Cell differentiation in Dictyostelium results in the formation of two cell types, stalk and spore cells. The stalk cells undergo programmed cell death, whereas spore cells retain viability. The current evidence suggests that stalk cell differentiation is induced by Differentiation Inducing Factor (DIF), while spore cell differentiation occurs in response to cAMP. We have discovered the first developmentally regulated Dictyostelium gene, the glycogen phosphorylase gene 2 (gp2) gene, that can be induced by both DIF-1 and cAMP, suggesting the possibility of a new group of developmentally regulated genes that have DIF-1 and cAMP dual responsiveness. The gp2 gene was found to be expressed in both prestalk/stalk cells and prespore/spore cells. The DIF-1 competence of the gp2 gene required uninterrupted development, whereas the cAMP-competence for the gene required only starvation. Both DIF-1 and cAMP induction of the gene could be inhibited by NH3, a factor that is thought to act as a developmental signal in Dictyostelium. Another developmental signal, adenosine, was found to repress the DIF-1 induction of the gp2 gene. Two introns in the gp2 gene were examined for their involvement in the regulation of the gene, but no regulatory function was detected. A model for the regulation of the gp2 gene during the development is proposed.

Key words: Dictyostelium, differentiation, glycogen phosphorylase gene, cAMP, DIF
been observed that initial prespore cell differentiation could be facilitated by NH₃ (Gross et al., 1983) and the expression of some prespore-specific genes could be enhanced by this molecule (Oyama and Blumberg, 1986b). However, it has also been reported that NH₃ did not appear to facilitate preferentially the expression of either the prespore marker gene D19 or the prestalk marker genes pDd63 and pDd56 (Berks and Kay, 1990).

Adenosine is another molecule that has been identified as a developmental signal in Dictyostelium. Adenosine appears to play an antagonistic role to cAMP, in that, during aggregation, it inhibits the binding of cAMP to the cAMP receptor (Theibert and Devreotes, 1984; Van Lookeren Campagne et al., 1986). Depletion of adenosine in intact slugs induces conversion of prestalk cells to prespore cells (Schaap and Wang, 1986).

In this report, we test the effect of DIF-1, cAMP, NH₃ and adenosine on the glycogen phosphorylase-2 gene. Glycogen degradation is essential for synthesizing the structural end products of differentiated Dictyostelium cells (Gustafson and Wright, 1972). The first step of the degradation is catalyzed by glycogen phosphorylase. Two glycogen phosphorylase isozymes (Brickey et al., 1990; Cloutier and Rutherford, 1987), which are encoded by two distinct genes (Rogers et al., 1992; Rutherford et al., 1992), have been found in D. discoideum. During vegetative growth, only glycogen phosphorylase-1 (gp1) is active. Gp1 enzyme activity decreases during differentiation and becomes inactive after 20 hours of development. The activity of glycogen phosphorylase-2 (gp2), on the contrary, is undetectable in vegetative cells, but appears at 8 hours and reaches a maximal level at 20 hours of development. Northern analysis of the gp2 gene showed that the gp2 gene can be induced by cAMP in a shaking cell suspension (Rutherford et al., 1992; Sucic et al., 1993). Involvement of other developmental signals in the gp2 gene regulation is expected, due to the assumption that proper temporal and spatial gene expression triggers are required during development. Due to the lack of data on gene regulation through the interactions of DIF, cAMP, NH₃ and adenosine, and lack of direct evidence that NH₃ and adenosine participate in gene regulation in Dictyostelium, investigating the effects of the four developmental signals on the gp2 gene was especially interesting. We report here that: (1) the gp2 gene is both DIF-1 and cAMP-inducible, (2) the DIF-1 induction of the gp2 gene requires prior cell-cell contact, while the cAMP induction does not, (3) DIF-1 inhibits the cAMP induction of the gp2 gene, (4) NH₃ inhibits both DIF-1 and cAMP induction of the gp2 gene and (5) adenosine inhibits the DIF-1 induction but not the cAMP induction of the gp2 gene. A model of the gp2 gene regulation during development is proposed.

