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Expression and Properties of the Regulatory Subunit of *Dictyostelium* cAMP-Dependent Protein Kinase Encoded by λgt11 cDNA Clones†

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ABSTRACT: λgt11 phages harboring five different cDNA fragments for the regulatory (R) subunit of *Dictyostelium discoideum* cAMP-dependent protein kinase (CAK) directed the synthesis of this protein in *Escherichia coli* cells. Crude bacterial extracts were probed with an antiserum against the *Dictyostelium* R subunit. The presence of specific epitopes for the R subunit in a given extract was compared with high-affinity cAMP-binding activity and with the ability to inhibit the catalytic (C) subunit through protein–protein interaction. The expression and the biochemical properties of these proteins were correlated with their cDNA nucleotide sequence. The results show that the *Dictyostelium* R subunit can be functionally expressed in *E. coli* cells either as a fusion protein with β-galactosidase or as a nonfusion protein. In both cases, the products of cDNA clones containing the entire coding sequence retained high-affinity cAMP-binding activity and the capacity to interact with the catalytic subunit. One of the fusions, lacking the 94 N-terminal residues, failed to inhibit catalytic activity, although it bound cAMP with an affinity similar to that of the native R protein from *D. discoideum*.

Dictyostelium has long been considered a model organism for the study of cell differentiation and morphogenesis [for review, see Loomis (1982)]. Moreover, the cellular slime molds may represent one of the most primitive forms of eukaryotic life, since they diverged from the main eukaryotic branch at the earliest point yet characterized by molecular phylogeny (McCaroll et al., 1983; Lane et al., 1985).

cAMP plays an important role in the control of cellular function in prokaryotic and eukaryotic cells. In bacteria, the nucleotide affects gene regulation directly via binding to the

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catabolite gene activator (CAP)[1] protein [for review, see de Crombrugghe et al. (1984)]; in eukaryotic cells, the intracellular effects of cAMP are mediated by cAMP-dependent protein kinases [for review, see Flockhart and Corbin (1982)]. These enzymes are composed of regulatory (R) and catalytic (C) subunits. In mammalian cells, the inactive holoenzyme is an R_2C_2 heterotetramer that, upon binding of two molecules of cAMP to each regulatory subunit, dissociates into an $R_2(cAMP)_4$ dimer and two monomeric catalytically active C subunits. Biochemical analysis and the determination of the amino acid sequence indicate that the functional properties of the R subunit (i.e., dimer formation, interaction with the C subunit, and cAMP binding) are contributed by structurally distinct domains on the protein (Takio et al., 1984b).

Although antisera against the R subunits from *Dictyostelium* do not react with bovine heart R subunit (Chevalier et al., 1986), functional homology of the two proteins is indicated by the observations that purified *Dictyostelium* R subunit can inhibit the catalytic activity of bovine C subunit in a cAMP-dependent manner (de Gunzburg & Véron, 1982) and that the holoenzymes from the slime mold and from mammals are activated in vitro by similar cAMP concentrations (de Gunzburg et al., 1984; Majerfeld et al., 1984).

Despite these homologies, the R subunit from the slime mold has a quaternary structure different from that of its mammalian counterparts since the holoenzyme is isolated as a heterodimer of one R and one C subunit (de Gunzburg et al., 1984). This particular structure of the *Dictyostelium* enzyme has recently been confirmed by the prediction of the primary structure of the R subunit from the cDNA sequence and has led us to propose that it was related to the phylogeny of the enzyme (Mutzel et al., 1987).

Crystallographic data on R subunits are not yet available, but theoretical considerations (Weber et al., 1987) based on comparisons of the primary structures of mammalian R subunits and the crystal structure of the bacterial CAP protein predict homologous tridimensional structures of the high-affinity cAMP-binding domains in R subunits and the low-affinity cAMP-binding domain of CAP.

