Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4746–4750, June 1990
Developmental Biology

Conditions that alter intracellular cAMP levels affect expression of the cAMP phosphodiesterase gene in *Dictyostelium*

(gene regulation/differentiation/8-bromoadenosine 3',5'-cyclic monophosphate/ammonia/caffeine)

BRUCE B. RILEY AND STEPHEN L. BARCLAY*

Department of Bacteriology and Program in Cell and Molecular Biology, University of Wisconsin–Madison, Madison, WI 53706

Communicated by T. Kent Kirk, April 16, 1990 (received for review July 24, 1989)

ABSTRACT **We examined expression of the *Dictyostelium* cAMP phosphodiesterase (PDE) gene under conditions that alter intracellular cAMP levels during *in vitro* differentiation of wild-type strain V12M2 and a sporogenous derivative, HB200. In control cultures, cellular PDE activity peaked at 6 hr and declined by 8 hr, while secreted PDE activity continued to increase through 8 hr. Lowering intracellular cAMP levels with caffeine or progesterone increased cellular and secreted PDE activities 2-fold, increased stalk cell differentiation, and inhibited spore differentiation. In contrast, exposure to 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP; a membrane-permeable cAMP analog) or ammonia (which promotes intracellular cAMP accumulation in V12M2 and HB200 cells) lowered PDE activities by as much as 45%, decreased stalk cell differentiation, and increased spore differentiation. Simultaneous exposure to 8-Br-cAMP and caffeine gave intermediate PDE activities as would be expected if 8-Br-cAMP entered the cell and bypassed the caffeine-mediated block to adenylate cyclase activation. In all cases, we observed commensurate changes in developmental PDE transcript levels. The developmental time course of expression was not significantly altered by these treatments. These results suggest that the magnitude of PDE gene expression is negatively regulated by intracellular cAMP levels and provide evidence for one of the earliest changes in gene expression that is consistent with cell-type specificity. These results are discussed in terms of a bistable switch employing intracellular cAMP as a regulator of cell fate.**

Differentiation of *Dictyostelium* amoebae culminates with the formation of fruiting bodies composed of stalk cells and spores. Simplified *in vitro* culture conditions permit analysis of signals that regulate the choice between stalk cell and spore differentiation. Stalk cell formation by wild-type V12M2 cells during *in vitro* differentiation requires two known secreted factors: cAMP and differentiation-inducing factor (DIF) (1–3). V12M2 cells cannot complete spore differentiation under these conditions, but mutants of V12M2 can be isolated that form both spores and stalk cells *in vitro* (3, 4). Such mutants are termed "sporogenous." All cells require exogenous cAMP to initiate differentiation. Later in development, high exogenous cAMP levels inhibit terminal stalk cell differentiation and promote spore differentiation (5–7). DIF antagonizes the effects of exogenous cAMP and induces several genes expressed only in prestalk and stalk cells (8, 9).

We are investigating the role of cAMP in differentiation. Extracellular cAMP binds to cell surface cAMP receptors, causing rapid accumulation of several intracellular second messengers, including intracellular cAMP (10, 11). There is growing evidence that intracellular cAMP influences the decision between stalk cell and spore differentiation. Caffeine and progesterone, which inhibit intracellular cAMP accumulation by different mechanisms (12, 13), inhibit spore and promote stalk cell differentiation in concentration-dependent manners (7). Conversely, conditions that raise intracellular cAMP levels by hyper-inducing adenylate cyclase activity increase spore and decrease stalk cell differentiation (14). Ammonia, which promotes intracellular cAMP accumulation by inhibiting its secretion (15), and 8-bromoadenosine 3',5'-cyclic monophosphate (8-Br-cAMP), a membrane-permeable cAMP analog with low affinity for the cell surface cAMP receptor (16), also increase spore and decrease stalk cell differentiation in cultures of sporogenous mutants (2, 14). Finally, high levels of 8-Br-cAMP induce wild-type V12M2 cells to form spores *in vitro* (17).

To investigate how intracellular cAMP levels might regulate differentiation, we are identifying and studying genes that regulate or are regulated by intracellular cAMP levels. In this report, we investigate regulation of cAMP phosphodiesterase (PDE). A single PDE gene in *Dictyostelium* encodes at least two distinct transcripts: a 1.8-kilobase (kb) mRNA expressed constitutively and a 2.2-kb mRNA expressed only during development (18). Starvation and extracellular cAMP are known to regulate developmental transcript levels (18, 19), but the intracellular second messenger(s) involved has not been identified. We provide evidence that changes in intracellular cAMP levels affect the magnitude, but not the developmental time course, of PDE gene expression.