MATERIALS AND METHODS

Plasmid construction

A multistep process was used to construct fusions of gp2 cDNA/or genomic region to a luciferase reporter gene. A cDNA fragment of the gp2 gene (corresponding to the genomic region +14 to +1012 bp from the translational initiation codon) was obtained by synthesizing the antisense strand of the cDNA by reverse transcription. Total RNA from slug stage cells and a primer corresponding to the region +14 to +34 were used in this process. The synthesis of the sense strand cDNA was done with Taq polymerase and a primer corresponding to the region of +991 to +1012 bp in a thermal cycler. The PCR amplification of the double-stranded cDNA was performed using the same two primers described above. The amplified cDNA was then cloned into pBluescript II SK+ (Stratagene) at the EcoRI site through EcoRI recognition sequences in the primers. A 5’ gp2 gene DNA fragment (∼1216 to +20 bp) from a gp2 genomic clone was fused to the cDNA fragment at the RsaI site (+16 to +20 bp) so that the connection between the promoter and the coding region is exactly the same as in the native gp2 gene. The orientation of the gp2 insert was then reversed at the flanking EcoRI sites. This provided a BamHI (5’)- HindIII (3’) gp2 fragment for the in-frame fusion with the luciferase reporter gene. The fusion was done with the BamHI-HindIII double-digested vector backbone of the plasmid Pha1.4LO0F (a generous gift from R. Firtel). The final construct was named pCL48-1. To construct a parallel plasmid to pCL48-1, but with the coding region containing the two gp2 introns, a gp2 genomic DNA fragment was amplified by PCR using the same pair of the primers as described for the gp2 cDNA amplification above. The amplified genomic DNA then was processed exactly as described above for pCL48-1. This generated a final plasmid construct named pGL16, which is exactly the same as pCL48-1 except it contains two introns.

For the gp2-lacZ fusion, a Kpn1 (5’)-BamHI (3’) fragment containing the same gp2 gene fragment as described for the two gp2-luciferase constructs was fused in-frame to the E. coli lacZ reporter gene in the Kpn1-BamHI double-digested lacZ reporter vector pDdGal-16 (a generous gift from A. Harwood and R. Kessin). The resulting two parallel plasmids pCZ28 and pGZ27 are exactly the same except the former lacks introns and the latter contains the two gp2 introns.

Transformation

Transformation was performed as described previously (Nellen et al., 1984), with a few modifications. After 2 days of G418 selection at 20 µg/ml in H5L, the recipient AX3K cells were plated on DM agar (Podgorski and Deering, 1980) containing 60 µg/ml of G418 in association with E. coli B/r 1-1 cells (a G418 resistant strain) (Hughes et al., 1992). Single colonies of transformants that formed on the agar plates were streaked on the same DM/G418 agar to ensure obtaining pure, independent clones.

Cell growth

All transformants were first grown on DM plates (containing 60 µg/ml of G418) on E. coli B/r 1-1 cell lawn. The vegetative cells then were transferred to axenic medium H5L (Firtel and Lodish, 1973) containing 5 µg/ml of G418. The growth temperature for all cultures was 21°C.

Shaking conditions

Cells used for shaking experiments were predeveloped on MES-LPS (7 mM MES, 20 mM KCl, 5 mM MgSO₄, pH 6.5) non-nutrient agar until early aggregation stage (streaming) which was either 4 hours at 21°C or 16 hours at 4°C. For the experiments with DIF including parallel samples with cAMP, NH₃ and adenosine, cells were predeveloped on the MES-LPS agar until the loose mound stage, which was either 6-8 hours at 21°C or 24-30 hours at 4°C. After this predevelopment, the cells were harvested in MES-LPS, passed through a 18.5 gauge needle to disassociate cell aggregates, pelleted and resuspended at 2x10⁶ cells/ml in MES-LPS. A 10 µl sample of the cell suspension, with appropriate concentrations of cAMP, DIF, NH₄Cl and adenosine as mentioned in figure legends was shaken at 180 revs/minute at 21°C. TS buffer (10 mM Tris, 2 mM NaCl, 2 mM KCl, 1 mM MgSO₄, 1 mM CaCl₂; pH 7.5) was used for some of the shaking experiments with NH₄Cl.

