reenter the cell cycle and the kinetics of that process were examined. In the experiment shown here, the S-49 cyc⁻ cells were treated for 24 h with 50 μM dmPGA₁. The cells were then rinsed and resuspended in media containing 0.2 mM dBcAMP and samples taken every 2 h. DNA synthesis (●—●) resumed within hours and was essentially complete by 13 h, the cell number (○—○) doubled within 13 h. Results shown are from one of 2 separate experiments. Shown are average results from duplicate sets of flasks.

TABLE II. Oncogene mRNA Expression in Prostaglandin Arrested Cells*

μg mRNA (height)	c-fos (peak height)		c-myc (peak)	
	Control	dmPGA₁	Control	dmPGA₁
64	66	216	57	131
	89	197	48	163
32	65	109	7	48
	42	111	15	83
16	—	30	23	84
	—	53	29	86
8	—	24	14	51
	—	—	—	53

*S-49 cells were grown with and without 30 μM dmPGA₁ for 24 h. Messenger RNA was extracted, blotted, and probed as described in Methods. Video scanning densitometry of band peak height minus background is expressed in relative arbitrary units.

of the cells were arrested in G₁. Messenger RNA was isolated as discussed in Methods. As seen in Table II, the c-fos signal is higher in the prostaglandin treated cells than the control. The same effect was seen in the c-myc probe of the control and treated cells. The visualized c-fos and c-myc messages were quantitated with video scanning densitometry. Calculating area by relative

height of the peaks scanned (Table II), we found that both the c-fos and the c-myc signals were from 2–3 fold higher than that of rapidly growing control cells.

DISCUSSION

Prostaglandins stimulate the synthesis of adenosine 3'5' cyclic monophosphate (cAMP), and, therefore, it has been suggested that the growth retardation caused by prostaglandins is mediated by this increase in intracellular cAMP level [Santoro and Jaffe, 1979; Bourne et al., 1975; Johnson et al., 1980]. Many investigators have shown that prostaglandins inhibit cell

growth [Ramwell and Shaw, 1970; Wiley (Hughes-Fulford) et al., 1983; Honn et al., 1981; Thomas et al., 1974; Bhuyan, 1986; Ikai et al., 1991; Santoro and Jaffe, 1979; Hughes-Fulford et al., 1984] in normal and malignant cells. However, Wiley (Hughes-Fulford) et al. showed that prostaglandins per se can inhibit both DNA synthesis and cell growth independent of increased cAMP generation [Wiley (Hughes-Fulford) et al., 1983; Hughes-Fulford et al., 1984, 1985]. Although it has been suggested that the prostaglandin inhibition is mediated through cAMP alone, previous studies did not establish a cAMP mechanism for the prostaglandin induced inhibition of cell growth. Recent reports of the induction of *gadd153* mRNA in prostaglandin cell cycle arrest have shown that protein kinase inhibitor 2-aminopurine decreased PGA_2 induction of *gadd 153*. It was suggested that the PGA_2 mediated growth arrest was working through a protein kinase since the use of the broad spectrum protein kinase inhibitor reduced the *gadd 153* signal [Choi et al., 1992]. These observations have caused renewed interest in the prostaglandins' mechanism of action and their role as growth regulators.

The results presented in this paper confirm our previous hypothesis of a non-cAMP mechanism for prostaglandin induced inhibition of DNA synthesis. In this study we show that $dmPGA_1$ inhibits DNA synthesis in both wild type and adenylate cyclase variant S-49 lymphoma cells. In the cyc⁻ type S-49 cell the DNA synthesis was inhibited by 46% despite the lack of adenylate cyclase activity in the cyc⁻ cells. This prostaglandin effect on DNA synthesis may be distinct from the arrest of the cell cycle, since we saw no significant change in the distribution of the cell cycle after 2 h of exposure to prostaglandins, even though there was a significant reduction ($P < .02$) in DNA synthesis.

The specificity of prostaglandin action is also of interest. Depending on their structures prostaglandins regulate DNA synthesis in different ways. Some of the prostaglandins tested were inhibitors of thymidine incorporation into DNA, with dimethyl PGA_1 being more effective than PGE_1 and PGB_1. Since PGE_1 can be dehydrated to PGA_1 in biological systems, all of the prostaglandins that inhibit cell growth share a common ring structure of cyclopentenone which has been noted by this laboratory and others [Honn et al., 1981; Hughes-Fulford et al., 1984]. There was a significant increase of thymidine incorpo-

ration into DNA caused by dimethyl PGE_2. The inhibition of cell growth and DNA synthesis by $dmPGA_1$ is probably not secondary to toxicity for two reasons. First, cell membrane integrity, as measured by dye exclusion, is not changed even after several days of prostaglandin treatment. Secondly, even after 26 h exposure, the effect of $dmPGA_1$ is reversible after removal of the prostaglandin from the media. Within hours the cells proceed through the cell cycle at the same rate as the controls.