MATERIALS AND METHODS

Cells of strains V12M2 and HB200 were grown on nutrient agar in association with *Klebsiella pneumoniae* at 22°C and harvested when cell density reached $\approx 8 \times 10^5$ cells per cm^2. Cells were prepared for *in vitro* differentiation as described (14) except that the buffer contained 5 mM cAMP, and cells were developed at 2.5×10^5 cells per cm^2. HB200 is a spontaneous sporogenous mutant of wild-type strain V12M2 (7). V12M2 and HB200 aggregate to form mounds by 6 hr and form standing slugs by 10–12 hr of development on agar. However, HB200 cells terminally differentiate by 12–14 hr (compared to 20–22 hr for V12M2) and never form migrating slugs.

The PDE assay has been previously described (20). Minor modifications are noted in the legend to Fig. 1.

Total RNA was isolated and purified by lysing cells with guanidinium thiocyanate and spinning lysates over $CsCl_2$. For Northern analysis, 10 μg of total RNA was size fractionated on 1.3% agarose/6% formaldehyde gels, electroblotted onto GeneScreen*Plus* (NEN), and hybridized with the cloned inserts of p1.2 (PDE cDNA; ref. 19) or pLK326 (a constitutive cDNA generously provided by H. L. Ennis,

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Abbreviations: 8-Br-cAMP, 8-bromoadenosine 3',5'-cyclic monophosphate; PDE, phosphodiesterase; DIF, differentiation-inducing factor.

*To whom reprint requests should be addressed.

Roche Institute of Molecular Biology) labeled with an oligolabeling kit (Pharmacia). Hybridization and washing of blots were performed according to procedures described elsewhere (21). Washed blots were autoradiographed using Kodak XAR-5 film with intensifying screens at −80°C.

RESULTS

Quantitation of PDE Enzyme Activities and Cell Fate. Experimental manipulation of gene expression during differentiation of *Dictyostelium* amoebae is difficult under natural conditions because the cells are not in direct contact with the medium and are enclosed by a slug sheath whose permeability to exogenous agents is unknown. Differentiation of sporogenous amoebae in tissue culture dishes has the advantage that cells remain in contact with the surrounding medium and complete terminal differentiation as stalk cells and spores. For this study, we used sporogenous strain HB200 because a thorough study showed that its regulation of cAMP metabolism is indistinguishable from that of its wild-type parent, V12M2 (14). During *in vitro* differentiation of HB200 or V12M2 cells, levels of cellular PDE enzyme activity began to rise by 4 hr, peaked at 6 hr, and declined by 8 hr (Fig. 1). This time course is considerably faster than that reported for AX3 cells, which complete aggregation and reach peak PDE transcript levels by 12–14 hr (18, 19). However, V12M2 and HB200 cells complete aggregation on nonnutrient agar by 6 hr (see *Materials and Methods*), and the time course of PDE accumulation is the same during *in vitro* differentiation and development on agar (B.B.R., unpublished data). Thus, the accelerated time course of PDE gene expression seen here reflects the generally rapid development of V12M2-derived strains.

In the following experiments, we tested whether conditions previously shown to alter intracellular cAMP levels and cell fate also alter PDE gene expression. First, we used caffeine to inhibit activation of adenylate cyclase (12). Five millimolar caffeine lowers intracellular cAMP levels, inhibits spore formation, and stimulates stalk cell formation during *in vitro* differentiation of HB200 cells (refs. 7 and 14; Table 1). HB200 or V12M2 cells exposed to 5 mM caffeine accumulated twice as much cellular PDE activity as control cells (Fig. 1 *A* and *B*). These changes could be mediated by low intracellular cAMP levels in caffeine-treated cells or, alternatively, caffeine could have pleiotropic effects on other second messenger systems (12). To address this, we added 8-Br-cAMP to caffeine-treated cultures. 8-Br-cAMP is a membrane-permeable cAMP analog that has high affinity for the regulatory subunit of cAMP-dependent protein kinase (23) but not for the cell surface cAMP receptor (16). This allows 8-Br-cAMP to enter the cell, mimic endogenous cAMP, and bypass the caffeine-mediated block to adenylate cyclase activation. Exposure to 1 mM 8-Br-cAMP restores spore formation to near normal levels in caffeine-treated cultures of HB200 (ref. 14; Table 1). Similarly, 1 mM 8-Br-cAMP returned cellular PDE activity to near normal levels in caffeine-treated cultures of HB200 and V12M2 (Fig. 1 *A* and B). Without caffeine, 1 mM 8-Br-cAMP nearly doubled spore formation in HB200 cultures (Table 1) and reduced cellular PDE activity by as much as 40% in HB200 and V12M2 cells (Fig. 1 *A* and *B*). Thus, changing intracellular cAMP levels is sufficient to alter cellular PDE accumulation, with low cAMP levels stimulating maximal PDE accumulation.