 Luciferase assay

Cells harvested from shaken suspension were pelleted and resus-
Histochemical stain

Cells were spread on Whatman no. 1 filters supported by absorbent pads saturated with MES-LPS and developed to different stages at 21°C in the dark. Staining was performed as described previously (Dingermann et al., 1989) except the cells were treated with 0.1% NP40 for 15 minutes before the fixation step with 1% glutaraldehyde. The color development was completed in 15 to 60 minutes at 37°C.

RESULTS

Plasmid constructions

A fragment containing the entire 5′ non-coding region and 0.8 kb of the 5′ coding region of the gp2 gene from a gp2 cDNA segment was fused in frame to the luciferase reporter gene in the vector backbone of the plasmid Pha1.4L00F (a generous gift from R. Firtel). This resulted in the plasmid pCL48-1 (Fig. 1A,B). A parallel plasmid, pGL16, was constructed in the same way except that the gp2 coding region segment was incorporated. The only difference between pCL48-1 and pGL16 is that the latter construct contains two native gp2 introns. Both plasmid constructs contain the intact 5′ non-coding region of the gp2 gene. Two gp2-lacZ gene fusion plasmids, pCZ28 and pGZ27 (Fig. 1C,D), were constructed using the same gp2 gene fragments as found in the gp2-luciferase gene fusions. The only difference between the two constructs is that pCZ28 lacks all introns while pGZ27 contains both native introns of the gp2 gene. The availability of these plasmids allows us to study the regulatory sequences that direct the expression of the gp2 gene during cell differentiation.

The constructs with/without introns in both gp2-luciferase fusion and gp2-lacZ fusion, enabled us to examine the two gp2 introns for their possible roles as enhancers. The gp2 introns, like many other Dictostelium introns, are located in the 5′ region of the gene. The close proximity of the introns to the 5′ non-coding regulatory region makes them candidates for possible regulatory sequences. However, to date there has been no documented attempt to examine any Dictostelium intron for a possible regulatory function. Introns have been reported to act as transcriptional enhancers in mammals (Bornstein et al., 1988), Drosophila (Schultz et al., 1991), and Caenorhabditis elegans (Andrew Fire, personal communication). The constructs shown in Fig. 1, in which a portion of the gp2 coding region was fused to the luciferase gene, allowed us to test the stability of the luciferase mRNA and protein in Dictostelium cells, as compared to gp2-luciferase fusion constructs without native sequences attached to them. Also, the N-terminal region might contain information for correct compartmentalization and/or processing of the native gp2 protein and, if so, the fusion protein would retain these signals.

DIF-1 induces gp2 gene expression

We have shown previously by northern analysis of the gp1 and gp2 mRNAs that both genes are cAMP-inducible (Sucic et al., 1993, Rutherford et al., 1992). Gp1 protein is present only in prestalk cells of the early culmination stage (Rogers et al., 1993) and is enzymatically inactive. The gp2 enzymatic activity is not detectable in vegetative cells but appears during the later stages of development where it reaches the maximal activity at 20 hours of development. The gp2 mRNA and protein appears earlier at the aggregation stage. Proteolytic cleavage of the protein at 18 hours of development is required for activation. Because both cell types appear to require active glycogen phosphorylase for terminal differentiation, we thought it is possible that the gp2 gene is expressed in both cell types and perhaps regulated by both cAMP and DIF, the spore and stalk morphogen, respectively. We examined the gp2 gene for DIF inducibility using the gp2-luciferase gene fusion, and we employed the gp2-lacZ gene fusion to observe the expression pattern in the two cell types. To test the possible DIF induction of the gp2 gene, AX3K cells were transformed with either the pCL48-1 (without introns) or the pGL16 (with introns) construct. Two independent clones from each transformation were used; CL15-16 from the pCL48-1 transformation and GL15-16 from the pGL16 transformation. Cells that had reached early aggregation stage (streaming) on non-nutrient MES-LPS agar were dispersed at 2×10^6 cells/ml in MES-LPS buffer, either with or without 100 nM of DIF-1. The cell suspension was then incubated at 21°C on a rotary shaker at 180 revs/minute. A portion of the cell suspension was harvested at 2, 4, and 6 hours and assayed for luciferase activity. Fig. 2 shows the results from a representative experiment. Little or no luciferase activity was observed in the cells from T0 or 2 hour time points either in the presence or absence of DIF-1. However, after 4 hours, clearly higher luciferase activities were seen in the +DIF-1 cells as compared to the −DIF-1 cells and, by 6 hours, 7- to 30-fold higher activities were observed in the +DIF-1 cells. There was no significant difference between the CL and GL clones, suggesting that the introns had no effect on the DIF-1-mediated induction of the gp2 gene at the tested conditions.

