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Two Divergently Transcribed Genes of *Dictyostelium discoideum* Are Cyclic AMP-Inducible and Coregulated during Development

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The cysteine proteinase 1 (CP1) gene of *Dictyostelium discoideum* encodes a developmentally regulated sulfhydryl proteinase. We characterized the DNA sequences upstream of the CP1 gene and found a second developmentally regulated gene, which we term DG17. The translational open reading frame of the DG17 gene encoded a 458-amino-acid cysteine- and lysine-rich protein of unknown function. In several regions, the cysteine and lysine residues were arranged in a manner characteristic of the zinc-binding domains found in proteins which interact with nucleic acids. During normal development, the DG17 and CP1 genes are coordinately activated late in aggregation. The addition of exogenous cyclic AMP (cAMP) induced the premature expression of both mRNAs. By measuring the rate of specific mRNA synthesis in isolated nuclei, we showed that cAMP acted at the transcriptional level to activate both genes. The two genes were separated by 910 nucleotides and were divergently transcribed. The intergenic region was predominantly composed of A+T residues except for four short G-rich regions. These sequences coincided with the positions of four nuclease-hypersensitive sites, which appear during aggregation when the DG17 and CP1 genes are transcribed (J. Pavlovic, E. Banz, and R. W. Parish, Nucleic Acids Res. 14:8703–8722, 1986). Two of the G-rich regions formed the core of two almost identical 80-nucleotide repeats located 220 and 320 nucleotides upstream of the CP1 gene. Using the *Dictyostelium* transformation system, we showed that a restriction fragment containing the intergenic region was capable of directing bidirectional transcription in a cAMP-dependent manner.

In response to starvation, single cells of *Dictyostelium discoideum* form aggregates which develop into multicellular fruiting bodies consisting of stalk and spore cells. Aggregation occurs in response to pulsatile emissions of cyclic AMP (cAMP) from a signalling center (22). As multicellular aggregates form, there are major changes in gene expression to yield the new proteins required for cellular differentiation, and approximately 2,000 to 3,000 mRNAs are induced (1, 4, 5). In addition to its role as a chemoattractant, the rise in extracellular cAMP concentration during aggregation also plays a role in regulating gene expression. The addition of exogenous cAMP to developing cells represses the expression of a number of genes active early in development and induces the premature expression of a group of genes which are active late during aggregation (13, 21, 26, 28, 40, 46). Although it has been shown that cAMP can alter the rate of transcription of specific genes in *D. discoideum* (21, 46), the precise mechanism of action of cAMP is not understood. Pharmacological studies suggest that the effects of extracellular cAMP on gene expression are mediated by the cell surface cAMP receptor (12, 24, 31). In the postaggregative stage, cAMP plays an additional role in controlling gene expression by specifically stabilizing a subset of developmentally regulated mRNA sequences (6, 23).

During development there is extensive breakdown of cellular protein, which provides a source of energy and free amino acids (10). We have previously isolated two genes encoding cysteine proteinases which may play a role in autodigestion (34, 44, 45). The cysteine proteinase 1 (CP1) and cysteine proteinase 2 (CP2) genes are precisely coregulated during development. Both mRNAs are first expressed late during aggregation, are inducible by the addition of exogenous cAMP, and are enriched in prestalk over prespore cells (34, 45). Restriction enzyme mapping shows that the two genes are not closely linked in the genome (34).

To identify upstream sequences important in regulating the expression of the CP1 gene, we have determined 2.6 kilobases (kb) of DNA sequence upstream of the start site of transcription. We have identified another gene, which we term DG17, located 910 base pairs (bp) upstream of the CP1 cap site and established its complete nucleotide sequence. The DG17 gene encodes a translational open reading frame of 458 amino acids which does not show significant homology to any known protein. The DG17 and CP1 mRNA sequences appear coordinately at aggregation during normal development, and both genes are transcriptionally activated by exogenous cAMP. We have determined the nucleotide sequence of the intergenic region and identified four G-rich elements which may be positive regulatory elements. Using DNA-mediated transformation, we show that a restriction fragment containing the intergenic region can direct bidirectional transcription which is developmentally regulated and cAMP-inducible.

MATERIALS AND METHODS

Cell culture. Axenic cells of the AX-2 strain (ATCC 24397; from J. Ashworth) were grown at 22°C in shaking suspension in axenic medium (43). NC4 cells were grown at 22°C on SM agar (39). Cells were developed on agar (39) or in suspension (44) as described previously.