The expression of a plasmid-encoded cDNA clone for bovine RI subunit has recently been reported (Saraswat et al., 1986). The ability to prepare large amounts of R subunits from phylogenetically distinct organisms should allow physicochemical studies to analyze the evolution of their structural and functional properties. We therefore analyzed the expression and the properties of λgt11 encoded cDNA clones for the *Dictyostelium* R subunit in *Escherichia coli*. Our results show that the *Dictyostelium* R subunit can be functionally expressed in bacteria either as a fusion protein with *E. coli* β-galactosidase or in its nonfusion form.

Experimental Procedures

Materials. Materials and chemicals were obtained from the following sources: M13 sequencing kit, [^{3}H]cAMP (42 Ci/mmol), [α-^{35}S]dATP (400 Ci/mmol), and [γ-^{32}P]ATP (>3000 Ci/mmol), Amersham; restriction enzymes and IPTG, Boehringer Mannheim; DEAE-Sephacel, Pharmacia P-L Biochemicals; Kemptide and bovine serum albumine (fraction V), Sigma; BA85 nitrocellulose sheets and filter circles, Schleicher & Schuell; prestained MW marker proteins, BRL. M13 mp19 sequencing primer was obtained from the Unité de Chimie Organique, Institut Pasteur. Purified *E. coli* β-galactosidase, anti-β-galactosidase antiserum, and ^{125}I protein A from *Staphylococcus aureus* were generous gifts from Dr. Nicole Guiso.

Isolation and Nucleotide Sequence Analysis of cDNA Clones. cDNA clones for *D. discoideum* R subunit were isolated and their nucleotide sequences determined as previously described (Mutzel et al., 1987). Briefly, a λgt11 cDNA library prepared from *D. discoideum* AX3 polyA RNA (Lacombe et al., 1986) was screened with specific antiserum against *Dictyostelium* R subunit as described (Young & Davies, 1983). Subcloning into M13 (Messing, 1983) and nucleotide sequence analysis according to the dideoxynucleotide chain termination method (Sanger et al., 1977) were performed as described (Mutzel et al., 1987).

Preparation of Bacterial Extracts. E. coli Y1089 cells (Δ*lac* U169, *pro*A$^+$, Δ*lon*, *ara* D139, *str*A, *hfl*A, [chr::*Tn*10], pMC9) were lysogenized either with nonrecombinant λgt11 or with λgt11 clones containing cDNA inserts as described by Huynh et al. (1984). Bacteria were screened for lysogeny by their inability to grow at 42 °C. For the preparation of crude bacterial extracts, parallel cultures of each strain were grown at 32 °C. To induce phage multiplication, the logarithmically growing cultures were rapidly heated to 42 °C and maintained at this temperature for 20 min. Then, in one of the two samples, *lac*-expression was induced by addition of 10 mM IPTG, and both cultures were further incubated at 37 °C for 30–60 min. No cell lysis was observed during this time. One milliliter samples of the cultures were centrifuged 30 s at room temperature in an Eppendorf centrifuge, and the bacterial pellets were either immediately frozen in liquid nitrogen and stored at −60 °C or resuspended in 0.5 mL 20 mM potassium sodium phosphate, pH 7.5, frozen in liquid nitrogen, and stored at −60 °C. Lysis of the bacteria occurs upon thawing, yielding a crude total extract. For assay of cAMP-binding activity and interaction with C subunit the resuspended bacteria were thawed on ice, and the lysate was used without further treatment. For immunoblots, the frozen pellets were resuspended in hot (90 °C) gel-sampling buffer (Laemmli, 1970), boiled 2 min, and subjected to SDS–polyacrylamide gel electrophoresis.

Partial Purification of the Catalytic Subunit of Dictyostelium cAMP-Dependent Protein Kinase. Crude extracts from *Dictyostelium* cells were prepared as described (de Gunzburg et al., 1984) and applied to a DEAE-Sephacel column equilibrated with 10 mM MOPS, pH 7.5, containing 2 mM EDTA. The column was briefly washed with the same buffer, and RC holoenzyme was eluted with a linear KCl gradient (0–0.3 M). Fractions containing holoenzyme (as assayed by stimulation of kinase activity upon addition of cAMP) were pooled, diluted 5-fold, and rechromatographed on a DEAE-Sephacel column in the presence of 0.1 mM cAMP to dissociate R and C subunits. Free C subunit was recovered in the flow through from this column, whereas R subunit remained attached to the ion exchanger.

