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Opposite effects of adenosine on two types of cAMP-induced gene expression in *Dictyostelium* indicate the involvement of at least two different intracellular pathways for the transduction of cAMP signals

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Adenosine promotes the cAMP-induced increase of mRNAs, probed with the cDNAs D11 and D14, which are preferentially expressed in prestalk cells, while it inhibits cAMP-induced prespore gene expression. Half-maximal inhibition of prespore gene expression occurs at about 300 μM, while prestalk stimulation by adenosine occurs at about 100-fold lower concentrations and requires the presence of cAMP. These results indicate that adenosine interferes with the transduction of cAMP to gene expression and suggest the involvement of two different adenosine target sites. Our data furthermore indicate that the transduction of extracellular cAMP to prespore gene or prestalk gene expression occurs via divergent pathways.

cyclic AMP; Adenosine; Signal transduction; Gene expression; (*Dictyostelium*, Prespore/prestalk)

1. INTRODUCTION

During *Dictyostelium discoideum* development, starving amoebae aggregate to form multicellular slugs which display a simple antero-posterior pattern of prespore and prestalk cells. The slugs ultimately form fruiting bodies, consisting of a droplet of spores supported by a column of stalk cells.

Several compounds have been identified which can induce cell type specific gene expression in vitro. Micromolar cAMP concentrations induce the synthesis of prespore specific gene products and of gene products which are preferentially present in the prestalk region of slugs [1-4]. A recently identified stalk differentiation inducing factor (DIF [5,6]) induces the synthesis of stalk specific proteins, which appear during early culmination [7], and the synthesis of a stalk specific mRNA, which appears after slug formation [8]. Adenosine, a cAMP hydrolysis product, is probably involved in the regulation of the prestalk/prespore pattern in slugs; this compound prevents the regulation of purified prestalk cells to normal prestalk/prespore proportions [9] and inhibits the cAMP-induced synthesis of prespore proteins [10]. It was furthermore found that a reduction of endogenous adenosine levels in slugs induces the redifferentiation of prestalk into prespore cells [10].

As a first step to investigate the mode of action of adenosine on cell type specific gene expression, we compared the effects of adenosine and cAMP on the levels of a prespore specific mRNA, probed with D19 cDNA, with the effects of these compounds on the levels of two mRNAs, probed with respectively D11 and D14 cDNA, which are preferentially present in prestalk cells of slugs [11].

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2. MATERIALS AND METHODS

2.1. *Materials*

[α-^{32}P]dCTP (3000 Ci/mmol) was obtained from Amersham (England) and Gene Screen was from New England Nuclear (USA). The cDNA probes D11, D14 and D19 were kind gifts from Dr S. Cohen.

2.2. *Culture conditions*

D. discoideum NC4 was cultured on glucose-peptone agar in association with *Escherichia coli* 281 [12]. Vegetative cells were separated from bacteria by repeated washing with 10 mM Na/K phosphate buffer, pH 6.5 (PB). Aggregation competent cells were obtained by starving vegetative cells at 2×10^6 cells/cm^2 on non-nutrient agar (1.5% agar in PB) during 16 h at 6°C [3]. The cells are then confined within aggregation territories, but have not yet started to aggregate.

2.3. *Incubation conditions*

Aggregation competent cells were resuspended in PB to 5×10^6 cells/ml. The cell suspension was shaken at 150 rpm and 21°C. Cyclic AMP was added every hour as a single dose, adenosine being added at the onset of the incubation period. At 2 h intervals, the cells were collected by centrifugation (1 min, $150 \times g$) and resuspended in fresh PB, or PB with respectively cAMP and/or adenosine. This was done to avoid excessive accumulation of cAMP hydrolysis products or cellular secretion products in the incubation medium.

2.4. *Semiquantitative measurement of cell type specific mRNA levels*

Cytoplasmic RNA was isolated from 3×10^7 cells and purified by phenol extraction and ethanol precipitation [13]. RNA (20 μg) was size fractionated on 1.5% agarose gels, containing 2.2 M formaldehyde, and transferred to Gene Screen [14]. Northern transfers were hybridized at 41°C in 50% formamide and 1% SDS in 5×SSPE (0.9 M NaCl and 5 mM EDTA in 50 mM Na_2HPO_4, pH 7.7) to cDNA probes, which were labeled with [α-^{32}P]dCTP by means of nick-translation [15]. The blots were washed 4 times during 5 min at room temperature with 0.5×SSPE and twice during 15 min at 60°C with 1% SDS in 5×SSPE and exposed to X-ray films. The amount of ^{32}P-labeled cDNA probes hybridized to specific mRNAs was semiquantitatively determined by measuring the absorbance of specific bands on autoradiographs by means of an Ultroscan XL densitometer (LKB).