Cloning and sequencing. The pDG17 genomic clone was isolated from a library of *Sau*3A partial digestion fragments of *Dictyostelium* genomic DNA from AX-2 cells. The fragments were inserted into the *Bam*HI site of pAT153 (42). Because of a lack of suitable restriction enzyme sites, an exonuclease III deletion series was constructed to sequence the pDG17 clone (15). The deletion clones were sequenced by a combination of the chemical cleavage method (25) and the dideoxy chain termination method with double-stranded

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DNA as a template (14). The nucleotide sequence was determined in both strands.

Nucleic acid analysis. Total cellular nucleic acid was isolated by phenol extraction as described previously (16). Primer extension was performed with either the M13 universal 17-mer sequencing primer or a synthetic oligonucleotide complementary to the coding strand of the DG17 gene as a primer. Oligonucleotides were kinase labeled and annealed to 10 μg of total cellular RNA, and primer extension products were analyzed on a 7.5% acrylamide–urea gel (8). RNAs were analyzed by Northern transfer (RNA blot) with 10 μg of total cellular RNA as described previously (45). Southern transfers were performed with 5 μg of genomic DNA (38). Hybridization probes were labeled with [α-^{32}P]dATP by the random priming method (9).

Transcription in isolated nuclei. In vitro nuclear transcription was performed as previously described (17) with the following modification. Nuclei were incubated for 30 min at 22°C at 10^8 nuclei per ml in a solution containing 40 mM Tris, pH 7.9, 200 mM NaCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, 5% glycerol, 0.25 mM ATP, 0.25 mM GTP, 0.25 mM CTP, 5 μM UTP, and 25 μCi of [α-^{32}P]UTP. Incorporation ranged from 2×10^7 to 5×10^7 cpm/10^8 nuclei and was not affected by the addition of cAMP. Approximately 35 to 50% of the total RNA synthesis was sensitive to 12.5 μg of α-amanitin, which inhibits RNA synthesis by RNA polymerase II, per ml. Equal numbers of α-amanitin-sensitive, trichloroacetic acid-precipitable counts were hybridized to nitrocellulose filters as described (21).

DNA-mediated transformation. The transformation vector pB10ST is a derivative of the pB10 vector described by Nellen et al. and contains the bacterial neomycin resistance gene (*neo*) under the control of the actin 6 promoter (30). The pB10ST vector also contains the polylinker, M13 sequencing priming site, and F1 region of pEMBL8 (A. Early and J. Williams, Gene, in press). DNA was transformed into AX-2 cells, and stable transformants were selected in G418 (10 μg/ml) as described previously (29, 30; Early and Williams, in press).

RESULTS

pDG17 genomic clone hybridizes to two developmentally regulated mRNA sequences. The pDG17 genomic clone contains almost the entire coding region of the CP1 gene, extending from nucleotide +1530 to a *Sau*3A site approximately 2.6 kb upstream from the start site of transcription (34). A restriction map is shown in Fig. 1. To determine whether other genes were present in the pDG17 clone, total cellular RNAs were isolated from NC-4 cells developing on agar and analyzed by Northern transfer. We have previously shown that a CP1 cDNA clone hybridizes to a 1,150-nucleotide mRNA which is expressed late in aggregation (45). The pDG17 genomic clone hybridized to both the CP1 mRNA and to a second, developmentally regulated mRNA approximately 1,600 nucleotides in length, referred to as the DG17 mRNA (Fig. 2A). Both the DG17 and CP1 mRNAs were absent from vegetative cells and first became detectable at 6 h of development. The CP1 mRNA was expressed at high levels from the time of tip formation and throughout subsequent development. The concentration of the DG17 mRNA peaked at the time of tip formation and began to decline at the first-finger stage. The DG17 mRNA was similar in abundance to the CP1 mRNA, which has been estimated to represent 1% of the total cellular mRNA at the time of maximal expression (35).

cAMP activates transcription of the DG17 and CP1 genes. Both the DG17 and CP1 mRNAs were first detected during aggregation, when the concentration of intracellular cAMP increases. We have previously shown that the addition of exogenous cAMP during very early development induces premature expression of the CP1 mRNA (46). The DG17 gene was also cAMP-inducible (Fig. 2B). Vegetative AX-2 cells were starved for 1.5 h to initiate development, and then cAMP was added to 1 mM. Both the DG17 and CP1 mRNAs became detectably expressed within 2.5 h of the addition of cAMP, approximately 2 h earlier than in the absence of cAMP.