To test this further, we altered cAMP levels with agents that inhibit or stimulate cAMP secretion. First, progesterone lowers intracellular cAMP levels by stimulating its secretion and, like caffeine, inhibits spore and promotes stalk cell formation (7, 13, 14). Continuous exposure to 100 μM progesterone increased cellular PDE activity by 50% (relative to peak activity in the control) but delayed the onset of PDE accumulation by 4–6 hr (not shown), making these results difficult to interpret. We were able to eliminate this delay by removing the progesterone after 1 hr. Exposing HB200 cells to 100 μM progesterone for only the first hour in *in vitro* differentiation blocked spore formation, stimulated stalk cell formation, and increased cellular PDE activity by 2-fold (Table 1, Fig. 1*C*). Second, we have recently found that ammonia raises intracellular cAMP levels in wild-type and sporogenous cells by inhibiting its secretion (ref. 15; Table 1). HB200 cells treated with 15 mM NH_4Cl formed twice as many spores and accumulated 45% less cellular PDE activity than control cells (Table 1, Fig. 1*C*). The same results were obtained with V12M2 cells (B.B.R., unpublished data). In summary, agents that elevate intracellular cAMP levels (8-Br-cAMP, ammonia) reduce cellular PDE accumulation, whereas agents that lower intracellular cAMP levels (caffeine, progesterone) increase cellular PDE accumulation.

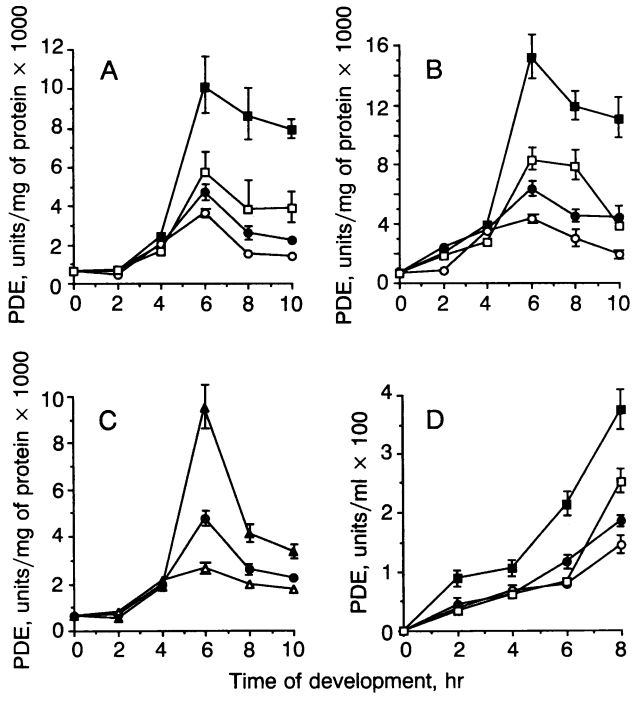

FIG. 1. Effects of caffeine and 8-Br-cAMP on cellular PDE activities in HB200 (*A*) and V12M2 (*B*) cells. Cells (5×10^6) were plated for *in vitro* differentiation on 6-cm tissue culture dishes with 5 mM caffeine (■), 1 mM 8-Br-cAMP (○), 1 mM 8-Br-cAMP plus 5 mM caffeine (□), or no drug (●). At the indicated times, extracellular medium was drawn off, and cells were resuspended in 1 ml of ice-cold distilled water, centrifuged twice to remove contaminating exogenous cAMP, and finally resuspended in 100 μl of cold distilled water. Cells were lysed by sonication, and 5–10 μl of cell lysate was assayed at 37°C for 20 min in a 200-μl volume containing 10 mM Tris (pH 7.6), 1 mM $MgCl_2$, 100 μM cAMP, and 0.13 μM [^{3}H]cAMP (31.2 Ci/mmol; 1 Ci = 37 GBq). Protein was determined by the Bradford assay (22). (*C*) Effects of progesterone and ammonia on cellular PDE activities in HB200 cells. Assays were performed as above except that cells were treated with 100 μM progesterone (▲), 15 mM NH_4Cl (△), or no drug (●). Progesterone was removed after 1 hr. (*D*) Secreted PDE activities in HB200 cultures. Chilled extracellular medium from the cultures in *A* was centrifuged to remove cells and dialyzed against 50 mM Tris, pH 7.6/5 mM $MgCl_2$ for 24 hr, during which the dialysis buffer was changed twice. One-hundred microliters of dialyzed medium was assayed as described above. One unit of PDE degrades 1 pmol of cAMP per min. Data are the means ± standard deviations of two or more experiments. Data points without error bars have standard deviations of <200 units/mg of protein (*A–C*) or 10 units/ml (*D*). Differences between PDE activities in control cultures and cultures exposed to caffeine, progesterone, 8-Br-cAMP, or ammonia were highly repeatable and statistically significant ($P < 0.01$).

