Isolation and characterization of cDNA clones encoding polypeptides related to a *Dictyostelium discoideum* cyclic AMP binding protein

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By screening a cDNA library with a cDNA encoding the *Dictyostelium discoideum* cAMP-binding protein CABP1, under conditions of reduced stringency, we have isolated clones which code for two closely related molecules. Hybrid selection experiments indicated that these cDNAs encoded polypeptides with molecular masses of 34 (p34) and 31 (p31) kDa, both of which were recognized by anti-CABP1 monoclonal antibodies. Sequence analysis revealed that the clones were identical except for the presence of a 102 nucleotide segment inserted in-frame in the p34 cDNAs, just downstream of the translation initiation codon. DNA blot analysis suggested that p34 and p31 were encoded by the same gene. This hypothesis was strongly supported by the observation that both polypeptides were generated when a single cDNA was expressed under the control of the actin 15 promoter in *D. discoideum* cells. RNA blot analysis indicated that the cDNAs were complementary to three developmentally regulated transcripts of sizes 1.15 kb, 1.25 kb and 1.4 kb. Comparison of the derived amino acid sequences of p34 and p31 with those of the two subunits of CABP1 indicated that these polypeptides were very closely related, and that the corresponding genes probably arose by duplication followed by sequence divergence. Finally, the carboxy termini of these four polypeptides demonstrated 50% similarity to two polypeptides encoded by a bacterial plasmid which confers resistance to tellurium anions.

Introduction

The cellular slime mould *Dictyostelium discoideum* grows vegetatively as single-celled amoebae. Starvation triggers a process of development and differentiation, which ultimately leads to the formation of a multicellular fruiting body composed of two distinct cell types, spores and stalk cells (see Loomis, 1982, for a review). The molecule cAMP plays a pivotal role in the developmental cycle of *D. discoideum*. It acts as a chemoattractant to direct the aggregation of individual amoebae after development has begun, regulates the differentiation of both spores and stalk cells, and controls the expression of many developmentally regulated genes (reviewed in Gerisch, 1987). While most of these effects are mediated

by the interaction between extracellular cAMP and a cell-surface cAMP receptor (reviewed in Firtel *et al.*, 1989), it also appears that intracellular cAMP plays an important role in some of these processes (Kimmel, 1987; Kay, 1989; Riley *et al.*, 1989).

In an attempt to identify possible pathways which could mediate the effects of intracellular cAMP on the development of *D. discoideum*, we have isolated and characterized a novel cAMP binding protein, CABP1 (Tsang & Tasaka, 1986). This protein consists of two polypeptide subunits, CABP1A and CABP1B. Western blot analysis using anti-CABP1 monoclonal antibodies indicates that there are several CABP1-related polypeptides present in developing cells (Kay *et al.*, 1987; Tsang *et al.* 1988).

Two of the polypeptides which crossreact with the anti-CABP1 antibodies are of particular interest because of their developmental regulation and intracellular localization. These molecules, which have apparent molecular masses of 34 kDa (p34) and 31 kDa (p31), are not detected in vegetatively growing cells, but appear early in development (Kay *et al.*, 1987). They are localized primarily in the nucleus (Kay *et al.*, 1987). In this paper, we describe the molecular cloning and

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characterization of cDNAs encoding p34 and p31. We show that these two polypeptides are very closely related to the two subunits of CABP1 and provide evidence that they are the products of a single gene.

Methods

Isolation of cDNA clones. A cDNA library was constructed according to standard procedures (Maniatis *et al.*, 1982) from a mixture of polyA⁺ RNAs prepared from NC4 cells that had developed for 12 and 20 h as described by Kay *et al.* (1987). The library was screened under conditions of low stringency with a nick-translated cDNA clone encoding a portion of CABP1 (Grant & Tsang, 1990) as described by Maniatis *et al.* (1982). To isolate full-length cDNAs, a λ gt11 cDNA library, kindly provided by Dr Peter Devreotes (Klein *et al.*, 1988), was screened under high stringency conditions using a nick-translated cDNA isolated from the cDNA library described above.

DNA sequencing. The nucleotide sequences of the cDNAs were determined by the chain termination method (Sanger *et al.*, 1977) using [³⁵S]dATP (Amersham) as the radiolabel.

