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Inositol trisphosphate and diacylglycerol can differentially modulate gene expression in *Dictyostelium*

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ABSTRACT **We have previously shown that several genes expressed during *Dictyostelium* development could be induced in shaking culture by exogenous cAMP, even though the accumulation of intracellular cAMP was inhibited. The use of selected cAMP analogs indicated that the exogenous cAMP functioned by activating the cell surface cAMP receptor and not by interacting with the regulatory subunit of the intracellular cAMP-dependent protein kinase. Although some genes in *Dictyostelium* appear to be regulated by intracellular cAMP, these data suggest that this is not the case for all genes regulated by cAMP. Intracellular second messengers other than cAMP may, therefore, promote the expression of these other genes. Here, we have examined inositol trisphosphate and diacylglycerol as candidates for such mediators of signal transduction. We have studied three genes that exhibit disparate modes of temporal and spatial expression during development of *Dictyostelium*. In shaking cultures, maximal levels of expression of each are dependent on the accumulation of or exposure to extracellular cAMP. We show that the addition of inositol trisphosphate and/or diacylglycerol to cells in shaking culture has distinct effects on the expression of each gene and, under specific conditions, can bypass the requirement for extracellular cAMP. These data suggest that extracellular cAMP interacting with its cell surface receptor may promote synthesis of inositol trisphosphate and diacylglycerol to regulate gene expression and aspects of differentiation in *Dictyostelium*.**

Receptors on the surface of eukaryotic cells interact with specific extracellular signals to activate a variety of intracellular pathways (1–3). Activated receptors coupled to guanine nucleotide-binding regulatory proteins (G proteins) can promote the synthesis of intracellular cAMP by stimulating adenylate cyclase. The second messenger cAMP is an activator of cAMP-dependent protein kinase. Receptor–G protein complexes may also be linked to phospholipase C, a membrane-bound phosphodiesterase that cleaves phosphoinositol bisphosphate into inositol 1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG). IP_3 and DAG are themselves activators of certain intracellular signaling systems. IP_3 can mobilize Ca^{2+} from membrane pools and thereby activate a Ca^{2+}/calmodulin-dependent protein kinase; DAG can activate or down-regulate protein kinase C. DAG and inositol derivatives have been suggested to interact with other intracellular regulatory pathways (4, 5). This variety of transduction pathways points to the complexity of mechanisms through which eukaryotic cells respond to certain extracellular signals to ultimately modulate gene expression and differentiation.

Dictyostelium is an excellent organism for studying cellular responses to transmembrane signaling (6, 7). Soon after the initiation of development an intercellular signaling system becomes established. Gradients of extracellular cAMP are secreted and receptors specific for cAMP appear at cell surfaces. The *Dictyostelium* cAMP receptor is structurally related to the family of cell surface receptors that interact with G proteins to stimulate intracellular signaling pathways (8, 9). In *Dictyostelium* stimulation of receptors promotes a chemotactic response as well as the activation of adenylate cyclase. cAMP is synthesized and secreted to propagate the original extracellular cAMP signal. Cells then become transiently unresponsive to continued stimulation by cAMP. Extracellular levels of cAMP decline, cells regain responsiveness, and another gradient of extracellular cAMP is established. Several hours into development pulsed waves of cAMP propagate from aggregation centers at 6- to 10-min intervals; later in development, higher concentrations of extracellular cAMP may accumulate during the differentiation of prestalk and prespore cells (10). It should be emphasized that in *Dictyostelium* extracellular cAMP is a functional analog of molecules involved in hormonal and sensory stimulation in vertebrate systems. In contrast, intracellular cAMP in *Dictyostelium*, which also accumulates during development, is a true second messenger. These various classes of cAMP pools differentially affect expression of specific genes (11–13).

In addition to the fluctuations observed for cAMP in *Dictyostelium*, other intracellular messengers, such as IP_3, Ca^{2+}, and cGMP, accumulate after cAMP stimulation of its cell surface receptor (6, 7, 14). These and other data suggest that by analogy with vertebrate systems there is a receptor–G protein-coupled stimulation of phospholipase C to produce IP_3 and DAG. Further, cGMP and to an extent Ca^{2+} may not accumulate as a direct consequence of receptor stimulation but rather as a secondary response to the production of intracellular IP_3.