Assay of Kinase Activity. The catalytic activity of *Dictyostelium* C subunit was assayed by measuring the transfer of radioactivity from [γ-^{32}P]ATP to Kemptide essentially as described (de Gunzburg et al., 1984). Assays were carried out at 30 °C in a final volume of 50 μL containing 50 mM MOPS buffer, pH 7.5, 5 mM $MgCl_2$, 0.5 mM EGTA, 20 mM NaF, 1 mg/mL bovine serum albumin, 0.2 mM Kemptide, 0.2 mM [γ-^{32}P]ATP (0.15 μCi/nmol), 20–30 milliunits of *Dictyostelium* C subunit, and the indicated additions. The

[1] Abbreviations: CAK, adenosine cyclic 3′,5′-phosphate dependent protein kinase; R subunit, regulatory subunit; C subunit, catalytic subunit; CAP, catabolite gene activator; IPTG, 1-methylethyl 1-thio-β-D-galactopyranoside; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MW, molecular weight; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

Table I: Expression of High-Affinity cAMP-Binding Activity in *E. coli* Y1089 Harboring Various λgt11 Lysogens[a]

clone	cAMP-binding activity (pmol/mg of protein)	
	−IPTG	+IPTG
λgt11	0.07[b]	0.02[b]
1.1	0.25[b]	19.9[b]
2.1	0.14[b]	14.7[b]
2.2	0.13	14.3
8.2	0.05	3.9
10.1	1.42[b]	0.9[b]

[a] cAMP binding was measured with 2×10^{-7} M [^{3}H]cAMP as described under Experimental Procedures. [b] Reproduced from Mutzel et al. (1987).

reaction was initiated by the addition of [γ-^{32}P]ATP and stopped after 15 min with 25 μL of glacial acetic acid. The phospho-Kemptide was separated from unreacted [γ-^{32}P]ATP on phosphocellulose paper and counted for radioactivity.

Assay of cAMP-Binding Activity. High-affinity cAMP-binding activity in crude bacterial extracts was measured by the filtration assay using [^{3}H]cAMP (Mutzel et al., 1987). Binding data were corrected for background radioactivity and nonspecific binding in the presence of a 10^4-fold excess of the nonradioactive ligand.

SDS–Polyacrylamide Gel Electrophoresis and Immunoblotting. Crude bacterial extracts (50 μg per lane) were chromatographed on polyacrylamide gels in the presence of 0.1% SDS as described by Laemmli (1970). Proteins were blotted from the gels to nitrocellulose by the method of Towbin et al. (1979) as modified by Kyhse-Andersen (1984). The blots were reacted with either antiserum against *E. coli* β-galactosidase or *Dictyostelium* R subunit, labeled with ^{125}I protein A, and subjected to autoradiography on Kodak XAR film. In order to remove antibodies cross-reacting with *E. coli* proteins, antiserum against R subunit was preadsorbed with a crude extract of *E. coli* BTA282 (λAp3) as previously described (Mutzel et al., 1987).

Miscellaneous. Protein was measured according to the method of Bradford (1976).

Results

Expression of λgt11 Encoded R Subunit. The expression of the protein products of five different recombinant λgt11 clones was measured in crude extracts of bacteria lysogenized with these phages. Since the cDNA inserts have been cloned into the *Eco*RI restriction site located 53 base pairs upstream from the *lac*Z translation termination signal (Huynh et al., 1984), their protein products are expected to be expressed as fusion proteins with β-galactosidase. The expression of these *lac*Z–cDNA protein fusions should be controlled by the *lac* promoter, i.e., inducible by the *lac* inducer, IPTG.