DNA blot hybridization. Genomic DNA was isolated from strain AX2 by centrifugation through CsCl density gradients (Firtel & Bonner, 1972), and Southern blotting was performed as described by Maniatis *et al.* (1982). The filters were prehybridized and hybridized at either 37 °C (moderate stringency) or 42 °C (high stringency). They were washed briefly in 2 × SSC, 0.1% SDS at room temperature and then twice for 30 min in 2 × SSC, 0.1% SDS at 65 °C (moderate stringency) or 0.1 × SSC, 0.1% SDS at 68 °C (high stringency).

RNA blot hybridization. Five micrograms of polyA⁺ RNA isolated from NC4 cells at various times of development was fractionated on 1.5% (w/v) agarose formaldehyde gels and transferred to Genescreen membrane (New England Nuclear) (Maniatis *et al.*, 1982). The membrane was probed using the high-stringency conditions employed for DNA blot analysis as described above.

Construction of actin 15-cDNA fusion. Oligonucleotide-directed mutagenesis (Kunkel, 1985) was used to introduce a *Hind*III site at the translation initiation codon of the clone pDdM34. The presence of the desired mutation was confirmed by DNA sequence analysis (Sanger *et al.*, 1977). Digestion of the resulting plasmid with *Hind*III released a fragment containing the entire coding region of the cDNA plus 3' untranslated sequences. This fragment was then inserted into the expression vector pB10Act15BKH (obtained from Dr J. Williams, Imperial Cancer Research Fund, Clare Hall Laboratories, U.K.).

Transformation of *D. discoideum* Plasmid DNA was introduced into strain AX2 by the calcium phosphate coprecipitation technique (Early & Williams, 1987). Approximately 150 independent transformants were pooled for analysis.

Hybrid selection, cell-free translation, and immunoprecipitation. These techniques were performed as described previously (Grant & Tsang, 1990).

Metabolic labelling. Bacterially-grown cells were harvested, washed three times with KKP (20 mM-potassium, phosphate, pH 6.2), and then resuspended to a final density of 5×10^7 cells ml⁻¹ in KKP. One millilitre of this suspension was spread over the surface of a 47 mm diameter polycarbonate membrane filter (Nuclepore) supported on two layers of filter paper (Whatman no. 1) saturated with KKP. After 3 h incubation at 22 °C, the filters were transferred to a clean, dry Petri dish and 20 μ Ci (740 KBq) [³⁵S]methionine was added directly to them. After a 3 h labelling period, the cells were washed off the filters, lysed and analysed by immunoprecipitation as described previously (Tsang & Tasaka, 1986).

Fig. 1. Identification of pC5D9 by hybrid selection of RNA. PolyA⁺ RNA, isolated from cells that had developed for 17 h, was hybridized under stringent conditions to denatured, filter-bound pC5D9 DNA. After washing the filters to remove the nonspecifically bound material, RNA that had hybridized to pC5D9 was eluted and translated *in vitro* using a rabbit reticulocyte lysate. The translation products were analysed by immunoprecipitation with an anti-CABP1 monoclonal antibody followed by SDS-PAGE. As a positive control, the CABP1 cDNA pC6H6 was also used to select RNA. Lane 1, immunoprecipitated products from the translation of 1 mg polyA⁺ RNA; lane 2, no exogenous RNA added to translation reaction; lane 3, immunoprecipitated products from the translation of RNAs complementary to pC5D9; lane 4, immunoprecipitated products from the translation of RNAs complementary to pC6H6. The two subunits of CABP1, 1A and 1B, as well as p34 and p31, are labelled.

Results

Isolation of cDNAs encoding CABP1-related polypeptides

To isolate clones encoding related polypeptides, we screened a cDNA library with a 0.67 kb cDNA containing a portion of the CABP1 coding sequence (Grant & Tsang, 1990) under conditions of reduced stringency. Besides CABP1 cDNAs, this approach led to the isolation of a single related cDNA species, termed pC5D9. In order to identify this clone, messenger RNAs complementary to pC5D9 were isolated by hybrid selection, translated *in vitro*, and analysed by immuno-

Fig. 2. CABP1-related cDNAs. cDNAs isolated from the phage library were subcloned into the *Eco*RI site of the plasmid vector Bluescript. The clone isolated from the plasmid library, pC5D9, is contained in the *Pst*I site of pBR322. The open reading frame in each clone is represented by an open rectangle; untranslated sequences are indicated by a line. The restriction fragment used to probe the Southern blot (Fig. 4) is labelled probe 1, while the segment used to probe the Northern blot (Fig. 5) is designated probe 2. The strategy used to determine the nucleotide sequences of the clones is indicated by the arrows. The restriction map of the cDNA clones is located at the top of the figure. A, *Alu*I; S, *Sau*3A; B, *Bam*HI; H, *Hinc*II.

precipitation followed by SDS-PAGE. As Fig. 1 shows, under stringent conditions pC5D9 selects RNA species which encode polypeptides with molecular masses of 34 and 31 kDa, both of which are recognized by the anti-CABP1 antibody. This observation suggests that pC5D9 contains sequences complementary to the transcripts coding for p34 and p31, and that these two transcripts share substantial similarity at the nucleotide level.