Differentiating *Dictyostelium* exhibit patterns of gene expression at distinct developmental stages (15, 16). Many of these changes are dependent upon cellular stimulation by cAMP (11–13, 17–21). Specific genes can be induced in shaking culture if *Dictyostelium* are exposed to cAMP. This laboratory has shown (11–13, 22) that the induction of several of these genes was not dependent upon accumulation of intracellular cAMP. These genes could be induced by added extracellular cAMP acting on the cell surface receptor in cells that were unable, either due to mutation or drug treatment, to stimulate adenylate cyclase. These data suggested that intracellular second messengers other than cAMP were responsible for modulating the expression of certain genes during *Dictyostelium* development. We now present data indicating that the second messengers IP_3 and DAG can bypass the requirement for extracellular cAMP to promote expression of these genes, suggesting a critical role for these second messengers in *Dictyostelium* development.

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Abbreviations: IP_3, inositol 1,4,5-trisphosphate; DAG, diacylglycerol; G protein, guanine nucleotide-binding regulatory protein; IP_2, inositol 1,2-bisphosphate.

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MATERIALS AND METHODS

Growth and Manipulation of Cells. Wild-type cells were used for all developmental studies on solid substrata. Axenic (Ax-3) cells (see ref. 12) were used for differentiation in shaking culture. Vegetative cells were grown to 2×10^6 cells per ml and resuspended at 2×10^7 cells per ml in DB or PDF medium (12). cAMP was added either as pulses (30 nM cAMP) at 10-min intervals for 1.5 hr after 3 hr of differentiation or as a single dose to 1 mM after 7 hr in culture. IP_3 (Amersham) was added as pulses (3 μM IP_3) with the same timing intervals that were used for cAMP pulsing. The intracellular concentration of IP_3 in *D. discoideum* is estimated to be $\approx$3 μM; a transient increase to 5.5 μM is observed after stimulation with cAMP (23). 1,2-Dioctanoyl-sn-glycerol (Avanti Polar Lipids) was added to 200 μM at 45-min intervals from 3 to 4.5 hr in culture. Unless otherwise noted IP_3 was added to cells permeabilized in 0.1% saponin (Sigma) (24). After treatment with IP_3 cells were washed into fresh buffer. Cells were inhibited for normal signaling with 20 mM succinate (pH 7.2). Succinate is reported to inhibit signal relay by restricting cAMP secretion (25).

Isolation and Hybridization of RNA. Total RNA was prepared by phenol or guanidine hydrochloride extraction as described (11, 26). RNA was separated by electrophoresis in formaldehyde/agarose gels, blotted on nitrocellulose, and hybridized to radiolabeled probes (11).

RESULTS

Developmental Expression of Genes 14-E6, 10-C3, and 2-H6. We studied three genes that have distinct patterns of spatial and temporal expression (12, 15, 27–29). RNAs for each are not expressed in growing cells but accumulate during development in response to cAMP signaling. Transcripts of gene 14-E6 are found predominantly in prespore cells, 2-H6 mRNAs are enriched ($\approx$5-fold) in prestalk cells, whereas 10-C3 mRNAs are expressed at similar levels in both cell types (15, 27). In addition to distinct spatial patterns, the three genes have different temporal patterns of expression during development on solid substrata (ref. 29 and Fig. 1). Gene 10-C3 was induced at $\approx$5 hr into development, 2-H6 transcripts appeared at $\approx$10 hr, at the time of cellular aggregation, and 14-E6 mRNA appeared at $\approx$15 hr. Levels of 10-C3 and 2-H6 mRNAs decreased late in development (Fig. 1).