The CP1 gene is transcriptionally regulated during normal development (35), which suggests that cAMP acts at the transcriptional level to induce its expression. We measured the relative rates of transcription of both the DG17 and CP1 genes in nuclei isolated from vegetative cells and from cells developed in the absence or presence of cAMP. Nuclei were incubated in vitro with [^{32}P]UTP. Under these conditions, previously initiated RNA chains are elongated but there is no initiation of new RNA chains by RNA polymerase II (17). Thus, the radiolabeled RNA synthesized in vitro reflects the rate of specific gene transcription in vivo at the time of nuclear isolation.

The radiolabeled RNAs were hybridized to Southern blots of restriction digests of plasmid DNAs. The IG7 cDNA clone, derived from a constitutively transcribed mRNA, was included as a control. As expected, nuclei isolated from all stages of development synthesized an equivalent amount of IG7 RNA (Fig. 3A). Nuclei isolated from vegetative cells or from cells during the first 4 h of development yielded no detectable RNA transcripts which hybridized to the DG17 or CP1 clones. However, when cAMP was added at 1.5 h of development, transcripts of both the DG17 and CP1 genes could be detected at 4 h. The addition of cAMP also activated transcription of the CP2 gene (Fig. 3A). In these experiments, there was a 2.5-h lag period between the addition of cAMP and gene transcription. When cAMP was added at 3 h of development, all three genes were detectably transcribed within 60 min (Fig. 3B).

Determination of the transcriptional polarity and nucleotide

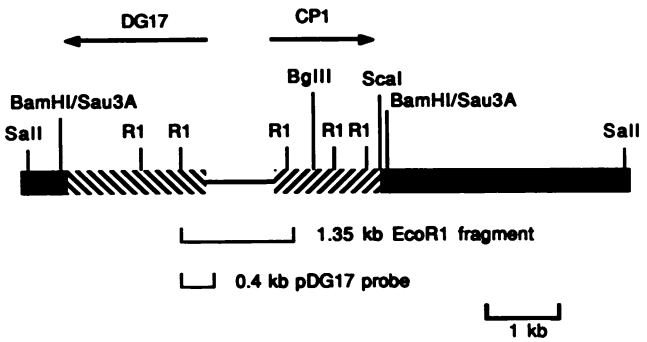

FIG. 1. Restriction enzyme cleavage map of the pDG17 clone. The pDG17 clone was isolated from a library of partial *Sau*3A fragments of *Dictyostelium* genomic DNA cloned into the *Bam*HI site of pAT153 (42). The thick solid lines indicate vector sequences, the hatched lines show the coding regions of the CP1 and DG17 genes, and the thin solid line shows the intergenic region. The direction of transcription of the DG17 and CP1 genes is indicated by the arrows. Below the map are shown the 1.35-kb *Eco*RI fragment which was cloned into the pB10ST transformation vector (Fig. 7A) and the 0.4-kb fragment from the 5′ end of the DG17 gene which was used as a hybridization probe (Fig. 7C). R1, *Eco*RI site.