Parts A and B of Figure 1 show an analysis by immunoblotting of the expression of both β-galactosidase and R subunit in crude bacterial extracts using antisera against the two proteins. The presence of multiple bands in several lanes (see Figure 1A,B, lanes 4 and 6) results from partial proteolysis of the fusion proteins. In these cases, only the presence of the bands with the highest MW will generally be discussed.

Figure 1A demonstrates that the expression of proteins recognized by anti-β-galactosidase antiserum was *lac* dependent: neither the parental λgt11 phage nor any of the recombinants directed the synthesis of β-galactosidase without prior induction by IPTG (lanes 1, 3, 5, 7, 9, and 11). Upon addition of the inducer, antigenic material was detected in all of the extracts (lanes 2, 4, 6, 8, 10, and 12). The amount of protein varied with the strain (see Table I) but allowed detection of the high MW fusion products on Coomassie Blue stained gels (data not shown). Nonrecombinant λgt11 directed the synthesis of β-galactosidase of the same MW as the authentic protein (compare lanes 2 and 13 in Figure 1A). Clones 1.1, 2.1, and 8.2 synthesized immunoreactive material of considerably higher MW than β-galactosidase (lanes 4, 6, and 10). Although in clone 2.2 the predominant band comigrated with authentic β-galactosidase, a band of M_r 155 000 was seen on overexposed films (not shown). In contrast, clone 10.1 synthesized no antigenic material of higher MW than β-galactosidase (lanes 11 and 12).

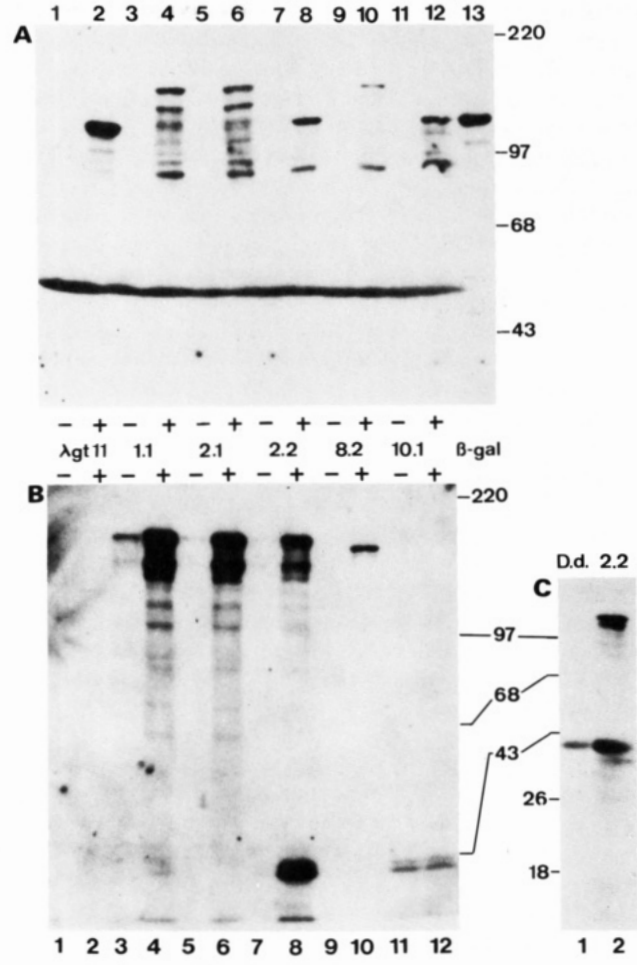

FIGURE 1: Immunoblotting of crude extracts from *E. coli* Y1089 harboring different λgt11 lysogens. Extracts from IPTG-induced (+) or noninduced (−) cultures were prepared as described under Experimental Procedures. Aliquots corresponding to 50 μg of total protein were subjected to electrophoresis on 7.5% (A, B) or 10% (C) polyacrylamide gels in the presence of 0.1% SDS and electrophoretically blotted to nitrocellulose. The blots were reacted with antiserum, labeled with ^{125}I protein A, and autoradiographed. (A) Reaction with anti-β-galactosidase antiserum. Lane 13 (designated β-gal) corresponds to 1 μg of purified β-galactosidase. (B) Reaction with anti-R subunit antiserum. (C) Comigration of the low MW band from clone 2.2 with authentic R subunit in *Dictyostelium*. (Lane 1) A crude extract from 10^6 *D. discoideum* cells starved for 8 h was prepared as in de Gunzburg et al. (1984); (lane 2) crude extract from clone 2.2 as in (B). The gel was probed with anti-R subunit antiserum.