The insert in pC5D9 is only about 400 nucleotides long. To isolate full-length cDNAs for p34 and p31, a λ gt11 cDNA library, kindly provided by Dr Peter Devreotes (Klein *et al.*, 1988), was screened under highly stringent conditions using pC5D9 as a probe. Twelve positive clones were obtained after screening approximately 200000 plaques.

Sequence analysis of cDNAs

To facilitate further analysis, the longest cDNAs were subcloned into Bluescript plasmids. Fig. 2 describes the resulting clones and their restriction map as well as the strategy employed to determine their nucleotide sequences.

The two longest cDNAs, pDdM34 and pDdM31, have been sequenced completely on both strands. Each clone contains only a single long open reading frame flanked by 5' and 3' untranslated sequences. The nucleotide and deduced amino acid sequences of the cDNAs are illustrated in Fig. 3.

Remarkably, pDdM34 and pDdM31 are completely identical except for the presence of a 102 nucleotide

segment located at the 5' end of pDdM34, just downstream of the translation initiation codon. This element does not disrupt the open reading frame, thereby leading to the production of a protein containing an extra 34 amino acids compared to the polypeptide encoded by pDdM31.

DNA blot analysis

Southern analysis was performed using the 3' *Bam*HI-*Eco*RI fragment common to both pDdM34 and pDdM31 as a probe (probe 1 in Fig. 2). As Fig. 4(a) shows, only a single band hybridized to the probe under conditions of high stringency for all enzymes tested. Even if the stringency of hybridization was reduced, the only additional bands recognized by this probe contain the CABP1 gene (Fig. 4b). Therefore, the transcripts complementary to pDdM34 and pDdM31 appear to be derived from a single gene.

RNA blot analysis

To examine the pattern of expression of the transcripts complementary to pDdM34 and pDdM31, polyA⁺ RNA isolated from cells at various times of development was analysed by RNA blotting. Highly stringent conditions were employed to prevent hybridization to CABP1 RNAs. As is shown in Fig. 5, three transcripts with lengths of approximately 1.15 kb, 1.25 kb and 1.4 kb were detected during growth and at all times of development, although their relative amounts were variable.