3. RESULTS

3.1. *Effects of cAMP and adenosine on prestalk and prespore mRNA levels*

The effects of adenosine on cAMP-induced differentiation reported so far occurred only at rather high concentrations (K_i about 300 μM) and were counteracted by high cAMP concentrations [10]. Therefore, we first investigated the effect of 1 and 5 mM adenosine on the increase of prestalk and prespore mRNA levels induced by fairly low cAMP concentrations (10 and 30 μM).

Fig.1 shows that the accumulation of a prespore mRNA, D19, requires the presence of cAMP and is inhibited by 1 mM adenosine and more strongly by 5 mM adenosine. The levels of the prestalk mRNA, D11, also increase during incubation with cAMP. However, instead of being inhibited by 1 and 5 mM adenosine, the cAMP-induced increase of D11 mRNA is more pronounced in the presence of adenosine. Adenosine alone does not induce an increase in D11 mRNA levels. Another prestalk RNA, D14, is already present in aggregation competent cells. D14 mRNA levels only show a moderate increase during incubation with 10 or 30 μM

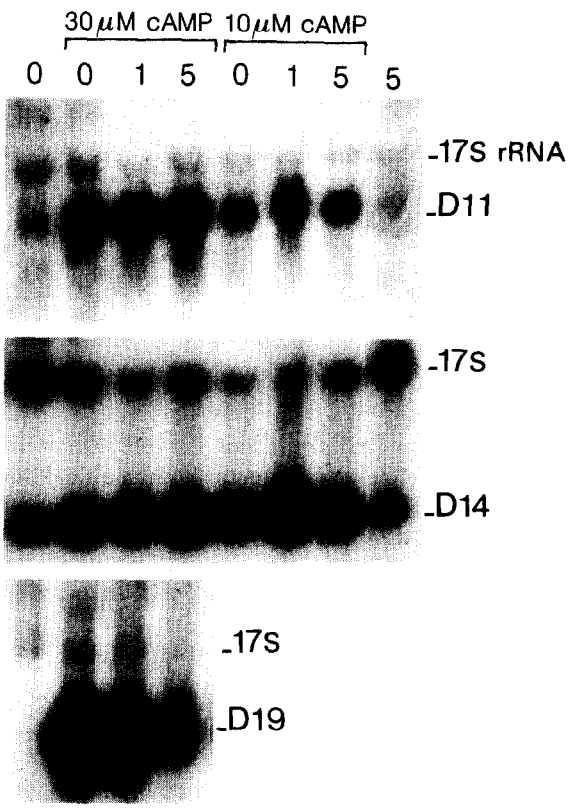

Fig.1. Effects of cAMP and adenosine on prestalk and prespore mRNA levels. Aggregation competent cells were shaken at 5×10^6 cells/ml during 6 h in PB to which 10 or 30 μM cAMP and 1 or 5 mM adenosine were added as is indicated. After 6 h cytoplasmic RNA was extracted and size fractionated. Northern transfers were probed with either D11, D14 or D19 cDNA. Similar results were obtained in two other independent experiments.

cAMP, but also in this case adenosine seems to further promote this increase. In the absence of cAMP, adenosine does not affect D14 mRNA levels.

3.2. *Dose dependency of the effects of adenosine on prestalk and prespore mRNA levels*

As an initial step to establish whether the inhibitory and stimulatory effects of adenosine on cAMP-induced mRNA accumulation are mediated by the same target site, we compared the dose dependency of the effects of adenosine on the increase in D11, D14 and D19 mRNA levels induced by 30 μM cAMP. Fig.2 shows that cAMP-induced prespore gene expression is not affected by adenosine concentrations up to 100 μM and is half-maximally inhibited at about 500 μM of adenosine. The stimulatory effect of adenosine on the cAMP-induced increase in D11 and D14 mRNA levels is already evident at 1 μM of adenosine and is saturated between 10 μM and 100 μM of adenosine. It thus appears that the adenosine concentrations required for prestalk stimulation are more than 100-fold lower than those required for prespore inhibition.

4. DISCUSSION

4.1. *Target sites for the effects of adenosine*

It was shown previously that the effects of cAMP on prespore and probably also on prestalk gene expression are mediated by a protein with the specificity of the cell surface cAMP receptor [3,4,16,17]. For adenosine, two possible cell surface target sites have been identified. A high-affinity α-receptor with a K_d of about 1 μM and a low-affinity β-receptor with a K_d of about 400 μM [18,19]. It was previously shown that adenosine in-