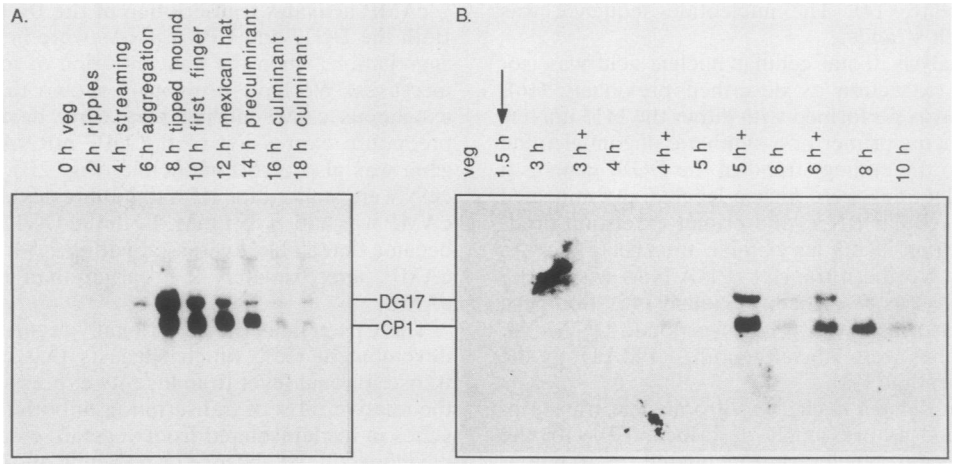

FIG. 2. Time course of the accumulation of the DG17 and CP1 mRNAs during development and in response to cAMP. (A) Bacterially grown NC4 cells were developed on agar plates (38) and harvested at the indicated times during development. Total cellular RNAs were isolated (16), separated on a 1.5% formaldehyde gel, and transferred to nitrocellulose (45). The filter was hybridized to the pDG17 genomic clone. veg, Vegetative. (B) Exponentially growing AX-2 cells were developed in shaking suspension (44) with 1 mM cAMP added at 1.5 h after starvation. Total cellular RNA was isolated at the indicated times during development and analyzed by Northern transfer as described above.

sequence of the DG17 gene. Analysis of Northern blots with various restriction fragments of the pDG17 clone indicated that the DG17 gene was situated approximately 1 kb upstream from the start site of transcription of the CP1 gene. We determined the polarity of transcription of the DG17 gene by hybridizing strand-specific probes to Northern blots (data not shown) and found that the two genes were divergently transcribed. This was confirmed by nucleotide sequence analysis of the DG17 gene.

The nucleotide sequence and the predicted amino acid sequence of the DG17 gene are shown in Fig. 4. The open reading frame was interrupted by a single, short intron which was bounded by GT at its 5′ end and AG at its 3′ end. The intron had a very high (>95%) A+T content and contained 27 tandem copies of the trinucleotide sequence TTA (data not shown). This was similar to the first intron in the CP1 gene, which contains 18 copies of TTA (34). There was a putative polyadenylation signal, AATAAA, approximately 80 nucleotides downstream of the termination codon and preceding 140 nucleotides of presumably intergenic sequence with an extremely high A+T content. Because we have not isolated a cDNA clone from the DG17 mRNA, we were unable to identify the exact 3′ end of the gene.

To identify the 5′ end of the DG17 mRNA, we performed primer extension with a synthetic oligonucleotide complementary to the coding strand. There were multiple start sites of transcription, which mapped to the 10-nucleotide region underlined in Fig. 4. The cap site was preceded by a run of 23 T residues, which is characteristic of start sites in other *Dictyostelium* genes (20). If the first ATG is assumed to be

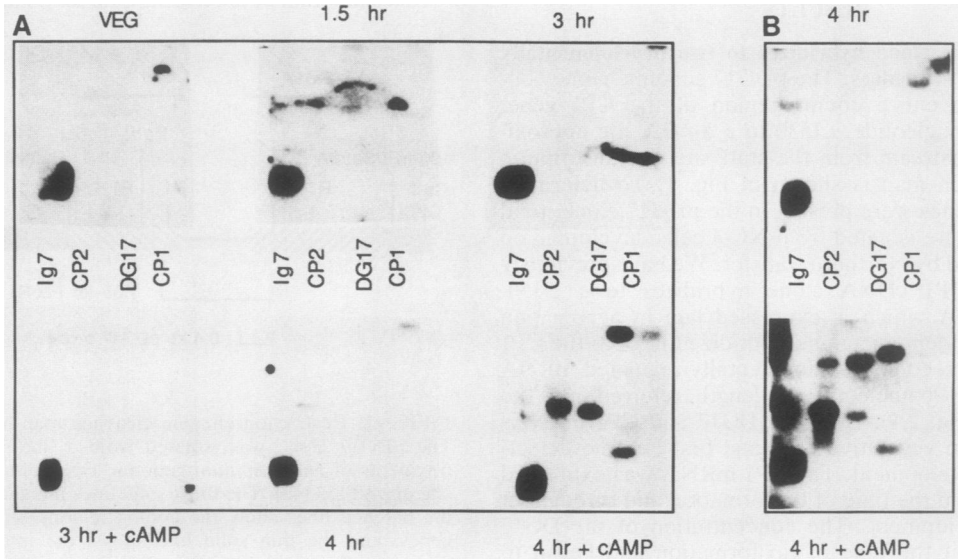

FIG. 3. Analysis of the transcription of the DG17 and CP1 genes in isolated nuclei. AX-2 cells were developed in suspension with 1 mM cAMP added at 1.5 h (A) or 3 h (B) as described in the legend to Fig. 2. Nuclei were isolated at the indicated times during development and incubated in vitro with [^{32}P]UTP to label nascent RNA chains as described in Materials and Methods. Plasmid DNAs were restricted, electrophoresed on a 1% agarose gel, and transferred to nitrocellulose (38). The filters were hybridized to the radiolabeled RNA transcripts as described previously (21).