The expression of all of these genes can also be induced in shaking cultures (12, 15); cells inhibited in cAMP signaling (synthesis and/or secretion) express these genes only if exposed to exogenous (>100 μM) cAMP (12). Studies using analogs of cAMP indicate that interactions with the surface receptor for cAMP and not with the intracellular regulatory subunit of the cAMP-dependent protein kinase were causal in induction of expression of these genes. This suggested to us

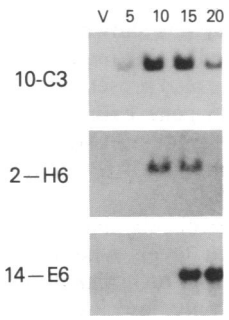

FIG. 1. Developmental expression of genes 10-C3, 2H-6, and 14-E6. Equal amounts of poly(A)$^+$ RNA from vegetative (lane V) cells and cells developed for 5, 10, 15, and 20 hr (lanes 5, 10, 15, and 20, respectively) were separated by gel electrophoresis and blotted for hybridization.

that intracellular second messengers other than cAMP were responsible for promoting the receptor-dependent expression of these developmentally regulated genes. Based upon vertebrate studies, IP_3 and DAG seemed reasonable candidates for second messengers that might mediate this regulation (2, 3).

IP_3 and DAG Can Induce 14-E6 Gene Expression. To examine the potential roles of IP_3 or DAG in regulating 14-E6 gene expression, five parallel cell cultures were established. Control cultures initially received no additional treatment. Other separate cultures received either pulses of 20 nM cAMP at 10-min intervals to mimic normal cAMP signaling, pulses of 200 μM 1,2-dioctanoyl-*sn*-glycerol for DAG, pulses of 3 μM IP_3, or a combination of DAG and IP_3. Pulse periods were chosen that are maximal for endogenous cAMP signaling during development and, hence, for IP_3 and DAG synthesis. After 7 hr each culture was split in half. One half received cAMP to 1 mM and the other received no cAMP. All of the cultures were shaken for an additional 13 hr. There was little degradation of the exogenous cAMP during this period. RNA was isolated, separated by gel electrophoresis, blotted, and hybridized with a probe specific for gene 14-E6.

Under these shaking conditions, gene 14-E6 was expressed only in control cultures exposed to exogenous cAMP at 1 mM (Fig. 2*A*). Neither endogenous nor exogenous pulsing of cAMP during early differentiation was able to promote expression of gene 14-E6 unless high continuous levels of cAMP were given later. Neither DAG nor IP_3 alone was able to remove the requirement for exogenous cAMP. Interestingly, cells treated with the combination of DAG and IP_3 in the absence of exogenous cAMP expressed 14-E6 mRNA. These results suggest that receptor-mediated stimulation of the IP_3/DAG pathway promotes the expression of gene 14-E6 during *Dictyostelium* development.

To examine the effect of IP_3 and DAG in more detail, we looked at the expression patterns of 14-E6 mRNA at earlier times. Cultures were established and RNAs were isolated at 8 and 13 hr. As is seen in Fig. 2*B*, gene 14-E6 was expressed only in 13-hr control cells exposed to exogenous cAMP. Similar data were obtained for the cultures pulsed with cAMP, DAG, and IP_3. However, the cultures treated with DAG and IP_3 accumulated 14-E6 mRNA in the absence of cAMP at a time similar to control cells and developing cells. We note that exogenous cAMP did appear to potentiate the effect of DAG and IP_3 on 14-E6 gene expression.

In a series of control experiments, we monitored the possible effects of saponin and DAG in the absence of IP_3 and of DAG and IP_3 in the absence of saponin. The results in Fig. 3*A* confirmed that only saponized cells exposed to DAG and IP_3 (lane DAG/IP_3+S) could accumulate 14-E6 mRNA. Control cells (lane C), saponized cells (lane S), saponized cells treated with DAG (lane DAG+S), and nonsaponized cells treated with DAG and IP_3 (lane DAG/IP_3-S) were unable to accumulate 14-E6 mRNA under the described conditions. We also show that the combination of DAG and inositol 2,4-bisphosphate (IP_2) (Calbiochem) had a minimal effect on the induction of 14-E6 gene expression in permeabilized cells relative to that of cultures treated with DAG and IP_3 (Fig. 3*B*). Overexposure of the autoradiograph indicated that low levels of 14-E6 mRNA accumulated in control (untreated) cultures (lane C).