Table 1. Effects of various treatments on intracellular cAMP accumulation and cell fate in HB200 cultures

Culture conditions	Intracellular cAMP, pmol/mg (peak levels)	Final spore/stalk ratio
Control	33 ± 3	32/64
5 mM caffeine	13 ± 2	1/94
+ 1 mM 8-Br-cAMP	Indeterminate	25/75
1 mM 8-Br-cAMP	Indeterminate	58/42
100 μM progesterone	11 ± 1	1/95
15 mM NH_4Cl	43 ± 3	61/32

Intracellular cAMP levels (pmol/mg of protein) were measured by radioimmunoassay as described (14). Effective cAMP levels in cells treated with 8-Br-cAMP cannot be determined because cellular cAMP-binding proteins differ in their affinities for the analog relative to endogenous cAMP (16, 23). To quantitate changes in cell fate, cells were cultured under each set of conditions for 24 hr at 22°C and scored by phase-contrast microscopy for the percentage of cells forming spores, stalk cells, and amoebae. Phase bright spores and highly vacuolated stalk cells are easily distinguishable and, unlike amoebae, are resistant to 0.5% Triton X-100. The ratios shown are percent spore/percent stalk cell.

None of the conditions used here altered the time course of cellular PDE accumulation (Fig. 1 *A–C*).

The relative contributions of membrane-bound and cytoplasmic PDE activities to total cellular PDE activity are shown in Table 2. At 6 hr of *in vitro* differentiation, when cellular PDE activities were maximal, 41–45% of total cellular activity was present on the surface of intact cells (membrane-bound) in all cultures. The remainder, by inference and by direct measurement, comprised cytoplasmic PDE activity. Thus, treatments that change total cellular PDE activities affect both cellular compartments to roughly the same degree.

These treatments also affected accumulation of secreted PDE activity. Caffeine increased secreted PDE activity by 2-fold, 8-Br-cAMP decreased activity by 25–30%, and simultaneous exposure to caffeine and 8-Br-cAMP gave intermediate activities (Fig. 1*D*). These data suggest that activity levels of cytoplasmic, membrane-bound, and secreted PDE correlate inversely with intracellular cAMP levels.

Quantitation of PDE Transcript Levels. During *in vitro* differentiation of HB200 cells, levels of the developmentally regulated 2.2-kb PDE transcript began to rise by 2 hr (not shown), peaked by 4 hr, and declined sharply by 8–12 hr (Fig. 2). Note that cellular PDE enzyme activities peak several hours after 2.2-kb transcript levels (compare Figs. 1 and 2), a lag that agrees with previous observations (19). Because peak levels of 2.2-kb transcript in control cultures were 15–20 times higher than 1.8-kb constitutive transcript levels, hybridization to constitutive transcript is not visible in the exposures used in Fig. 2*A*. Overexposures showed that 1.8-kb transcript levels were relatively constant at all times and in all cultures (not shown), confirming previous reports that its expression is constitutive (18).

Table 2. Effects of caffeine and 8-Br-cAMP on membrane-bound and cytoplasmic PDE activities in HB200 cells

Cellular PDE fraction	Developmental conditions			
	Control	Caffeine	8-Br-cAMP	Caffeine + 8-Br-cAMP
Total cell lysate	2830	5500	2320	4170
Membrane-bound	1270	2330	940	1900
Inferred cytosolic	1560	3170	1380	2270
Measured cytosolic	1710	3000	1670	2190

All PDE activities (expressed as units/mg of protein) were assayed as described in the legend for Fig. 1 except that they were measured at 22°C (and are therefore lower than those seen in Fig. 1). Total cell assays were of sonicated cell lysates and membrane-bound assays were of intact cells. Cytosolic activities were determined by inference (total activity minus membrane-bound activity) and by direct measurement as described (24). Data are averages of duplicate determinations.

Drugs that alter intracellular cAMP levels affected accumulation of 2.2-kb PDE transcript in the same manner that they affected PDE enzyme activity. Relative to the control, 5 mM caffeine increased 2.2-kb transcript levels 3-fold (Fig. 2). Addition of 1 mM 8-Br-cAMP to caffeine-treated cultures returned 2.2-kb transcript levels to near normal levels, and 8-Br-cAMP by itself reduced transcript levels by nearly half. Treating cells with 100 μM progesterone for the first hour of development increased 2.2-kb transcript levels by >2 fold, whereas 15 mM NH_4Cl reduced transcript levels by more than half. Reprobing Northern blots with constitutive probe pLK326 (Fig. 2*A*) showed that the observed changes in 2.2-kb PDE transcript levels were not due to loading errors. These data suggest that the magnitude of developmental PDE transcript accumulation correlates inversely with intracellular cAMP levels, as does cellular and secreted PDE enzyme activities (Fig. 1).

In contrast to PDE, the treatments used here did not affect expression of developmental genes D14, D18, D19, or PL1 (B.B.R., unpublished data). This suggests that intracellular cAMP regulates PDE gene expression by a selective process, rather than by globally affecting all developmental genes.