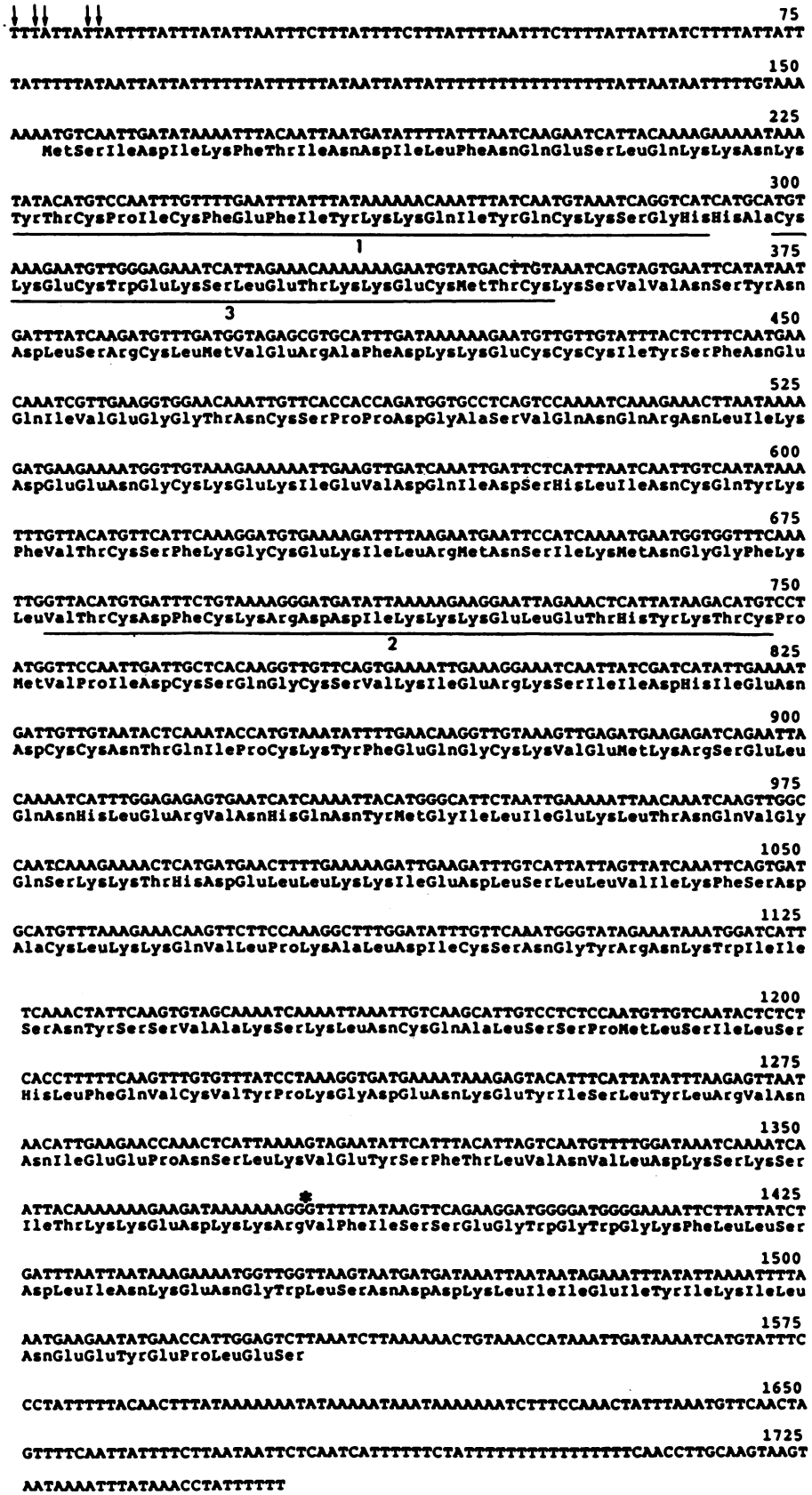

FIG. 4. Nucleotide and predicted amino acid sequence of the DG17 gene. An exonuclease III deletion series of the pDG17 clone was constructed (15), and the nucleotide sequence of both strands of the deletion clones was determined as described in Materials and Methods. The multiple start sites of transcription at positions +1 to +10, indicated with arrows, were identified by primer extension and S1 mapping. The position of the intron is indicated by the asterisk. The putative polyadenylation signal (AATAA) is underlined. The positions of the cysteine-rich regions 1, 2, and 3 are shown.

the initiation codon, the DG17 mRNA has a 5′ untranslated region of 150 nucleotides with an A+T content of 97%. The predicted size of the DG17 mRNA, from the transcription start site to the putative polyadenylation signal, is 1,640 nucleotides.

The open reading frame defined by the first ATG could potentially encode a polypeptide of 458 amino acids, which would have a minimum molecular weight of 53,015. The DG17 protein was unusually rich in cysteine (6.4%) and lysine (12.4%) residues, particularly in the amino terminus of the molecule. There were two cysteine-rich regions in which the cysteine residues were arranged in the sequence Cys-X_2-Cys-X_{10-12}-His/Cys-X_{2-3}-Cys (regions 1 and 2 in Fig. 4), where X represents any amino acid other than cysteine or histidine. The core of this sequence contained a lysine-rich fragment flanked by hydrophobic residues and showed homology (Fig. 5) to the zinc-binding domains in transcription factor TFIIIA (27) and a number of other known or supposed nucleic acid-binding proteins (reviewed in reference 3). It has been proposed that the interaction of the cysteine and histidine residues with zinc results in the formation of a finger domain which is stabilized by the