It is noteworthy that the cells used in the experiments were not pretreated with exogenous cAMP prior to the exposure to DIF-1. It has been reported that the pretreatment of Dictostelium cells (Strain V12M2) with cAMP was necessary for the induction of some prestalk cell-specific genes by DIF-1 (Williams et al., 1987; Berks and Kay, 1990). We did find, however, that the cells had to reach the streaming aggregate stage (6-8 hours) before exposing the cells to DIF-1 in order to observe the DIF-1 induction.

Prerequisites for gp2 gene expression by DIF-1 and cAMP

In contrast to the DIF-1 induction requirements mentioned above, cAMP-mediated induction of the gp2 gene in cell suspension could be demonstrated with cells that had previously developed for a shorter period of time on non-nutrient agar (16 hours at 4°C or 4 hours at 21°C). To determine if the different time length requirement for the DIF-1 and cAMP responsiveness was due to the length of time that the cells were starved, or to the formation of stable cell-cell contact on a solid surface, vegetative cells from two GL clones, GL17 and GL18, were starved in shaken flasks of MES-LPS buffer at 180 revs/minute for 10 or 14 hours. Under these conditions, the cells are unable
to form stable cell contacts. The cells then were pelleted, resuspended in fresh MES-LPS buffer containing either 100 nM DIF-1 or 1 mM cAMP, then shaken for another 6 hours. Fig. 3 shows the activation of the luciferase gene by cAMP under these conditions, but not by DIF-1. This indicates that starvation is sufficient for the cAMP-mediated gp2 gene induction,
while cell-cell contact appears to be necessary for DIF-1 induction of the gp2 gene.

**DIF-1 inhibits the cAMP induction of the gp2 gene**

DIF-1 and cAMP are believed to function as antagonists during the Dictyostelium cell differentiation (Williams, 1988). The DIF-1 induction of a prestalk B cell marker gene, ecmB (pDd57), has been shown to be repressed by cAMP. A prespore cell marker gene, D19, is cAMP inducible and the induction is strongly inhibited by DIF-1 (Berks and Kay, 1990). An exception is the prestalk marker gene ecmA (pDd63). The DIF-1 induction of this gene was enhanced in the presence of cAMP (Berks and Kay, 1990). The gp2 gene, as described in this report, presents a unique situation because of its dual inducibility by both DIF-1 and cAMP. This provided the opportunity to test current ideas regarding the antagonistic nature of cAMP and DIF. We again performed shaking experiments as described above, but included both DIF-1 and cAMP in the cell suspension. Fig. 4 shows the results from a representative experiment in which 4 GL (with introns) clones were starved for 4 hours then tested. It should be noticed that, in these 4-hour cells, the gp2 gene can respond to cAMP, but not to DIF-1. Compared to the cells treated with cAMP alone, 10-fold inhibition was observed from the cells that were given a combination of DIF-1 and cAMP. The 10-fold inhibition was very consistent in five replications of the experiment. It should be noted, however, that this was only a partial inhibition of the luciferase activities of the DIF-1+cAMP cells, in that there were 5-10×10^5 LU/mg. We also tested CL clones at the same conditions (data not shown). However, no differences between the CL and GL clones were found in the experiments.

**Effects of NH3 on gp2 gene expression**

NH3 is thought to play an important role during Dictyostelium development in that it has been shown to favor spore cell differentiation in monolayer cell assay (Town, 1984; Bradbury and Gross, 1989), and to repress prestalk cell differentiation (Inouye, 1988; Wang and Schaap, 1989). Thus, NH3 is thought to antagonize DIF during cell differentiation. However, NH3 has also been reported to repress the cAMP effect during the aggregation stage (Schindler and Sussman, 1977b, 1979; Williams et al., 1984). This raises the question as to how NH3 facilitates prespore cell differentiation while it also inhibits the effect of the prespore cell morphogen cAMP (Williams, 1988).
Is the NH₃ facilitation of spore cell differentiation due to an enhancement of a cAMP effect or an inhibition of a DIF effect? Does NH₃ regulate DIF- and cAMP-mediated gene expression differently? Due to the fact that the \(\text{gp2}\) gene can be induced by both DIF-1 and cAMP, the effects of NH₃ on both DIF-1 and cAMP inductions of the gene can be studied in one system.