IP_3 and DAG Promote Repression of Genes 10-C3 and 2-H6. The initial approach to examine the effects of IP_3 and DAG on the regulation of 10-C3 gene expression was to use the identical RNAs described for the 14-E6 gene expression studies. Genes that are not prespore-specific (e.g., genes 10-C3 and 2-H6) require lower levels of cAMP for their expression in culture than do prespore-specific genes (e.g., gene 14-E6) (19). Gene 10-C3 expression is less sensitive to subtle deficiencies in endogenous cAMP signaling; hence, these cultures are able to express gene 10-C3 in the absence

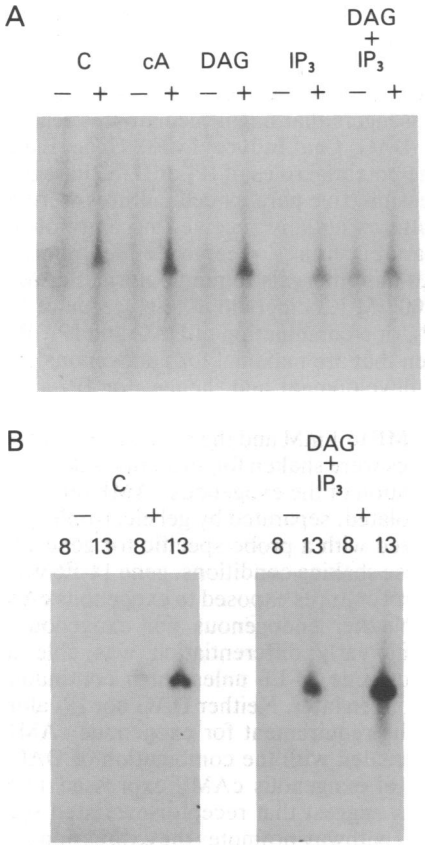

FIG. 2. Regulation of 14-E6 gene expression by cAMP, DAG, and IP_3, or both DAG and IP_3. (*A*) Cells in shaking culture received pulses of cAMP (lanes cA), DAG, or IP_3 as indicated. IP_3-treated cells were permeabilized with 0.1% saponin. Untreated control (lanes C) cultures were also included. After 7 hr cultures were split. Lanes: +, cultures received 1 mM cAMP; −, cultures received no cAMP. RNA was isolated at 20 hr and equal amounts (5 μg per lane) were blotted for hybridization to a 14-E6 probe. (*B*) RNA was isolated from control cultures (lanes C) and cultures treated with DAG and IP_3 (lanes DAG + IP_3) after 8 or 13 hr (lanes 8 and 13, respectively) in the presence (lanes +) or absence (lanes −) of cAMP.