DISCUSSION

We have quantitated PDE enzyme and transcript levels during *in vitro* differentiation. Under these conditions, cells are continuously exposed to 5 mM exogenous cAMP. This causes receptor adaptation and down-regulation in derivatives of wild-type strain NC4 but not in derivatives of wild-type strain V12M2 (5, 25, 26). Thus, it is possible to manipulate intracellular cAMP levels (or other second messengers) under the conditions used here. Furthermore, exogenous cAMP levels are super-saturating, so raising or lowering intracellular cAMP levels cannot affect gene expression by altering cAMP relay.

Using this system, we determined whether conditions that alter intracellular cAMP levels affect PDE gene expression. Drugs that lower intracellular cAMP levels (caffeine and progesterone) elevated PDE enzyme activity and transcript levels. Conversely, drugs that elevate intracellular cAMP levels (ammonia, 8-Br-cAMP) lowered PDE enzyme activity and transcript levels. 8-Br-cAMP reversed the stimulating effect of caffeine on PDE gene expression, indicating that caffeine-dependent stimulation occurs only if intracellular cAMP levels remain low.

From these results, we propose that synthesis or stability of the 2.2-kb transcript of the PDE gene (or expression of factors that regulate these functions) is sensitive to intracellular cAMP levels during development and is maximal when those levels are low. Accumulation of 2.2-kb PDE transcript varies 6- to 7-fold between conditions of high and low concentrations of intracellular cAMP. While this manuscript was in preparation, another report showed that PDE gene expression increases 3-fold or more when *Dictyostelium* amoebae overexpress a mutant mammalian regulatory subunit of cAMP-dependent protein kinase (27). This regulatory subunit fails to bind cAMP and constitutively blocks kinase activation. Taken together, both experiments suggest that synthesis or stability of the 2.2-kb PDE transcript is negatively regulated by cAMP-dependent phosphorylation. However, this cannot be the only mechanism controlling PDE gene expression because the overall time course of expression was the same in all cultures, regardless of intracellular