Fig. 5 shows that NH₃ inhibits the cAMP induction of the \(\text{gp2}\) gene. Almost no cAMP induction appeared in both GL and CL clones (data not shown for CL clones) in the presence of 20 mM NH₄Cl in TS buffer at pH 7.5, indicating that the cAMP induction of the \(\text{gp2}\) gene can be repressed by NH₃. However, when MES-LPS buffer, pH 6.5, was used instead of TS buffer, pH 7.5, there was no effect of NH₃ on cAMP induction of the \(\text{gp2}\) gene (data not shown). This pH dependence of NH₃ effects has been observed in previous studies and has been demonstrated to be due to decreased NH₃ concentration at the lower pH (Berks and Kay, 1990; Schindler and Sussman, 1977b, 1979; Williams et al., 1984). At 20 mM NH₄Cl, the concentrations of NH₃ at pH 6.5 and 7.5 are 40 \(\mu\)M and 400 \(\mu\)M, respectively (pK=9.2 for \(\text{NH}_4^+\rightleftharpoons \text{NH}_3 + \text{H}^+\)). Thus, a 10-fold change in the level of cellular NH₃ results in major changes in the ability of cAMP to regulate the \(\text{gp2}\) gene expression.

We also found that NH₃ inhibits the DIF-1 induction of the \(\text{gp2}\) gene with both GL and CL clones (data not shown for CL clones). In Fig. 6, 20 mM NH₄Cl at pH 7.5 inhibited the DIF-1 induction of the \(\text{gp2}\) gene. No NH₃ inhibition of the DIF-1 response was detected with MES-LPS buffer, pH 6.5, as seen above for the effect of NH₃ on the cAMP induction.

**Effects of adenosine on \(\text{gp2}\) gene expression**

It has been proposed that the prespore cell differentiation can be repressed by adenosine due to a decreased cell responsiveness to cAMP (Weijer and Durston, 1985; Schaap and Wang, 1986; Van Lookeren Campagne et al., 1986). Thus, adenosine is thought to be one of the factors that favor prestalk cell differentiation. The \(\text{gp2}\) gene provided a unique opportunity to test for adenosine enhancement of the DIF-1 induction and/or repression of cAMP induction of the \(\text{gp2}\) gene, as might be predicted from previous studies at the cellular level.

We found that adenosine has no effect on the cAMP induction of the \(\text{gp2}\) gene with both GL and CL clones (data not shown for CL clones). Fig. 7 shows the results from a representative experiment, in which two GL clones were treated separately with adenosine (2 mM), adenosine+cAMP (2 mM+1 mM), cAMP (1 mM), or no additions (negative control). See Fig. 2 legend for T₀ and LU/mg. Ad, adenosine.
observed but, interestingly, if both DIF-1 and adenosine were present, there was no induction by DIF-1. Thus adenosine can repress the DIF-1-mediated induction of the gp2 gene, but has no effect on cAMP induction of the gene.

The gp2 gene is expressed in both cell types

Elucidation of the DIF-1 and cAMP dual inducibilities for the gp2 gene suggested the possibility that the gene is expressed in both cell types since the two molecules are stalk and spore cell morphogens, respectively. AX3K cells were transformed with two parallel gp2-lacZ gene fusions, pCZ28 (no intron) and pGZ27 (with two gp2 introns), resulting in CZ and GZ clones. Cells from CZ and GZ clones were developed to various stages on filters before applying histochemical stain with X-gal. Fig. 9 shows examples of the staining pattern observed at the slug, culmination and fruiting body stages of development with a GL clone (data not shown for CZ clones). In numerous stages of four GZ and four CZ clones that have been studied, both prespore/spore and prestalk/stalk cells were stained, with the prespore region staining heavier than the prestalk region. The basal part of the stalk appeared to be stained heavier than the upper part of the stalk. No differences in staining pattern were observed between GZ and CZ clones at studied stages. These results show that the gp2 gene is expressed in both cell types. This agrees with the dual inducibility of the gp2 gene by both DIF-1 and cAMP. We also determined that the gp2 gene was expressed in both prespore/spore and prestalk/stalk cells. This finding is in agreement with the dual inducibility of the gp2 gene and suggests that the gp2 gene expression in prestalk/stalk cells is induced by DIF-1 and the expression in prespore/spore cells is induced by cAMP. This type of duality might also exist in other developmentally regulated Dictyostelium genes whose transcriptional or translational products have been previously detected in both cell types (Loomis, 1985; Schaap, 1986).