Since previous experience with both PGE_1 and PGB_1 had demonstrated degradation of biological activity of the prostaglandins over long periods of time, we used the most potent and degradation-resistant prostaglandin, $dmPGA_1$, to test the long-term effect of prostaglandin on growth. Cyc⁻ cells were chosen for these growth studies since this S-49 variant eliminated the possibility that $dmPGA_1$ was working through a cAMP mediated mechanism and the kinetics if their cell cycles are known. The S-49 cyc⁻ control cells exhibited the expected 17 h doubling time. Addition of $dmPGA_1$ stopped entry of the cells into S phase and, therefore, caused the cells to be arrested in G_1 of the cell cycle. When the $dmPGA_1$ block was removed after 26 h the effect of the prostaglandin was reversed within hours, with logarithmic growth re-established within 16 h. The α,β unsaturated ketone prostaglandins have been shown to block cell cycle in G_1 and inhibit entry into S-phase in L-1210 and B_{16} cells [Bhuyan et al., 1986], in S49 cells [Hughes Fulford, et al., 1984] and in BHK and Balb/c MC3T3 cells (unpublished observations).

Previous studies done in 1975 by Coffino et al. have shown that the S-49 cyc⁻ cell cycle is approximately 17 h long, with S-phase accounting for 12 h of the cell cycle. When cyc⁻ cells are exposed to prostaglandin over an extended period, we observe a block of cell growth. The cells arrested by the prostaglandin showed an increase of the G_1 phase population. This increase of cells in G_1 was coupled to a dramatic decrease of cells in S phase and in G_2/M. After 22 h of exposure, at least 80% or more of the cells were found to be in G_1, and this enrichment of the G_1 cell population was maintained throughout the 52 h of the study.

The time needed for cells to egress from the G_1 prostaglandin block can be estimated from the data in Figure 4. Cells were grown in $dmPGA_1$ for 22 h before removal, and media containing 0.2 mM dibutyryl cAMP was then

added (dibutyryl cAMP is known to block cell cycle in early G_1). If the dmPGA₁ block was before the dB-cAMP arrest, the cells would not have completed a cell cycle. These data demonstrate that the prostaglandin block of cell growth is different and distal to the site of the dB-cAMP arrest. Figure 5 shows a schematic of the location of the cell cycle block. Based on previous work by Gray et al. [1979] which showed an S phase of 12.1 h duration, and G_2/M of approximately 2.7 h, these data suggest that the dmPGA₁ block is very near the G_1/S interface. Assuming that the cells are at the G_1/S interface, it would take approximately 14.8 h for the cells to reach G_1, agreeing very closely with the approximate 15 h we observed in our studies.

During G_1, the cell is preparing for S phase DNA synthesis. Olashaw and Pledger have proposed two discrete subphases of G_1: (1) competence (which can be fulfilled by adding platelet derived growth factor, epidermal growth factor, or activation of c-myc gene); and (2), progression (which requires factors from platelet-poor plasma, insulin, or somatomedin C) [Olashaw and Pledger, 1985]. This hypothesis requires at least two molecular event switches to control progression into S-phase DNA synthesis. In recent years it has become apparent that regulation of cell cycle is a multi-phasic action of combined actions which can be receptor mediated, ion flux facilitated, and regulated at the level of gene expression. Our studies demonstrate that prostaglandins can increase or decrease DNA synthesis, depending on specific prostaglandin structure independent of cAMP. Other studies by this and other laboratories have demonstrated that prostaglandin E_2 acts as a growth factor both in vitro and in vivo [Ueno et al., 1984; Jee et al., 1985; Handler et al., 1990; Mori et al., 1992; Hughes-Fulford et al., 1992]. It is possible that the synthetic polar

α,β unsaturated prostaglandins like dmPGA₁ may be interfering with natural endogenous prostaglandins like PGE₂ that normally regulate growth in selected tissues. These data using the cyc⁻ adenylate cyclase variants also show that α,β unsaturated prostaglandin growth inhibition is not mediated through cAMP or cAMP dependent protein kinases. The mechanism of action for prostaglandin regulation of cell cycle remains to be defined. We are currently investigating specific prostaglandin receptors, phopholipases, the inositol phosphate pathway, and calcium channels as possible signalling mechanisms. Our data suggest that dmPGA₁ inhibits cell growth in late G_1 accompanied with enhanced c-fos and c-myc expression even 24 h after growth inhibition. DmPGA₁ inhibits the cell cycle in a non-toxic manner and blocks entry into S-phase DNA synthesis near the G_1/S boundary. This compound offers a powerful tool for the study of control of the cell cycle and regulation of initiation of DNA synthesis.

ACKNOWLEDGMENTS

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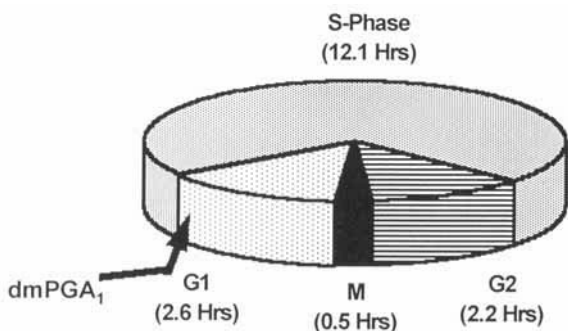

Fig. 5. Schematic of location of cell cycle block by dmPGA₁.

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