hydrophobic residues and allows the lysine and other positively charged amino acid residues to interact with DNA (27). There was a third potential metal-binding site in the pDG17 protein (region 3 in Fig. 4), although the homology was weaker in that the core lysine residues were not flanked by hydrophobic residues. Other than this structural homology with metal-binding domains, the predicted amino acid sequence of the pDG17 protein showed no significant homology with any proteins in the available databases.

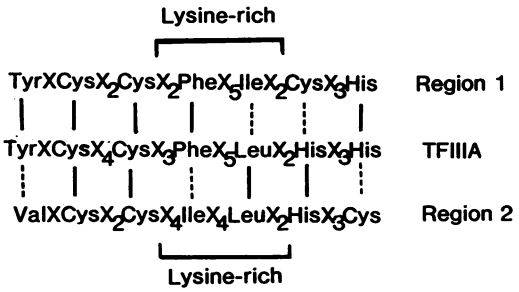

FIG. 5. Homology between the DG17 protein and the metal-binding domain of transcription factor TFIIIA. The positions of the cysteine-rich regions 1 and 2 in the DG17 protein are shown in Fig. 4. The consensus sequence of the zinc-binding domains of transcription factor TFIIIA (27) contains seven almost invariant amino acids. These are separated by amino acids which are variable and are represented by the letter X. However, there is a preponderance of DNA-binding amino acids in the central segment of the TFIIIA sequence (27), and this is also true for regions 1 and 2 of the pDG17 protein. The first two cysteine residues are separated by two amino acids in the pDG17 protein, compared with four amino acids in TFIIIA. However, in the Kruppel gene, a *Drosophila* gene closely related to TFIIIA, the first two cysteine residues are also separated by two amino acids (37). A solid vertical line indicates identity, and a broken line indicates similarity. Thus, cysteine and histidine are considered similar in that both are able to interact with zinc. In both the TFIIIA and Kruppel genes, the pair of zinc-binding residues are cysteines in the N-terminus and histidines in the C-terminus of the sequence (27, 37). However, there are proposed zinc fingers which do not display such symmetry (3).