In Figure 1B, identical immunoblots were reacted with antiserum against *Dictyostelium* R subunit. No signal was observed in extracts from lysogens of the nonrecombinant λgt11 phage (lanes 1 and 2). IPTG induction resulted in the production of large amounts of high MW immunoreactive material in clones 1.1, 2.1, 2.2, and 8.2 (lanes 4, 6, 8, and 10), whereas little or no signal was observed in these four clones without prior induction of the *lac* promoter. In addition, in

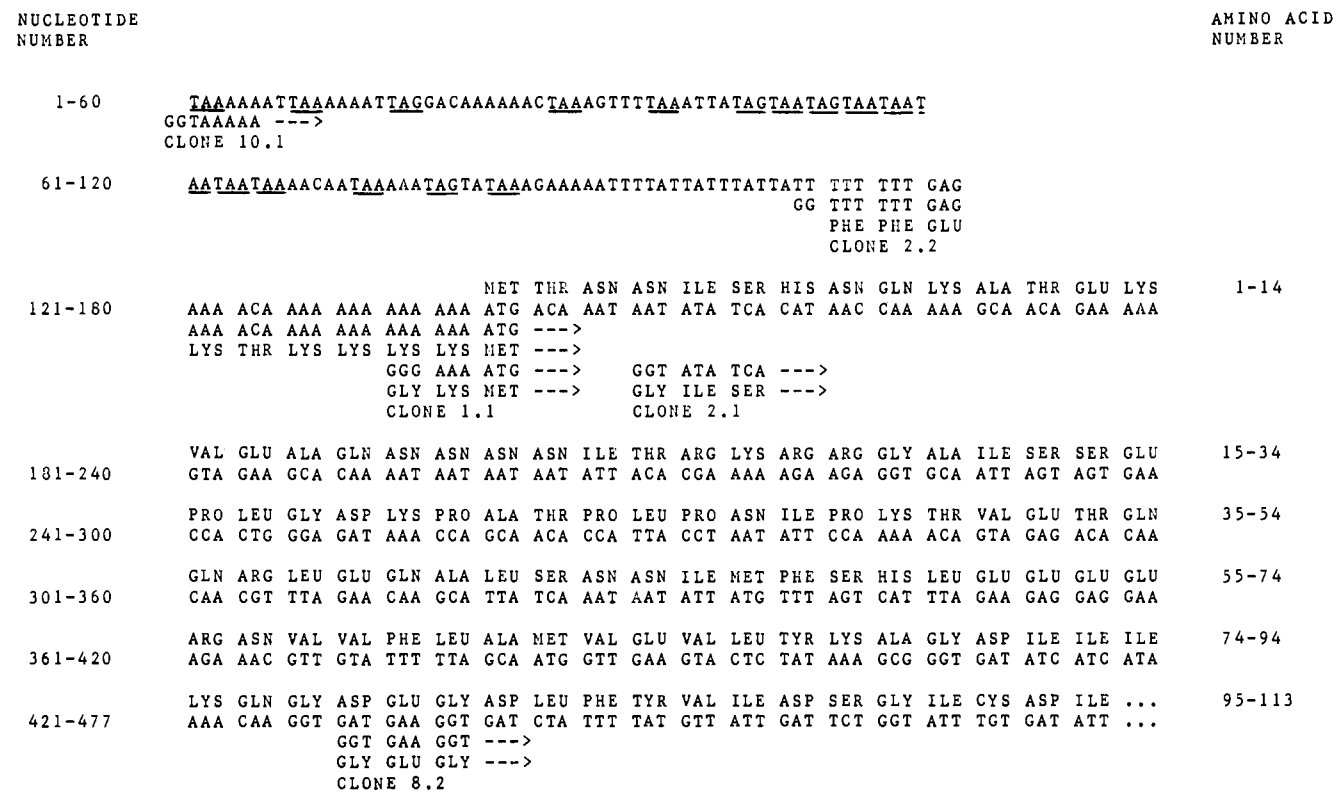

FIGURE 2: Nucleotide sequence analysis of the 5′ extremities of the λgt11 cDNA clones. The *Eco*RI inserts were subcloned into M13 mp19 and the 3′ and 5′ extremities of their DNA sequences determined according to the method of Sanger et al. (1977). All of the clones contained the 3′ end of the cDNA sequence coding for the C-terminal part of the R subunit (not shown). The corresponding predicted amino acid sequences are also shown. Non-sense codons are underlined. The entire cDNA sequence has been published elsewhere (Mutzel et al., 1987).

clone 2.2 a predominant band with lower MW was present (lane 8, Figure 1B). Figure 1C shows that this species co-migrated with the native R subunit from *D. discoideum* cells, likely resulting from specific proteolysis of the fusion protein (see below).

Expression of proteins cross-reacting with anti-R subunit antibodies was completely different in extracts from clone 10.1: low MW material was synthesized in the presence as well as in the absence of IPTG (lanes 11 and 12, Figure 1B). Even upon heavy overexposition of the autoradiograph, no high MW signal was observed, suggesting that the cDNA-encoded product in clone 10.1 is expressed in a nonfusion form and that its expression is entirely independent from that of the *lacZ* encoded product.

The expression of λgt11 encoded R subunits has also been followed by the direct measure of high-affinity cAMP-binding activity in the crude bacterial extracts before and after induction by IPTG (Table I). Comparison with the results of Figure 1B shows a close correlation between the level of cAMP-binding activity and the presence of proteins detected with anti-R antiserum.