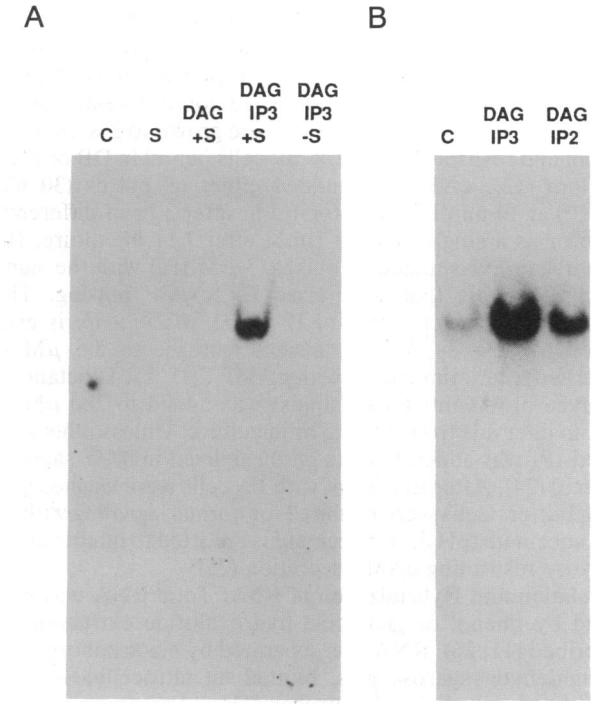

FIG. 3. Requirement of DAG and IP_3 for 14-E6 gene expression in saponized cells. (*A*) Cells in shaking culture received saponin (lane S), saponin plus DAG (DAG+S), saponin plus DAG plus IP_3 (lane DAG/IP_3+S) or DAG plus IP_3 in the absence of saponin (lane DAG/IP_3−S). Untreated control (lane C) cultures were also included. RNA was isolated at 17 hr and equal amounts (5 μg per lane) were blotted for hybridization to a 14-E6 probe. (*B*) RNA blots from control cells (lane C), saponized cells treated with DAG and IP_2 (lane DAG/IP_2), or saponized cells treated with DAG and IP_3 (lane DAG/IP_3).

of additional cAMP. However, if cells are blocked in cAMP signaling, gene 10-C3 is expressed only if the cultures are supplemented with cAMP (ref. 12; see also Fig. 6*A*). It must be emphasized that, although cells in shaking culture can be induced to express gene 10-C3, these cultures did not exhibit the developmental repression of 10-C3 gene expression that is normally seen during development.

In control and cAMP-pulsed cells, high levels of 10-C3 mRNA were observed that accumulated independently of the addition of higher cAMP levels (Fig. 4*A*). Similar mRNA levels were detected in DAG cultures; however, levels of 10-C3 RNA at 20 hr were less if the DAG cultures were exposed to continuous cAMP. Time-course studies using RNA isolated from cells after 8 and 13 hr of differentiation in culture (Fig. 4*B*) indicate that the decrease in RNA levels was not the result of a decrease in the initial accumulation of 10-C3 mRNA but rather the repression of 10-C3 gene expression subsequent to induction. Similarly, IP_3 cultures expressed low levels of 10-C3 mRNA after long periods (Fig. 4*A*) but initially accumulated 10-C3 mRNA similar to that of control cells (Fig. 4*C*). Thus repression of gene 10-C3 occurs in these cultures and mimics repression normally observed in cells that developed on solid surfaces. The combination of DAG and IP_3 gave a more complex pattern that resulted in the complete repression of 10-C3 gene expression in conjunction with cAMP (Fig. 4 *A* and *D*).

As with gene 10-C3, gene 2-H6 was expressed in cells that were able to signal normally but whose expression was dependent on exogenous cAMP in signal-inhibited cultures. Slight differences were seen with the control cultures or cultures pulsed with cAMP, DAG, or IP_3 with or without exposure to exogenous cAMP (Fig. 5*A*). Gene 2-H6 repression was observed after 20 hr in shaking cultures treated with DAG/IP_3 and subsequently exposed to cAMP (Fig. 5*A*). Time-course studies show that the initial accumulation of 2-H6 mRNA was not significantly different during early (8–13 hr) differentiation among these shaking cultures (Fig. 5*B*). These data indicate that 2-H6 gene expression can be repressed late during differentiation if shaking cultures are treated with DAG and IP_3 and subsequently exposed to cAMP (Fig. 5*A*).

IP_3 Can Induce Genes 10-C3 and 2-H6 in Signal-Inhibited Cells. This laboratory has shown (12) that genes 10-C3 and 2-H6 could be induced in shaking cultures inhibited in cAMP signaling if they were also presented with exogenous cAMP. Since 10-3 and 2-H6 gene expression in these cultures is dependent upon exogenous cAMP, these conditions would seem ideal for determining if IP_3 or DAG could bypass the requirement for cAMP.