	*pDdM34										*pDdM31																			
1	GTATAAAAAAAAAAAAAAAAAAAAAAAAAACAAATAATTAAGAAA																				ATG	TAT	AAT	CCA	CCA	CCA				
											M	Y	N	P	P	P		6												
71	CCA	TCC	GGT	TCA	CAA	GGT	AAC	AAT	AAT	TAT	TAT	AGA	CAA	CCA	TCA	TCC	ACA	CCG	GGT											
	P	S	G	S	Q	G	N	N	N	Y	Y	R	Q	P	S	S	T	P	G	25										
	*pDdM115																													
128	GTT	TCT	AAC	CCA	AAT	CCA	CAA	GCT	AAT	CAA	TTT	TTA	CCA	CCT	CCA	CCT	TCT	AAT	ACA											
	V	S	N	P	N	P	Q	A	N	Q	F	L	P	P	P	P	S	N	T	44										
185	CAA	GCT	CCA	AGA	CCA	GGA	TTT	CCA	CCA	AGT	GGT	CCA	CCA	CCA	AGT	GCC	CCA	GCA	GGA											
	Q	A	P	R	P	G	F	P	P	S	A	P	P	P	S	A	P	A	G	63										
242	CAA	TAC	AGT	ATG	CCA	CCA	CCA	CCA	CAA	CAA	CAG	CAA	CAA	GCA	GGA	CAA	TAT	GGT	ATG											
	Q	Y	S	M	P	P	P	P	Q	Q	Q	Q	Q	A	G	Q	Y	G	M	82										
299	CCA	CCA	CCA	CCA	TCA	GGT	TCA	GGT	ATA	GGT	ACA	GGT	GTT	TCA	TTA	GTA	AAA	GAT	CAA											
	P	P	P	P	S	G	S	G	I	G	T	G	V	S	L	V	K	D	Q	101										
356	CAA	ATT	TCA	TTA	AGT	AAA	GAG	GAT	CCA	TAT	CTT	AGA	AAA	TTA	ACA	GTA	GGT	TTA	GGT											
	Q	I	S	L	S	K	E	D	P	Y	L	R	K	L	T	V	G	L	G	120										
	*pC5D9																													
413	TGG	GAT	GTA	AAT	ACA	ACA	CCA	AGT	GCA	CCA	TTT	GAT	TTG	GAT	GCA	GTA	GTT	TTT	ATG											
	W	D	V	N	T	T	P	S	A	P	F	D	L	D	A	V	V	F	M	139										
470	TTG	GGT	GCA	AAT	GGT	ATG	GTT	CGT	CAA	CCA	GCA	GAT	TTT	ATT	TTT	TAT	AAT	AAC	AAA											
	L	G	A	N	G	M	V	R	Q	P	A	D	F	I	F	Y	N	N	K	158										
	*pDdM114																													
527	CAA	TCT	AGG	GAT	GGT	TCA	ATC	TTT	CAT	CAT	GGT	GAT	AAT	TTA	ACA	GGT	GCA	GGT	GAT											
	Q	S	R	D	G	S	I	F	H	H	G	D	N	L	T	G	A	G	D	177										
584	GGA	GAC	GAT	GAA	GTC	GTA	TCT	GTA	AAC	TTA	CAA	GCA	GTT	TCA	CCT	GAC	GTT	ACT	CGT											
	G	D	D	E	V	V	S	V	N	L	Q	A	V	S	P	D	V	T	R	196										
641	TTG	GTT	TTC	GCC	GTC	ACC	ATT	CAT	CAA	CCT	GAA	TTA	AGA	AGA	CAA	AAT	TTC	GGT	ATG											
	L	V	F	A	V	T	I	H	Q	P	E	L	R	R	Q	N	F	G	M	215										
	*pC5D9																													
698	GTT	CCA	AGA	GCT	TTC	ATT	CGT	ATT	GCA	AAC	CAA	GAA	ACA	ACT	AGA	AAT	ATA	TGT	AGA											
	V	P	R	A	F	I	R	I	A	N	Q	E	T	T	R	N	I	C	R	234										
755	TAC	GAT	CTA	ACC	AAT	GAA	GGT	GGT	ACA	AAT	ACT	GCT	ATG	ATT	GTT	GGT	GAA	GTT	TAT											
	Y	D	L	T	N	E	G	G	T	N	T	A	M	I	V	G	E	V	Y	253										
812	CGT	GAT	CCT	CAA	AAT	CCT	CAA	AAT	TGG	TCA	TTT	ATT	GCT	GTT	GGT	AAA	TCT	TTC	CCT											
	R	D	P	Q	N	P	Q	N	W	S	F	I	A	V	G	K	S	F	P	272										
869	GGT	GGT	TTA	CAA	TTC	CTT	TGT	CAA	ATC	TTT	GGT	GTA	AAT	GCG	TCA	TAA	ATTTTTTAATT													
	G	G	L	Q	F	L	C	Q	I	F	G	V	N	A	S					287										
	*pDdM115																													
928	TTAATTTTTCTTAGGCATTTTTGGCAAATTAGTTGGTTGGAATTTTCACTCATAATTTTGTATACAGATATTTT																													
	pDdM34*										*pDdM31										pDdM114*									
1003	TTTTTATTCCACATAACACAATAACAATCACTTTTTTTGTTCTAAAATTAAAAAAAAAAAAAAAAAAAAA																													

Fig. 3. Nucleotide and deduced amino acid sequences of cDNAs. The sequence of the two longest cDNAs, pDdM34 and pDdM31, was determined completely on both strands. The remaining clones have not been sequenced entirely on both strands. The 5' and 3' borders of all cDNAs are indicated. The 3' end of pC5D9 was not determined. The border that is marked represents the 3' end of our sequencing data for this clone. The sequence which is underlined represents the 102 nucleotide element which is absent from pDdM31. Nucleotide numbers are on the left while amino acid numbers are on the right.