Characterization of the intergenic region between the DG17 and CP1 genes. We were interested in identifying the *cis*-acting DNA sequences and *trans*-acting factors responsible for the coordinate regulation of the DG17 and CP1 genes. As a first step in this analysis, we determined the sequence of the intergenic region (Fig. 6A). Because of the lack of restriction sites, the sequence was determined from a series of overlapping deletion clones. The start sites of the DG17 and CP1 genes were separated by 910 bp, and this region was typical of *Dictyostelium* intergenic DNA, containing long homopolymeric runs of A and T residues with an overall A+T content of 86%. However, there were four short regions which were unusually rich in G+C residues (labeled boxes 1, 2, 3, and 4 in Fig. 6A). A striking feature of these four regions was their asymmetry, with G residues predominantly on the CP1 coding strand and C residues predominantly on the DG17 strand.

The G-rich regions in boxes 1 and 2 lay within two 80-nucleotide tandem repeats located approximately 200 and 300 nucleotides upstream of the CP1 gene (Fig. 6B). The two repeats were identical except for a single-base difference, a G residue within the G-rich core [GGAATGGGG(G/A)]. Each of the repeats contained two imperfect inverted repeats of 40 nucleotides. To investigate whether the 80-nucleotide repeats were the result of a very recent duplication event, we compared DNAs from several *Dictyostelium* strains by Southern blotting. Genomic DNAs were digested with *Sca*I, which cuts within the coding region of CP1, and then partially digested with *Hin*fI, which cuts within the core of the G-rich region of the repeat. Restriction fragments generated from the pDG17 clone were identical in size to those from the genomic DNAs of the wild-type strains V12M2 and NC4 and two axenic derivatives of the latter strain (data not shown).

The G-rich region of approximately 20 nucleotides located 150 nucleotides upstream of the DG17 gene (box 4) was an almost exact inverted copy of the core of the two 80-nucleotide repeats. The G-rich sequence 250 nucleotides upstream from the DG17 gene (box 3) was approximately 60 nucleotides long and contained several short stretches of alternating purine and pyrimidine residues with $(GT)_n$ on the CP1 coding strand and $(AC)_n$ on the DG17 coding strand.

The position and unusual G+C content of these four regions suggested that they might be important regulatory elements. More direct evidence for this came from studies on the structure of the chromatin flanking the CP1 gene. Four sites have been identified upstream of the CP1 gene at −220, −300, −670, and −770 relative to the start site of transcription, which became hypersensitive to nucleases during aggregation, when the CP1 gene is transcribed (32). When we compared the positions of these hypersensitive sites with the sequence of the intergenic region, these sites mapped to the four G-rich boxes (Fig. 6B).

Intergenic region is sufficient for developmental regulation and cAMP induction of the DG17 and CP1 genes. To begin to identify the DNA sequences required to regulate expression of the DG17 and CP1 genes, we used the recently described *Dictyostelium* transformation system (29, 30). The transformation vector pB10ST contains the bacterial *neo* gene under the control of the actin 6 promoter (Fig. 7A). To provide *Dictyostelium* termination and polyadenylation signals, a *Sau*3A fragment containing the 3′ end of the CP1 gene was fused to the *neo* gene (Early and Williams, in press). This restriction fragment also contains 650 nucleotides from the 3′ end of a constitutive gene of unknown function (gene Y), which is convergently transcribed relative to the CP1 gene (Fig. 7A).

To determine whether the intergenic region could direct regulated transcription, the 1.35-kb *Eco*RI fragment of the pDG17 clone (Fig. 1) was cloned into the polylinker of the