Permeabilized control cells that signal normally expressed gene 10-C3 at similar levels whether or not high levels of cAMP were added (Fig. 6*A*). Signal-inhibited permeabilized cultures expressed only low levels of gene 10-C3 unless exogenous cAMP was added. However, if IP_3 was added to signal-inhibited permeabilized cells, then a significant accumulation of 10-C3 mRNA was observed. Although this level is less than is observed in control cells, it could not be further induced by the addition of exogenous cAMP. Thus, IP_3

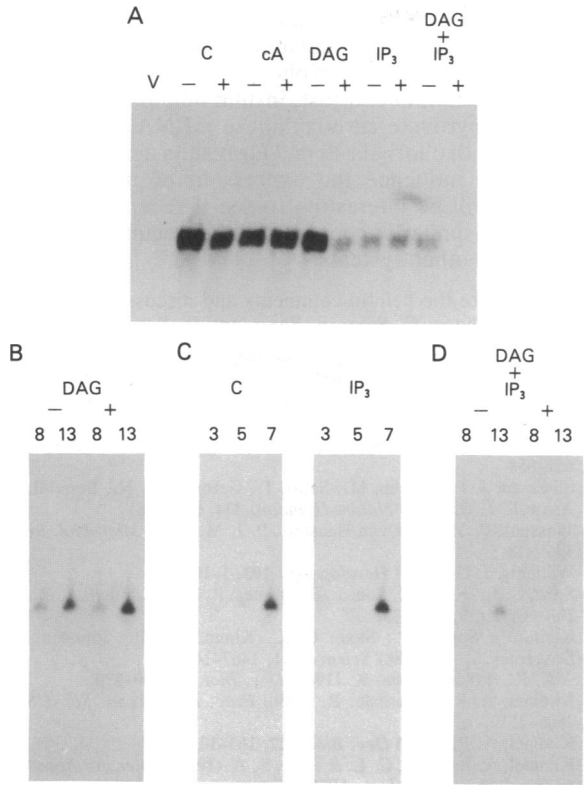

FIG. 4. Regulation of 10-C3 gene expression by cAMP, DAG, IP_3, or DAG plus IP_3. (*A*) RNA blots from cells as described in Fig. 2*A*. RNA from vegetative (lane V) cells is also included. (*B*) RNA from DAG cultures in the absence (lanes −) or presence (lanes +) of cAMP after 8 and 13 hr (lanes 8 and 13, respectively). (*C*) RNA from control (lanes C) and IP_3 (lanes IP_3) cultures after 3, 5, and 7 hr (lanes 3, 5, and 7, respectively). (*D*) RNA from cultures treated with DAG and IP_3 in the absence (lanes −) or presence (lanes +) of cAMP after 8 and 13 hr (lanes 8 and 13, respectively).

promotes the expression of gene 10-C3 in *Dictyostelium* independently of exogenous cAMP.

Similarly, 2-H6 (Fig. 6*B*) gene expression in signal-inhibited cells required exogenous cAMP. IP_3 in the absence of cAMP promoted expression of gene 2-H6 in these same

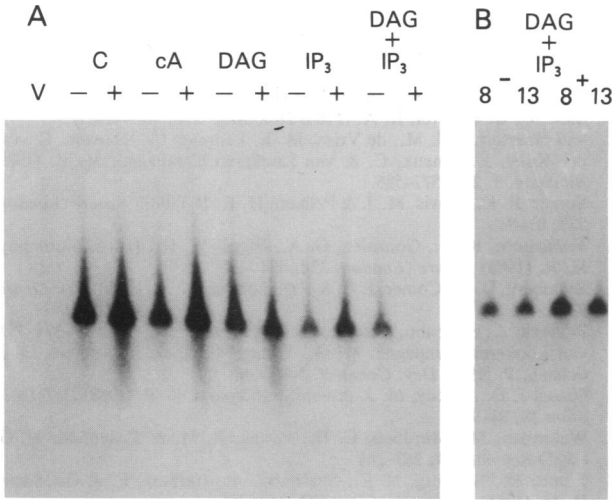

FIG. 5. Regulation of 2-H6 gene expression by cAMP, DAG, IP_3, or both DAG and IP_3. (*A*) RNA blots from cells are as described in Fig. 3*A*. (*B*) RNA from cultures treated with DAG and IP_3 in the absence (lanes −) or presence (lanes +) of cAMP after 8 and 13 hr (lanes 8 and 13, respectively; see Fig. 4*B*).