Fig. 4. DNA blot analysis. (a) Genomic DNA digested with a variety of enzymes, size-fractionated on an agarose gel, transferred to a nylon membrane, and then probed under conditions of high stringency with a restriction fragment common to both pDdM34 and pDdM31 (probe 1 in Fig. 2). E, *EcoRI*; D, *DraI*; H, *HinfI*; C, *ClaI*; X, *XhoII*. (b) Genomic DNA digested with *DraI* (D) or *HinfI* (H) and blotted as above. Identical blots were hybridized with either the probe used above or else with a restriction fragment corresponding to the 3' half of CABP1 under varying degrees of stringency as described in Methods.

Comparison of polypeptides encoded by pDdM34 and pDdM31 to CABP1 and related molecules

As described earlier, the polypeptides encoded by pDdM34 and pDdM31 are identical except for the presence of a 34 amino acid segment inserted near the amino terminus of the larger molecule. Similarly, we

Fig. 5. RNA blot analysis of the expression of RNAs complementary to pDdM34 and pDdM31. PolyA⁺ RNA isolated from cells which had developed for 0, 9, 12, or 16 h was fractionated by electrophoresis through an agarose formaldehyde gel and transferred to nitrocellulose. The blot was probed under stringent conditions with a restriction fragment from the cDNA clone pDdM115 (probe 2 in Fig. 2). The sizes of the transcripts which reacted with the probe are indicated.

have found that the two subunits of CABP1 are also identical, except for the presence of an additional 37 amino acids at the amino terminus of CABP1A. We have recently demonstrated that these subunits are the products of a single gene (Grant & Tsang, 1990), and that they are generated by an alternative splicing mechanism (Grant *et al.*, 1990). An alignment of the deduced amino acid sequences of the polypeptides encoded by pDdM34 and pDdM31 with those of CABP1A and CABP1B is shown in Fig. 6.

The similarity between these four molecules is remarkable. The amino-terminal sequences are virtually identical, with only two amino acid differences in the first 45 residues. Significantly, the sequence which is specific to the polypeptide encoded by pDdM34 almost exactly matches the region of CABP1 which is found only in CABP1A. Furthermore, the nucleotide sequence encoding this element is flanked precisely by the splice consensus signals GT/AG, suggesting that the transcript which is complementary to pDdM31 is generated by a splicing process very similar to the one which produces CABP1B (Grant *et al.*, 1990).

Immediately following this highly conserved amino terminus, the polypeptides diverge considerably. This region in CABP1, which is 94 amino acids in length, is very distinctive in its amino acid content. It contains a high proportion of proline and glutamine residues, as well as five copies of the nine amino acid repeat QPAGQYGAP (Grant & Tsang, 1990). In the molecules encoded by pDdM34 and pDdM31, however, this region contains only 46 residues. Even though it is considerably shorter than the same element in CABP1, its overall character remains very similar since it is also very rich in

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CABP1      MYNPPPPSGSQGNNNYRQPSSTPGVSNPNPQANQFLPPQPSNTTQTGGYPPQQQ
p34/31      .....P.....QAPRP.F.---

QRPTGAPQQPGGYPTPPPPGAPGGYPPQQQAGQYGAPPQQQAGQYGAPQAGQ
-----S...PSA.....SM.P.PQ.

YGAPQAGQYGAPQAGQYGAPPPPPGGAGISLVKNQQLSLTKEDPTLRKLTIGLG
---Q.Q.....M.P.-----SGSGI.T.V....D....S....Y....V...
ORF4                               MSV..S.GGNV..S.TA.SMKNVLV...
ORF5                               MAV....GGNV.....A.SMNVALV...

WDVNTTPTAPFDLDAVVFMLNAQGRVRSQDFIFYNNKVS RDNSVSHQGDNL TGQG
.....S.....G.N.M..QPA.....Q...G.IF.H.....A.
..ARS.DGQD.....SA.L.A.N.K..GDA.....LK.A.G..T.T...R..E.
..TRV.DGQA.....S..LVGEN.K.LSDSH.V....TT.P.GA.Q.....R..E.

EGDDEVVLVNLQAVSPDVTRLVFAVTIHLADERRQNFTMVPRAFIRVANQETGRNI
D.....S.....QPEL.....G.....I.....T...
D....SLKIK.D..PG..DKII.V....D.QA...S.GQ.SG....LV.DDNQTEV
D....Q.KID.TK.AA..KK.....E..S.K...G..SNS.M..V.ND..SE.