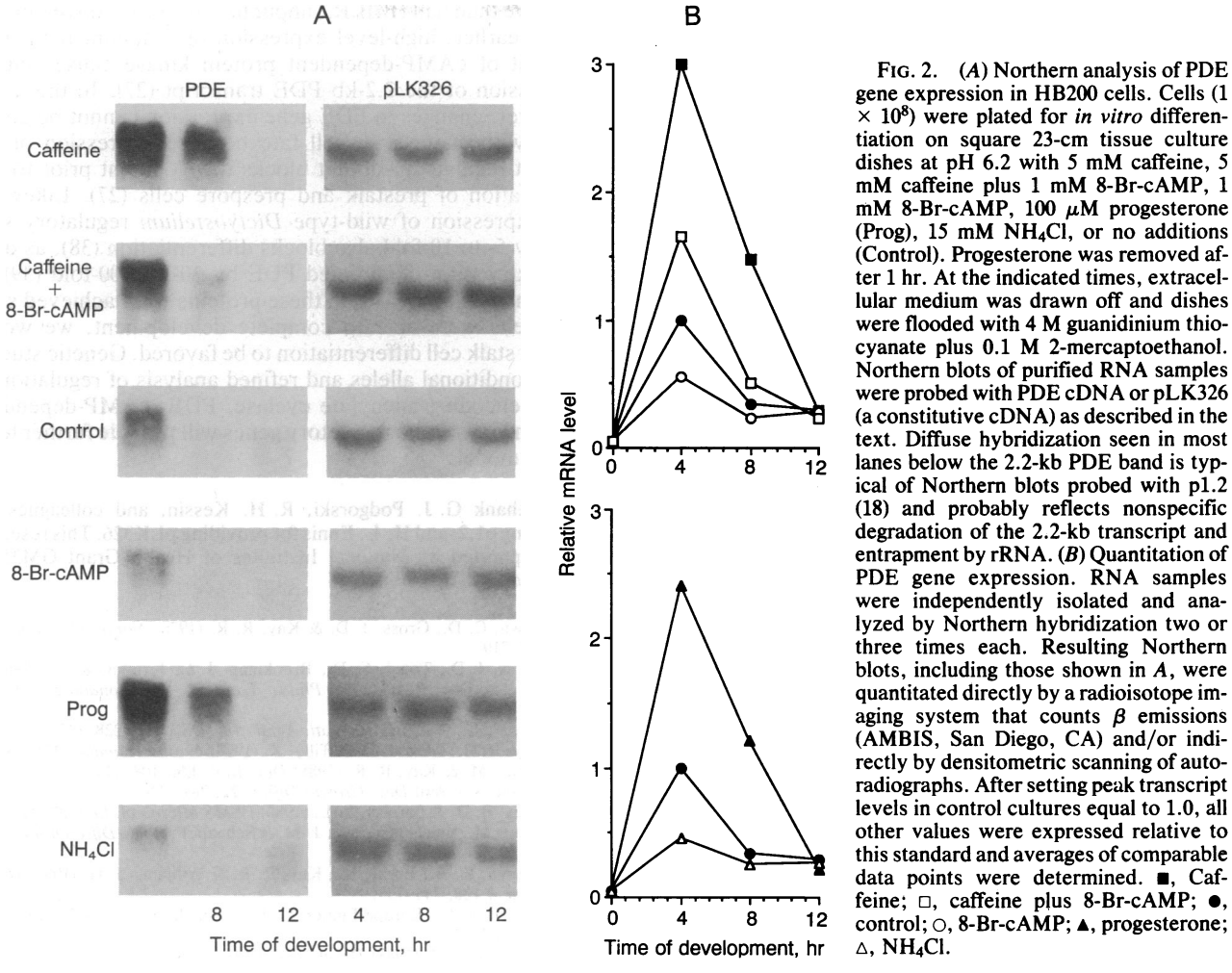

FIG. 2. (*A*) Northern analysis of PDE gene expression in HB200 cells. Cells (1×10^8) were plated for *in vitro* differentiation on square 23-cm tissue culture dishes at pH 6.2 with 5 mM caffeine, 5 mM caffeine plus 1 mM 8-Br-cAMP, 1 mM 8-Br-cAMP, 100 μM progesterone (Prog), 15 mM NH_4Cl, or no additions (Control). Progesterone was removed after 1 hr. At the indicated times, extracellular medium was drawn off and dishes were flooded with 4 M guanidinium thiocyanate plus 0.1 M 2-mercaptoethanol. Northern blots of purified RNA samples were probed with PDE cDNA or pLK326 (a constitutive cDNA) as described in the text. Diffuse hybridization seen in most lanes below the 2.2-kb PDE band is typical of Northern blots probed with p1.2 (18) and probably reflects nonspecific degradation of the 2.2-kb transcript and entrapment by rRNA. (*B*) Quantitation of PDE gene expression. RNA samples were independently isolated and analyzed by Northern hybridization two or three times each. Resulting Northern blots, including those shown in *A*, were quantitated directly by a radioisotope imaging system that counts β emissions (AMBIS, San Diego, CA) and/or indirectly by densitometric scanning of autoradiographs. After setting peak transcript levels in control cultures equal to 1.0, all other values were expressed relative to this standard and averages of comparable data points were determined. ■, Caffeine; □, caffeine plus 8-Br-cAMP; ●, control; ○, 8-Br-cAMP; ▲, progesterone; △, NH_4Cl.

cAMP levels. Clearly, some other mechanism regulates developmental timing of this gene.

We have also shown that conditions that alter intracellular cAMP levels affect cell fate, such that spore differentiation is favored when intracellular cAMP levels are high and stalk cell differentiation occurs when cAMP levels are low (refs. 7 and 14; Table 1). The relationship between intracellular cAMP levels, cell fate, and PDE gene expression during *in vitro* differentiation suggests the working hypothesis summarized in Fig. 3. PDE and cAMP are viewed as two components of a bistable switch that controls two metastable states. In state I, intracellular cAMP levels are low and PDE activity is high as a result of active transcription of the PDE gene. This state is self-sustaining because high cytoplasmic PDE activity will erase transient increases in intracellular cAMP levels, thereby permitting continued high-level expression of the PDE gene. Increased membrane-bound or secreted PDE activity will also lower intracellular cAMP levels by reducing signal relay between cells. Our results suggest that cells fated to become stalk cells pass through this state. In state II, intracellular cAMP levels are high and PDE activity is low because expression of the PDE gene is limited. In this context, the yeast *ADH2* gene, which is active unless its transcription factor (ADR1) is phosphorylated by a cAMP-dependent kinase, provides an example of transcription inhibition by cAMP (28). In our model, PDE gene expression could be limited by transcriptional or posttranscriptional processes, or both. Cells fated to become spores pass through state II, which is stable as long as cAMP levels remain high. State transitions can occur, but at differing rates. Transition from state II to state I will be rapid if intracellular cAMP levels are lowered by inhibition of adenylate cyclase. Transition from state I to state II will result from a sustained high rate of cAMP synthesis, but the transition will be slow because the rate of cAMP degradation is initially high. Thus, the rate of transition depends upon relative rates of PDE synthesis and decay.

The model is useful for organizing many other observations. First, prestalk cells of migrating slugs contain 2- to 3-fold higher levels of cellular PDE activity than do prespore cells (24, 29). Second, *Dictyostelium* differentiation occurs without determination and prespore and prestalk cell types interconvert during slug migration and after bisection of slugs (30, 31). Prestalk to prespore transitions are slow (usually requiring several hours) but prespore to prestalk transitions can occur within minutes (30, 31). Third, several secreted morphogens affect developing cells in ways that could stabilize one state or the other. For example, DIF, which induces stalk cell differentiation, inhibits the cell surface cAMP receptor and reduces cAMP signaling (8). Similarly, the cAMP hydrolysis product adenosine inhibits cAMP synthesis (32) and, when added to agar, increases the percentage of prestalk cells in migrating slugs (33). In contrast, ammonia increases intracellular cAMP levels in wild-type and sporogenous cells (ref. 15; Table 1) and favors spore differentiation (2, 15). Changing levels of any of these morphogens could induce a transition from one state to the other.