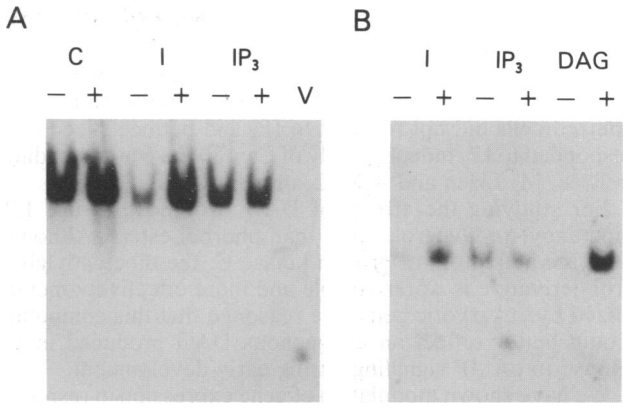

FIG. 6. Regulation of expression of genes 10-C3 and 2-H6 in signal-inhibited cells. (*A*) Gene 10-C3 expression in control (lanes C) cells, cells inhibited (lanes I) in signaling, and cells inhibited in cAMP signaling but pulsed with IP_3 (lanes IP_3). After 7 hr, cultures were split. Lanes: +, cultures received 1 mM cAMP; −, cultures received no cAMP. RNA was isolated at 20 hr and equal amounts (5 μg per lane) were blotted for hybridization to a 10-C3 probe. In addition, all cultures received 0.1% saponin. (*B*) Gene 2-H6 expression in saponized cells inhibited in signaling (lanes I) and cells inhibited in signaling but treated with IP_3 or DAG (lanes IP_3 and DAG, respectively) in the absence (lanes −) or presence (lanes +) of cAMP (see *A*).

cultures. Again the addition of cAMP did not increase 2-H6 mRNA levels. A similar experiment performed with DAG indicates that DAG alone could not induce 2-H6 gene expression but could potentiate its expression in the presence of added cAMP. Consistent with previous results, IP_3 and DAG are unable to promote 14-E6 gene expression without cAMP (Fig. 2 and ref. 28).

DISCUSSION

We have demonstrated that exogenous IP_3 and DAG modulate gene expression in shaking cultures of *Dictyostelium*. These data suggest that these signals act as bona fide intracellular second messengers, mediating the action of extracellular cAMP to promote developmentally regulated gene expression. Several critical points of control and experimental design must be detailed to substantiate these conclusions.

IP_3 is a highly charged molecule to which cells are not normally permeable (24). Therefore, we permeabilized cells prior to addition of IP_3 by treatment with saponin. Permeability was monitored with vital dyes. We (A.R.K. and K. Weijer, unpublished observations) have kept *Dictyostelium* cells in saponin solutions for >15 hr and observed little loss in viability or compromise in ability of cells to develop. In the experiments described here, cells were exposed to saponin for only 1.5 hr. The IP_3 concentration chosen is able to stimulate the synthesis of cGMP in *Dictyostelium in vivo* (24). We also monitored the ability of cells to metabolize exogenously added IP_3. [^{3}H]IP_3 was added to permeabilized and nonpermeabilized cells, extracts were taken at various times, and IP_3 metabolites were monitored by HPLC (A.R.K. and D. Cooper, unpublished observations). No significant metabolism of exogenous IP_3 was observed in nonpermeabilized cells. In contrast, saponin-treated cells were able to metabolize IP_3. These metabolites matched those that are synthesized by *Dictyostelium* (ref. 30; D. Cooper and A.R.K., unpublished observations).