CRYDLSQEGGPNTALIAGEVYRDPSPNPNWSFVAVGKGMQ GALPGLLQIFGCQ--
.....TN...T...M.V.....Q..Q....I....SFP.G.QF.C....VNAS
A....TEDASTE..MLF..L..HNGA---K.R...L.YA.G.ASVCAQY..INAS
A.F...EDASTE..M.F..L..HGAE---K.K...Q.FA.G.AA.ATQH..INI-

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Fig. 6. Alignment of the amino acid sequences of CABP1 and related polypeptides. The amino acid sequences of the polypeptides encoded by pDdM34 and pDdM31 were aligned with CABP1 and two related molecules, designated ORF4 and ORF5, encoded by a bacterial plasmid which confers resistance to tellurium anions, by pairwise comparison using the ALIGN program (Pearson & Lipman, 1988). The underlined sequences are absent from CABP1B and the polypeptide encoded by pDdM31. Identical amino acids are represented by dots, while dashes indicate gaps.

proline and glutamine residues. It also contains two degenerate versions of the nine amino acid repeat found in CABP1, APAGQYSMP and QQAGQYGMP, separated by a polyglutamine tract. However, in these polypeptides, the repeat has been extended to 12 amino acids by the addition of three proline residues to the carboxy terminus. Following this region, the similarity between CABP1 and the two related molecules increases again. The carboxy-terminal halves of these polypeptides possess 76% identity; this value increases to approximately 85% if conservative changes are included.

We have recently demonstrated that CABP1 is related to two polypeptides encoded by genes present on a bacterial plasmid which confers resistance to tellurium anions (Grant & Tsang, 1990). Unsurprisingly, the polypeptides encoded by pDdM34 and pDdM31 also show homology to these bacterial polypeptides (Fig. 6).

No other proteins whose sequences are present in the GenBank, EMBL or NBRF databases have been found to possess significant similarity to these molecules.

Expression of actin 15-pDdM34 fusion gene in Dictyostelium cells

Southern blot analysis (Fig. 4) suggests that the polypeptides encoded by pDdM34 and pDdM31 are the products of a single gene. To obtain further evidence for this hypothesis, the pDdM34 cDNA clone was expressed in *D. discoideum* cells under the control of the actin 15 promoter using the expression vector pB10Act15BKH. The expression construct codes for a translational fusion in which the amino-terminal 14 amino acids are contributed by vector sequences while the remainder is derived from the open reading frame present in pDdM34

Fig. 7. Expression of pDdM34 under the control of the actin 15 promoter. (a) The details of the actin 15-pDdM34 fusion are shown. The nucleotide sequences provided by the expression vector pB10Act15BKH are shown in small letters, while those present in pDdM34 are in capitals. The numbers above the sequence represent amino acid position in the fusion, while those below the sequence indicate the codon position in the open reading frame of pDdM34. After introducing the expression construct into *D. discoideum* cells, the transformants were analysed by metabolic labelling with [35 S]methionine followed by immunoprecipitation (b). The two subunits of CABP1, 1A and 1B, are labelled, along with p34 and p31. Lane 1, immunoprecipitated products obtained from control AX2 cells; lane 2, immunoprecipitated products obtained from cells transformed with the expression vector lacking the pDdM34 insert; lane 3, immunoprecipitated products obtained from cells transformed with the actin 15-pDdM34 fusion construct.

(Fig. 7a). Stable transformants were isolated and analysed by metabolic labelling with [35 S]methionine followed by immunoprecipitation with a monoclonal antibody which recognizes CABP1 as well as a number of antigenically related polypeptides (Tsang & Tasaka, 1986). As Fig. 7(b) clearly shows, transformants carrying this construct express large amounts of two polypeptides which migrate slightly more slowly than the endogenous CABP1-related p34 and p31 molecules. This result indicates that the information required to generate both these polypeptides appears to be present in pDdM34. Furthermore, it strongly supports the hypothesis that these two polypeptides are generated from one gene.

Attempts to use the overexpressing strain for a biochemical analysis of the polypeptides encoded by pDdM34 have not been successful. A more detailed examination of this strain has revealed that the overexpression of these polypeptides is not very stable. In addition, immunofluorescence experiments have demonstrated that the overexpressed molecules appear to be forming aggregates in the cytoplasm and therefore may not be functional (data not shown).