Nucleotide Sequence Analysis of the cDNA Clones. In addition to clones 2.1 and 10.1 whose cDNA nucleotide sequences had already been determined (Mutzel et al., 1987), the 5′ extremities of the cDNA sequences of clones 1.1, 2.2, and 8.2 were analyzed. As shown in Figure 2, the 5′ ends of clones 2.1 and 8.2 are located inside the coding region for the R subunit, and their sequences are in frame with the 3′ extremity of the truncated *lacZ* gene in λgt11 (Huynh et al., 1984). This readily explains the synthesis of β-galactosidase R fusion proteins. In contrast, the 5′ ends of clones 1.1, 2.2, and 10.1 are located at different positions in the 5′ noncoding region of the R cDNA. The expression of fusion proteins between β-galactosidase and R subunit in clones 1.1 and 2.2 is most probably due to cDNA cloning artifacts resulting in deletion or insertion of one or a few base pairs: Clone 2.2 is out of frame with respect to the *lacZ* coding sequence and the 5′ end in clone 1.1 contains three guanine residues instead of the two guanines derived from *Eco*RI linker ligation (Lacombe et al., 1986).

For clones 1.1 and 2.2, the predicted amino acid sequences of the encoded fusion proteins contain an N-terminal part of β-galactosidase and the entire R subunit separated by two and nine additional amino acids, respectively (Figure 2). In clone 2.2, the additional sequence consists mostly of lysines that could form a region highly susceptible to proteolytic cleavage. This explains the presence, in extracts from clone 2.2, of proteins with the MWs of both authentic β-galactosidase and *Dictyostelium* R subunit (lanes 8 in Figure 1A,B). In contrast, the fact that no fusion protein is detected in extracts from clone 10.1 is due to multiple non-sense codons in its 5′ extremity (underlined in Figure 2) that will stop translational read-through of any fusion mRNA. Therefore, a sequence somewhere in the 5′ noncoding region of clone 10.1 has to be recognized as a translational initiation signal.