DIF-1-induced expression of the gp2 gene did not require pretreatment with cAMP or the presence of exogenous cAMP. Instead, cells become DIF-1 responsive if they are subjected to uninterrupted cell-cell contact for 6 hours (or more) at 21°C or 24 hours (or more) at 4°C. This finding differs from all previously reported DIF-1-inducible genes; DIF-1 induction of other genes requires pretreatment with cAMP or the presence of cAMP during the DIF-1 induction (Williams et al., 1987; Berks and Kay, 1990). Thus, different genes or gene groups might have distinct prerequisites for their DIF-1-induced expression in order to exhibit precise temporal and spatial expression along the program of development. For the gp2

**DISCUSSION**

In this study, we have found that the gp2 gene can be induced by both DIF-1 and cAMP. We also determined that the gp2 gene was expressed in both prespore/spore and prestalk/stalk cells. This finding is in agreement with the dual inducibility of the gp2 gene and suggests that the gp2 gene expression in prestalk/stalk cells is induced by DIF-1 and the expression in prespore/spore cells is induced by cAMP. This type of duality might also exist in other developmentally regulated Dictyostelium genes whose transcriptional or translational products have been previously detected in both cell types (Loomis, 1985; Schaap, 1986).
gene, cell-cell contact appears to be a prerequisite for the DIF-1 responsiveness. However, it cannot be ruled out that cAMP that accumulated from an endogenous source during the 6-hour incubation on the rotary shaker, might have participated in the DIF-1-mediated induction. Alternatively, the disruption of the cell-cell signalling in the rapid shaking experiments might not be due to the lack of cell-cell contact, but the loss of the signalling by diffusible molecules such as cAMP.

We have shown that the gp2 gene responds to cAMP earlier in the developmental cycle than to DIF-1 (see Materials and Methods). Also, starvation of the cells in shaking suspension was found to be sufficient to induce the cAMP competence for the gp2 gene induction, but was not sufficient to induce the DIF-1 competence for the gene. Therefore, it is likely that the first appearance of gp2 gene expression at 6 hours (Brickey et al., 1990) is due to cAMP induction. Cyclic AMP might be the primary trigger for the gene through the aggregation stage, since the major rise in the level of DIF does not appear until formation of the tipped aggregates (Brookman et al., 1982).

According to this model, as the development proceeds to the tip formation stage, the gp2 gene in the tip (prestalk cells) would switch from cAMP-responsive to DIF-1-responsive phase, while the gene in prespore cells would continue to be induced by cAMP.

As mentioned previously, cAMP and DIF-1 are considered to function as antagonists during Dictyostelium cell differentiation (for a review, see Williams, 1988). We have also found in this study that DIF-1 partially inhibited the cAMP-mediated induction of the gp2 gene. The partial inhibition pattern between DIF-1 and cAMP has also been observed with the cell type-specific marker genes D19 and pDd56 (Berks and Kay, 1990).