Discussion

Based on the data presented here, it is very likely that pDdM34 and pDdM31 respectively encode the CABP1-related polypeptides p34 and p31, which cross-react with anti-CABP1 antibodies and also copurify with CABP1 (Tsang & Tasaka, 1986). First, hybrid selection analysis of the cDNA clone pC5D9 (Fig. 1) indicates that this sequence is complementary to mRNAs which encode these two molecules. Second, when a cDNA library was screened using this clone as a probe, two different classes of cDNAs were isolated, represented by pDdM34 and pDdM31. Third, the sizes of these two clones agree well with the sizes of two of the transcripts which are detected by Northern blotting (Fig. 5). Finally, these two polypeptides appear to be the products of a single gene (Figs 4 and 7) and are very similar to the two subunits of CABP1, which are also produced from a single gene by alternative splicing (Grant *et al.*, 1990). Taken together, these data strongly suggest that pDdM34 and pDdM31 are complementary to the mRNAs which encode p34 and p31.

RNA blot analysis demonstrated that three developmentally regulated transcripts with sizes of 1.15 kb, 1.25 kb and 1.4 kb are complementary to pDdM34 and pDdM31 (Fig. 5). The 100 bp difference in size between the two smaller transcripts, in conjunction with their overall length, suggests that the cDNA clones are derived from them. The precise origin of the 1.4 kb mRNA species is unknown at present. We do not believe that this transcript is generated from another closely related gene. Southern analysis demonstrated that only a single gene encodes p34 and p31 (Fig. 4a). Under low stringency conditions, p34/p31 probes crossreacted with only one additional gene, that which encodes CABP1 (Fig. 4b). CABP1 probes, however, do not hybridize to the 1.4 kb transcript under conditions of high stringency (Grant *et al.*, 1990). Therefore, this mRNA probably corresponds to a third transcript which is generated by the p34/p31 gene. The determination of its precise origin and function will require additional analysis.

The three transcripts detected by probes derived from pDdM34 and pDdM31 are present during both growth and development, although their levels are quite variable (Fig. 5). In contrast, p34 and p31 cannot be detected in growing cells by immunoblotting. They appear soon after starvation and then remain at relatively constant levels throughout development (Kay *et al.*, 1987). We do not know the reason for the discrepancy between the expression of the mRNAs and that of the polypeptides. The significance of the fluctuation in transcript levels during development is also not known.

Based on the high similarity between their polypeptide products (Fig. 6), it is probable that the CABP1 and

p34/31 genes arose by duplication. Subsequent sequence divergence has led to a major change in only one domain, with the remainder of the two genes maintaining close similarity. The virtual identity of the amino termini of p34/31 and CABP1 in contrast to the relatively weaker similarity displayed by the remainder of the proteins suggests that this region of the polypeptides is crucial for function. Furthermore, this area contains the segments which are removed from the CABP1 and p34/31 primary transcripts to generate CABP1B and p31. The strong conservation of this feature between the two genes implies that the ability to generate two polypeptides is also very important.

The high degree of similarity between p34/31 and CABP1 also raises the question of whether these proteins are functionally identical. We do not yet know whether p34 and p31 possess the cAMP binding activity displayed by CABP1. We have previously shown that the developmental regulation and intracellular distribution of these molecules are quite different (Kay *et al.*, 1987). These differences suggest that these molecules do not perform exactly the same functions in the cell.

The functional relationship between p34 and p31 is not clear. Since CABP1 operates as a heterodimer between CABP1A and CABP1B (Tsang & Tasaka 1986), it is possible that p34 and p31 also associate to form one functional protein. Alternatively, all four polypeptides may be required to form an active complex. More study is required to clarify whether or not this is the case.

The discovery of very high similarity between CABP1, p34/31 and two bacterial polypeptides encoded by a plasmid which confers resistance to tellurium anions (Jobling & Ritchie, 1988) (Fig. 7) was completely unexpected. This close similarity suggests that these molecules have a common origin. It also implies that they probably have similar functions. Unfortunately, the mechanism which imparts tellurium resistance in bacteria is not known, so we can gain no clues as to the function of p34/31 and CABP1. The results described in this paper directly demonstrate that the *D. discoideum* cAMP binding protein CABP1 belongs to at least a small family of related polypeptides. Additional distinct cDNA clones which encode other molecules which are recognized by anti-CABP1 monoclonal antibodies are currently being analysed in our laboratory. These studies should allow us to determine the precise size of the CABP1 family and may also provide some clues as to the functions of these polypeptides.

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