DNA restriction analysis (not shown) demonstrated that the cDNA in clone 10.1 is inverted with respect to the direction of *lacZ* transcription. cDNAs cloned in this direction have been reported to be transcribed under the control of the *lom* promoter (Chirala, 1986). This explains the lack of IPTG induction of the R–cDNA of clone 10.1 (see Figure 1B, lanes 11 and 12).

Functional Properties of the Dictyostelium R Subunit Expressed in E. coli. Since the R subunit synthesized in bacteria retained its cAMP-binding activity, it was of interest to measure the affinity for cAMP of the proteins encoded by the different λgt11 clones and to determine their capacity to interact with the catalytic subunit.

Linear Scatchard plots were obtained from cAMP-binding measurements in the crude bacterial extracts (not shown). As

Table II: Characterization of High-Affinity cAMP-Binding Activity in Crude Bacterial Extracts[a]

clone	K_D (nM)
1.1	30
2.1	45
2.2	30
8.2	19
10.1	57

[a] cAMP binding was measured in duplicate in a total of 50 μL of 20 mM potassium phosphate, pH 7.5, containing 20 μL of the extracts and 5×10^{-9} to 5×10^{-7} M [^{3}H]cAMP. Binding data were corrected for background and nonspecific binding in the presence of 10^{-4} M nonradioactive cAMP.

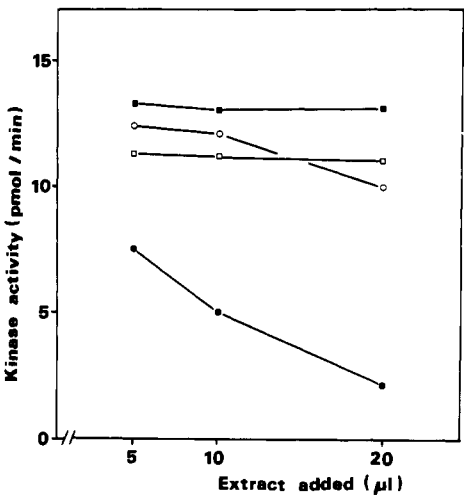

FIGURE 3: Inhibition of kinase activity by crude extract from clone 2.1. Kinase activity was measured as described under Experimental Procedures in the presence of the indicated volumes of extract from Y1089 cells lysogenized with λgt11 (□, ■) or clone 2.1 (○, ●) and with (□, ○) or without (■, ●) 0.2 mM cAMP. Assays contained 23 milliunits of kinase activity. Addition of as low as 2 μL of extract from the nonrecombinant λgt11 clone nonspecifically inhibited kinase activity by about 40%, but addition of up to 30 μL of extract from this clone did not further influence kinase activity.

shown in Table II, the K_Ds of the proteins encoded by either of the cDNA clones were all in the range of 20–60 nM, indicating that neither the fusion of R subunit with β-galactosidase (clones 1.1 and 2.1) nor the absence of the N-terminal third of the R subunit (clone 8.2) significantly affected the cAMP-binding properties of the enzyme.

Figure 3 shows that the *Dictyostelium* R subunit present in a crude extract from *E. coli* cells lysogenized with clone 2.1 inhibited the phosphorylation of Kemptide by *Dictyostelium* C subunit in a dose-dependent manner. The inhibition was abolished when 0.2 mM cAMP was added. As a control, the effect of adding increasing amounts of a crude extract from the nonrecombinant λgt11 lysogen is shown. An analgous cAMP-relieved inhibition of C activity was observed with IPTG-induced extracts from clones 1.1, 2.2, and 10.1 (data not shown). Consistent with the results described above, inhibitory activity in extracts from clone 10.1 was also present without prior IPTG induction. In contrast, no inhibition of the C subunit was observed with clone 8.2, even when high amounts of a crude extract were used, showing that the R subunit from clone 8.2, albeit cross-reacting with anti-R serum and binding cAMP with high affinity, is unable to interact with the C subunit.