The model predicts that prestalk cells or terminally differentiating stalk cells should have lower intracellular cAMP levels than prespore cells or terminally differentiating spores. In apparent contradiction, several studies have shown that total cAMP levels are slightly higher in the prestalk region of

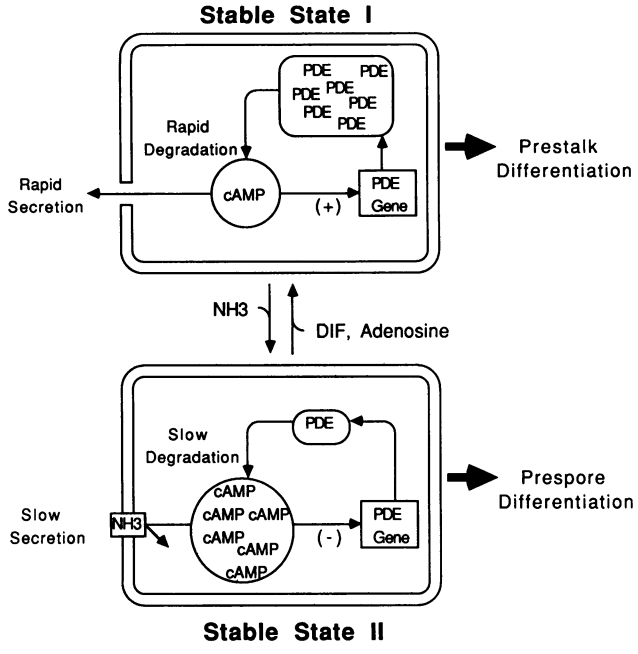

FIG. 3. A bistable physiologic switch. The size of the intracellular cAMP pool directly or indirectly affects the magnitude of developmental PDE gene expression. The resulting increase (+) or decrease (−) in PDE gene expression feeds back onto the intracellular cAMP pool, thereby stabilizing one of two possible physiologic states. Stabilization of intracellular cAMP levels then induces or represses downstream functions required for prespore/spore or prestalk/stalk cell differentiation. The two stable states are interconvertible, depending upon changes in rates of cAMP synthesis or secretion. The roles of ammonia, DIF, and adenosine are discussed in the text.

migrating slugs (34, 35). However, these studies did not distinguish between intracellular and extracellular cAMP, and much of the excess cAMP in the prestalk region could be extracellular. Indeed, prestalk sections of migrating slugs attract more aggregating amoebae than do prespore sections (36). Furthermore, measurements of cAMP relay in the absence of extracellular PDE activity indicate that prestalk cells secrete more cAMP than do prespore cells (24). It is possible that higher rates of secretion yield lower intracellular cAMP levels in prestalk cells. Consistent with this is the finding that the prespore morphogen ammonia raises intracellular cAMP levels by inhibiting its secretion (15).

Thus, measurement of average cAMP levels in slugs is not a suitable test for this model and may be misleading if cAMP levels oscillate rapidly as others suggest (24, 37). For example, without information about the amplitude and shape of cAMP waves in prestalk and prespore regions of slugs, one cannot infer how the concentration of cAMP and receptor occupancy change over time. Because rates of cAMP secretion and hydrolysis are higher in the prestalk region (24, 29), cAMP waves could have high peaks that are followed by rapid declines. In contrast, waves in the prespore region could have lower, but broader peaks. This could have the net effect of making time average receptor occupancy higher in the prespore region even though average cAMP levels are lower.

It may be more productive to manipulate the level or activities of adenylate cyclase, PDE, and cAMP-dependent protein kinase. Recently, one prediction of the model has been confirmed in this way. Conditions that hyperinduce GTP-dependent activation of adenylate cyclase increase spore production in V12M2 culminants and during *in vitro* differentiation of sporogenous mutants (14). These conditions also reduce accumulation of the 2.2-kb PDE transcript by more than half (B.B.R., unpublished data). Conversely, as noted earlier, high-level expression of a mutant regulatory subunit of cAMP-dependent protein kinase causes higher expression of the 2.2-kb PDE transcript (27). In this case, however, changes in PDE gene expression cannot be correlated with changes in cell fate because expression of the mutant regulatory subunit blocks development prior to differentiation of prestalk and prespore cells (27). Likewise, overexpression of wild-type *Dictyostelium* regulatory subunit by 5- to 10-fold also blocks differentiation (38), as does overexpression of secreted PDE by 30- to 100-fold (39). If more modest increases in these proteins were achieved such that cells were able to complete development, we would expect stalk cell differentiation to be favored. Genetic studies with conditional alleles and refined analysis of regulation of genes encoding adenylate cyclase, PDE, cAMP-dependent protein kinase, and regulatory genes will provide further tests of the model.