The clearest sets of controls for the effect of saponin on gene expression in *Dictyostelium* are shown in Figs. 3 and 5. Saponin alone or in conjunction with DAG was unable to promote gene expression. IP_2 given with DAG has a minimal, but detectable, effect on 14-E6 gene expression. We were also unable to rescue cAMP-dependent expression of gene 10-C3 with the 1,3,4-isomer of IP_3 (M. Eisen and A.R.K., unpub-

lished data). No significant differences in endogenous cAMP signaling or relay response to exogenous cAMP were observed in saponized cells compared to untreated cells (K. Weijer, M. Eisen, and A.R.K., unpublished observations). Nonpermeabilized cells did not respond to IP_3 and permeabilized cells responded to IP_3 independently of Ca^{2+} in the culture medium (ref. 28, M. Eisen and A.R.K., unpublished data).

For studying the effects of DAG, we chose to use 1,2-dioctanoyl-*sn*-glycerol rather than phorbol esters. Although both are activators of protein kinase C, the dioctanoylglycerol derivative is water-soluble and more effectively metabolized by eukaryotic cells. We reasoned that this compound would better reflect an endogenous DAG produced in response to cAMP signaling during early development.

We have shown modulation of gene expression in response to IP_3 or DAG and suggest that this reflects the use of these signal molecules during normal development. This laboratory has shown (11, 12) that the synthesis of intracellular cAMP was coupled to the developmental repression of another gene in *Dictyostelium*. Thus these findings define cAMP, IP_3, and DAG as three intracellular second messengers that differentially mediate the action of extracellular cAMP during development. Their mechanisms of action are not completely understood; they would appear to directly or indirectly regulate a series of protein kinases, such as the cAMP-, the Ca^{2+}/calmodulin-, and the cGMP-dependent protein kinases as well as protein kinase C (3). Alterations in protein phosphorylation patterns may ultimately modulate gene activity, as described for several eukaryotic transcription factors (31, 32). Alternatively, both DAG and inositol derivatives may regulate other cellular processes (4, 5).

Presently it remains unclear how enhancing intracellular signaling early in development affects subsequent gene regulation. With gene 14-E6, we are clearly not observing an immediate effect at the nuclear level. However, it is likely that signaling in shaking cultures may not be as compietely effective as in cells developing on a solid substratum but that it can be complemented by treating the cultures with high continuous levels of cAMP. The addition of IP_3 and DAG in the absence of cAMP may overcome a potential defect in signaling. Thus, it should be emphasized that we have been able to bypass the dependence of expression of certain genes on extracellular cAMP by focusing on the initial events of signaling. Not surprisingly, each gene we examined possesses a distinct expression pattern and differential response to DAG and/or IP_3, suggesting distinct intracellular regulatory pathways for each. Whether this is related to their specific temporal or spatial patterns is not yet known. It also remains to be determined if other genes within these expression classes are similarly regulated by DAG and IP_3. Our data also indicate a complex interrelationship among cAMP, DAG, and IP_3. Consistent with this, changes in gene expression after treating cells later in *Dictyostelium* development with cAMP and altering intracellular levels of Ca^{2+}, phorbol esters, or inositol derivatives have been observed (28, 33–35). It has been suggested that an elevated intracellular Ca^{2+} level promotes prespore-type gene expression whereas a submaximal level of Ca^{2+} leads to expression patterns more characteristic of prestalk cells (36).

In other organisms, cAMP and DAG are known second messenger regulators of many hormonally controlled genes. These include, for example, the genes for somatostatin, prolactin, human growth hormone, proenkephalin, metallothionein, phospho*enol*pyruvate carboxykinase, and the human vasoactive intestinal polypeptide (32, 37–41). In some cases the second messengers are believed to directly activate protein kinases to modulate gene expression or to act through other effectors, such as Ca^{2+} (32, 37). Additionally, some genes are under multihormonal control (40). For instance, glucagon stimulates intracellular synthesis of cAMP that is an apparent positive regulator of phospho*enol*pyruvate carboxykinase expression; in contrast, insulin inhibits expression of phospho*enol*pyruvate carboxykinase mRNA. A similar effect is seen with phorbol esters. Our results demonstrate that IP_3 can also influence the expression of the eukaryotic genome. It will be interesting to see if receptor-linked IP_3 synthesis is similarly coupled to the regulation of gene expression in other systems.

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