Discussion

We have analyzed the expression in *E. coli* of λgt11 encoded cDNA clones for the regulatory subunit of *Dictyostelium* cAMP-dependent protein kinase and studied antigenic and biochemical properties of their protein products. When synthesized as a fusion protein with *E. coli* β-galactosidase or when present in a nonfusion form, the R subunit retained its properties, namely, high-affinity binding of cAMP and cAMP-relieved interaction with the catalytic subunit. Both functions were expressed by those λgt11 phages that contained the entire (or almost entire) coding region. The presence of a large fragment of β-galactosidase fused to the N-terminus of the R subunit from clones 1.1 and 2.1 did not influence cAMP binding and inhibitory activity as compared to the R subunit from clone 10.1, which was expressed in a nonfusion form.

The fusion protein from clone 2.2 is interesting since it is partially cleaved into β-galactosidase and R subunit. Although we have no direct evidence that the proteolytic product corresponds to the complete R subunit, this is likely to be the case since (i) it comigrates on gels with authentic R subunit, (ii) it retains both cAMP-binding activity and the ability to inhibit the catalytic subunit, and (iii) the presence of five lysine residues in the sequence joining the β-galactosidase and the R subunit in the fusion protein (see Figure 2) provides a number of putative sites of proteolysis.

In contrast, extracts from λgt11 clone 8.2, which lacks the N-terminal 94 codons of the R subunit cDNA, were unable to inhibit the enzymatic activity of the C subunit both in the absence and in the presence of cAMP. This provides direct evidence that the site of interaction with the C subunit is carried by this N-terminal part of the polypeptide. In R subunits from mammals a short amino acid sequence that could serve as the site of interaction with the C subunit has been identified on the basis of its homology with the amino acid sequences around the phosphorylated sites in substrate peptides (Titani et al., 1984) and with the heat-stable inhibitor protein of mammalian CAKs (Scott et al., 1985). This sequence in R from *Dictyostelium* would correspond to residues 27–34 (see Figure 2). The recent determination of the primary structures of R subunits from widely different organisms (Jahnsen et al., 1986; Mutzel et al., 1987; Kunisawa et al., 1987) shows a total conservation of only three amino acids (-Arg-Arg-(X_5)-Glu) in this putative interacting sequence. The lack of considerable additional homology around this site, along with the possibility to reconstitute functional hybrid holoenzymes, suggests that the element required for R–C interaction may be restricted to this short sequence. The sequences surrounding this site in the different R subunits could have evolved independently, provided that accessibility of the inhibitory site was conserved.

Given the fact that polyclonal antisera against mammalian and *Dictyostelium* R subunits do not cross-react, one would have expected the different epitopes to reside in the N-terminal parts of the molecule that share low homology. A recent study by Weldon and Taylor (1985) using monoclonal antibodies suggests indeed that the antigenic determinants of bovine RII are located in the N-terminal third of the molecule. In the *Dictyostelium* R subunit, however, there should be (a) strongly antigenic region(s) in the C-terminal two thirds, since the protein products of all of the λgt11 clones, including clone 8.2, were equally recognized by the polyclonal antiserum used in this study (see Figure 1).

The possibility of expressing functional R subunit from *Dictyostelium* (this work) and from mammals (Saraswat et al., 1986) in *E. coli* provides a tool for further studies on cyclic nucleotide-binding proteins: The synthesis of high-affinity cyclic nucleotide-binding activity directed by phage or plasmid

expression vectors may be used to detect cDNA clones for other cNMP-binding proteins by in situ binding of the nucleotide (Lacombe et al., 1987). This should be especially useful in the cases where heterologous antisera or nucleotide probes fail to detect the desired clones and homologous probes are not available. A refined analysis of the structural properties of the R subunits from lower and higher eukaryotes together with the data already available for the prokaryotic CAP protein (Weber et al., 1987) should contribute to understanding the evolution of this set of functionally related proteins.

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Registry No. CAK, 9026-43-1; cAMP, 60-92-4; β-galactosidase, 9031-11-2.

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