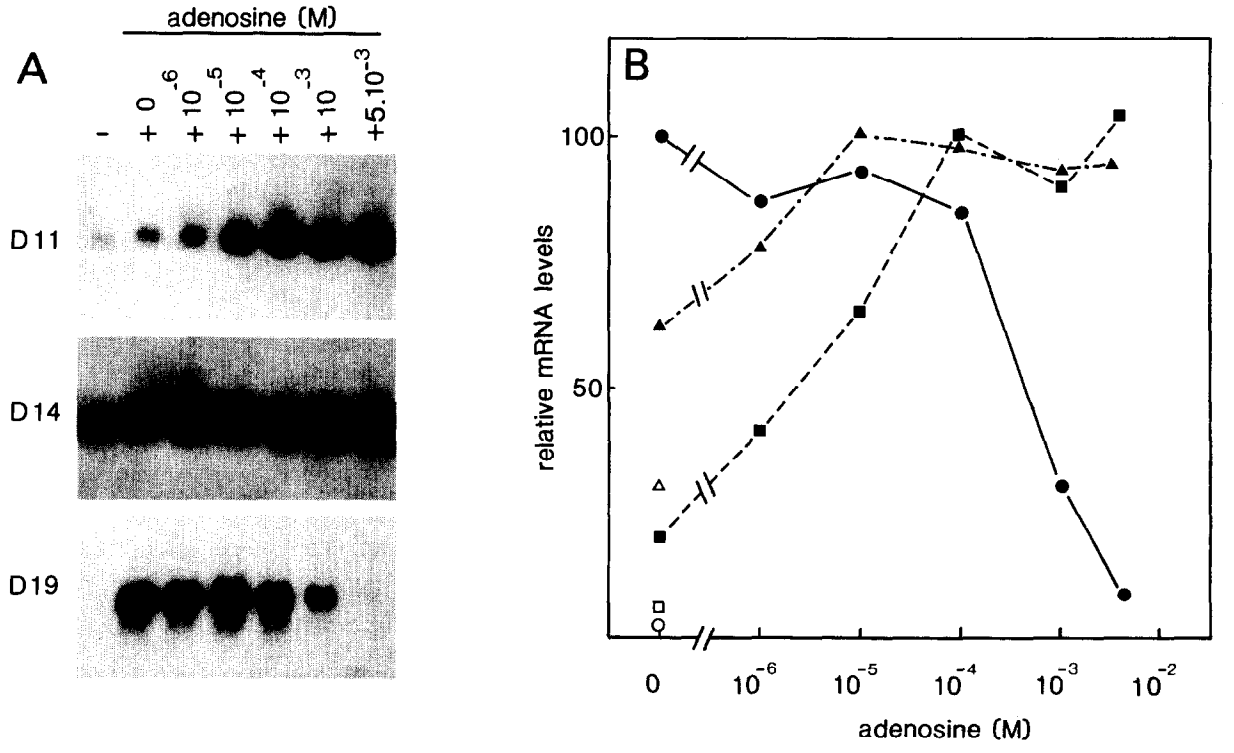

Fig.2. Dose dependency of the effects of adenosine on the cAMP-induced increase in prespore and prestalk mRNA levels. Aggregation competent cells were incubated in PB with various adenosine concentrations in the absence or presence of 30 μM cAMP (respectively indicated as − or + in A and as open or closed symbols in B). After 5 h of incubation, RNA was isolated, size fractionated and probed with D11, D14 and D19 cDNA. (A) Autoradiographs of Northern transfers of a typical experiment. (B) *A* values of densitometric scans of specific RNA bands expressed as percentage of the maximal *A* value reached during the experiment. (●,○) D19 mRNA; (■,□) D11 mRNA; (▲,△) D14 mRNA.

hibits the binding of cAMP to the chemotactic cell surface receptor [19–21]. The specificity and dose dependency of this inhibitory effect of adenosine were similar to the inhibition by adenosine of cAMP-induced prespore induction [10,22]; both inhibitory effects occurred half-maximally at about 300 μM, and were similarly sensitive to modifications in the adenosine molecule (both require an intact purine moiety). These data indicated that (i) the inhibition of cAMP binding as well as the inhibition of cAMP-induced prespore differentiation most likely result from interaction of adenosine with the low-affinity β-receptor; (ii) the inhibitory effect of adenosine on cAMP-induced prespore expression is probably caused by adenosine-induced inhibition of the binding of cAMP to its surface receptor.

The fact that stimulation of prestalk gene expression occurs at micromolar instead of millimolar adenosine concentrations indicates that this effect of adenosine is most likely not mediated by the low-affinity β-receptor, but suggests that this effect is mediated by the α-receptor with micromolar affinity.

4.2. *Implications for cAMP signal transduction*

The stimulatory effect of adenosine on the increase of D14 and D11 mRNA levels exhibits several interesting properties. Firstly, this effect of adenosine does not occur in the absence of cAMP, which suggests that also in this case adenosine interacts with the transduction of extracellular cAMP signals. Secondly, prestalk induction by cAMP is not inhibited by millimolar adenosine concentrations, which inhibit the binding of cAMP to the cell surface receptor. This means that either cAMP-induced prestalk gene expression is not mediated by surface cAMP receptors, or that this effect of cAMP is mediated by a subpopulation of surface receptors which are not affected by adenosine. In any case, the opposite effects of adenosine on cAMP-induced prespore and prestalk gene expression indicate that the transduction of cAMP to prestalk gene expression involves transmembrane and/or intracellular responses which are dissimilar to responses involved in the transduction of cAMP to prespore expression.

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