We thank G. J. Podgorski, R. H. Kessin, and colleagues for providing pl.2, and H. L. Ennis for providing pLK326. This research was supported by National Institutes of Health Grant GM35432 awarded to S.L.B.

1. Town, C. D., Gross, J. D. & Kay, R. R. (1976) *Nature (London)* **262**, 717–719.
2. Gross, J. D., Town, C. D., Brookman, J. J., Jermyn, K. A., Peacey, M. J. & Kay, R. R. (1981) *Philos. Trans. R. Soc. London Ser. B* **295**, 497–508.
3. Kay, R. R. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3228–3231.
4. Kay, R. R., Garrod, D. & Tilly, R. (1978) *Nature (London)* **271**, 58–60.
5. Berks, M. & Kay, R. R. (1988) *Dev. Biol.* **126**, 108–114.
6. Ishida, S. (1980) *Dev. Growth Differ.* **22**, 781–788.
7. Riley, B. B. & Barclay, S. L. (1986) *FEMS Microbiol. Lett.* **37**, 221–226.
8. Wang, M., Van Haastert, P. J. M. & Schaap, P. (1986) *Differentiation* **33**, 24–28.
9. Jermyn, K. A., Berks, M., Kay, R. R. & Williams, J. G. (1987) *Development* **100**, 745–755.
10. Newell, P. C., Europe-Finner, G. N. & Small, N. V. (1987) *Microbiol. Sci.* **4**, 5–11.
11. Williams, J. G. (1988) *Development* **103**, 1–16.
12. Brenner, M. & Thoms, S. D. (1984) *Dev. Biol.* **101**, 136–146.
13. Klein, C. & Brachet, P. (1975) *Nature (London)* **254**, 432–434.
14. Riley, B. B., Jensen, B. R. & Barclay, S. L. (1989) *Differentiation* **41**, 5–13.
15. Riley, B. B. & Barclay, S. L. (1990) *Development*, in press.
16. van Haastert, P. J. M. & Kien, E. (1983) *J. Biol. Chem.* **258**, 9636–9642.
17. Kay, R. R. (1989) *Development* **105**, 753–759.
18. Franke, J., Podgorski, G. J. & Kessin, R. H. (1987) *Dev. Biol.* **124**, 504–511.
19. Lacombe, M. L., Podgorski, G. J., Franke, J. & Kessin, R. H. (1986) *J. Biol. Chem.* **261**, 16811–16817.
20. Boudreau, R. J. & Drummond, G. I. (1975) *Anal. Biochem.* **63**, 388–399.
21. Holden, D. & Leong, S. (1989) *EMBO J.* **8**, 1927–1934.
22. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.
23. Dewitt, R. J. W., Hekstra, D., Jastorff, B., Stec, W. J., Baraniak, K. J., van Driel, R. & van Haastert, P. J. M. (1984) *Eur. J. Biochem.* **142**, 255–260.
24. Otte, A. P., Plomb, M. J. E., Arents, J. C., Jansens, P. M. W. & van Driel, R. (1986) *Differentiation* **32**, 185–191.
25. Rossier, C., Gerisch, G. & Malchow, D. (1978) *J. Cell Sci.* **35**, 321–338.
26. Town, C. D., Dominov, J. A., Karpinski, B. A. & Jentoft, J. E. (1987) *Dev. Biol.* **122**, 354–362.
27. Firtel, R. A. & Chapman, A. L. (1990) *Genes Dev.* **4**, 18–28.
28. Cherry, J. R., Johnson, T. R., Dollard, C., Shuster, J. R. & Denis, C. L. (1989) *Cell* **56**, 409–419.
29. Rutherford, C. L. & Brown, S. L. (1983) *Dev. Biol.* **96**, 296–303.
30. Raper, K. B. (1940) *J. Elisha Mitchell Sci. Soc.* **56**, 241–282.
31. Bonner, J. & Slifkin, M. K. (1949) *Am. J. Bot.* **36**, 727–734.
32. Theibert, A. & Devreotes, P. N. (1984) *Dev. Biol.* **106**, 166–173.
33. Schaap, P. & Wang, M. (1986) *Cell* **45**, 137–144.
34. Pan, P., Boner, J. T., Wedner, H. J. & Parker, C. W. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 1623–1625.
35. Brenner, M. (1977) *J. Biol. Chem.* **252**, 4073–4077.
36. Bonner, J. T. (1949) *J. Exp. Zool.* **110**, 259–271.
37. Schaap, P. (1986) *Differentiation* **33**, 1–16.
38. Simon, M. N., Driscoll, D., Mutzel, R., Part, D., Williams, J. & Veron, M. (1989) *EMBO J.* **8**, 2039–2043.
39. Faure, M., Podgorski, G. J., Franke, J. & Kessin, R. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 8076–8080.