As a catabolic product of protein and nucleic acid degradation, NH₃ has been shown to inhibit both the intracellular and extracellular accumulation of cAMP (Schindler and Sussman, 1977b, 1979) and to inhibit the cAMP relay (Williams et al., 1984). In the case of the gp2 gene, our data showed that 20 mM NH₄Cl, pH 7.5, repressed the cAMP-mediated induction. Due to the presence of a high concentration of exogenous cAMP (1 mM) in these experiments, reduced extracellular cAMP level and inhibited cAMP relay probably were not the factors that caused the gp2 gene inhibition. Although NH₃ inhibition of the cAMP-mediated gp2 gene induction has been found in the cells from early aggregation stage, it is possible that the inhibition is diminished in prespore cells during slug and culmination stages if the number of cAMP receptors increase during late development. This lower ratio of NH₃ to cAMP receptor at late stages may allow NH₃ to act as a down-regulator instead of a complete inhibitor. We have shown previously that, at the initial stages of the culmination process, endogenous NH₃ drops dramatically, then accumulates very quickly in prestalk/stalk cells, and more slowly in prespore cells (Wilson and Rutherford, 1978). The delayed NH₃ accumulation in prespore cells would maintain high levels of cAMP and thus result in continued cAMP activation of the gp2 gene. In the prestalk cell region, the cAMP-mediated gp2 gene induction is probably repressed due to a high NH₃ concentration and a possible low level of cAMP receptor sites.

The NH₃ inhibition of the DIF-1-mediated gp2 gene induction agrees with proposed antagonism between DIF-1 and NH₃ (Inouye, 1988; Wang and Schaap, 1989). The question arises, however, as to how gp2 gene expression and prestalk cell differentiation can be possible, if the DIF-1-mediated induction is inhibited by the high concentration of NH₃ in prestalk zone. It should be emphasized again that the cells used in the study were from early aggregation stage where the DIF-1-responsive components may just begin to appear and are at very low concentrations. Thus the NH₃ inhibition shown in the study was very strong due to the high ratio of inhibitor:target. But, as the prestalk and prespore pattern is established, it is possible that the DIF-1-responsive components in prestalk cells reach a high concentration. At this lower inhibitor:target ratio, the NH₃ is unable to shut down the DIF-1 induction completely. Thus, NH₃ may act as a down-regulator to balance the DIF-1 effects in the prestalk cells.

Adenosine has been shown to act as an antagonist to cAMP in regulating tip formation and in down-regulating the cAMP effect for prespore cell differentiation. This is due to the inhibitory effect of adenosine on the binding of cAMP to cAMP receptor (Schaap and Wang, 1986; Van Lookeren Campagne et al., 1986). Thus, adenosine was thought to favor prestalk cell differentiation in concert with DIF-1. However, there has been no direct evidence to support this idea, and the hypothesis was suggested before it was known that there are several developmentally regulated cAMP receptors. In our study, adenosine was found to inhibit the DIF-1-mediated gp2 gene induction, but had no effect on the cAMP-mediated gp2 gene induction with aggregation stage cells. This result seems to be in apparent contradiction with the proposed role for adenosine as a stalk cell morphogen. The observation that depletion of adenosine caused increased formation of tips (prestalk cells) (Schaf and Wang, 1986) might be due to the relief of adenosine-repressed DIF-1 effect rather than an effect of adenosine on cAMP-directed pathways. For the gp2 gene, adenosine had no inhibitory effect on the cAMP induction. This also seems to conflict with previous observations of the antagonistic relation between cAMP and adenosine (Schaap and Wang, 1986). However, multiple cAMP receptors have recently been discovered in different developmental stages (Johnson et al., 1992, 1993; Saxe et al., 1993), and it is possible that the signal for the cAMP-regulated gp2 gene induction is mediated through a cAMP receptor that is insensitive to adenosine inhibition.

Finally, we propose a model for the gp2 gene regulation during Dictyostelium development, based on the facts that: (1) both DIF-1 and cAMP can induce the gp2 gene expression, (2) the gp2 gene is expressed in both cell types, (3) DIF-1 reduces the cAMP-mediated gp2 gene induction, (4) NH₃ inhibits both DIF-1- and cAMP-mediated gp2 gene induction, (5) adenosine inhibits DIF-1-mediated gp2 gene induction, but does not inhibit the cAMP-mediated gp2 gene induction and (6) NH₃ is enriched in prestalk/stalk zone over prespore/spore zone during culmination (Wilson and Rutherford, 1978). The model also makes the following assumptions: (1) the cAMP receptor sites that are responsible for the gp2 gene induction are limited in the prestalk region, but are abundant in the prespore cell region, (2) the concentration of the DIF-1-responsive components that function in gp2 gene induction is very low in prespore cell region, but is very high in prestalk cell region, and (3) adenosine is enriched in prespore/spore zone over prestalk/stalk zone. In this model (Fig. 10), DIF-1 induces the
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