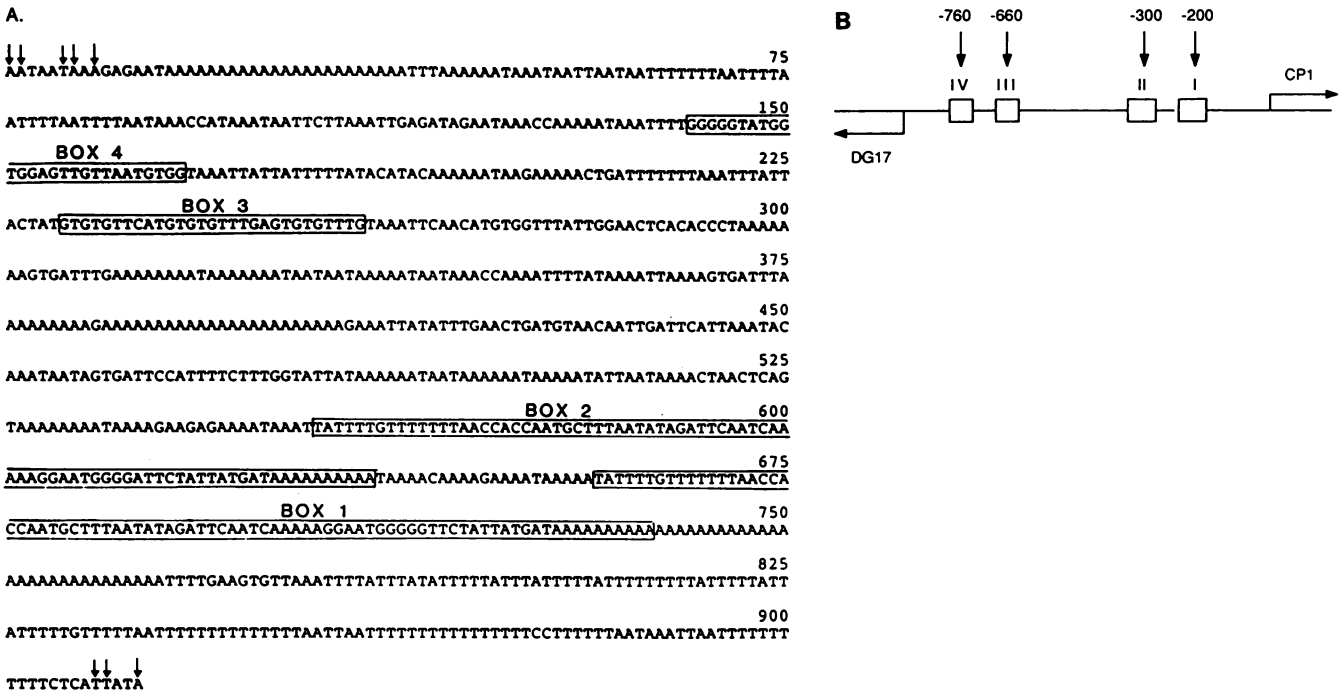

FIG. 6. Nucleotide sequence of the intergenic region. Exonuclease III deletion mutants of the pDG17 clone were constructed, and their nucleotide sequence was determined as described in Materials and Methods. (A) The nucleotide sequence shown is from the CP1 coding strand. The start sites of transcription of the DG17 gene (between nucleotides +1 to +10) and the CP1 gene (between nucleotides +909 to +913) are indicated by arrows. The positions of the four G-rich regions (boxes 1 to 4) are indicated. (B) Map of the intergenic region. The arrows indicate the position of the DNase-hypersensitive sites which are found at 8 h of development (32).

vector. This fragment contains the 910-bp intergenic region, 80 nucleotides of sequence encoding the CP1 mRNA, and 360 nucleotides of the DG17 mRNA. This created two fusion genes, with the 5′ end of the CP1 gene fused to the 3′ end of the Y gene and the 5′ end of the DG17 gene fused to the F1 region of the vector. This construct was transformed into AX-2 cells, and stable transformants were selected. When genomic DNA from the transformants was analyzed by Southern blotting, the vector DNA was integrated into the genome in tandem linear arrays at approximately 5 copies per cell (data not shown).

Pooled populations of transformants were starved in the absence or presence of exogenous cAMP, and total cellular RNAs were analyzed by Northern transfer. To analyze expression of the CP1-Y fusion gene, the Y gene was used as a hybridization probe (Fig. 7B). In addition to the 1,100-nucleotide endogenous Y mRNA, the Y probe hybridized to the 730-nucleotide CP1-Y fusion mRNA, which was cAMP-inducible. The RNAs also hybridized to a 0.4-kb fragment from the 5′ end of the DG17 gene (Fig. 1). The DG17 probe hybridized to the endogenous DG17 mRNA and to the DG17-F1 fusion mRNA, which was approximately 3,000 nucleotides long (Fig. 7C). Both of these mRNAs were coordinately induced by the addition of cAMP. The fusion mRNA also hybridized to a restriction fragment from the F1 region (data not shown) and terminated in vector sequences beyond the F1 region, although the exact 3′ end of the transcript has not been determined. The M13 sequencing primer site lay between the DG17 gene and the F1 region, and we used the sequencing primer in primer extension to confirm that the DG17-F1 fusion gene was transcribed from the correct start site (Fig. 7D). These results demonstrate that the sequences required for cAMP induction of the DG17 and CP1 genes lie within the 1.35-kb *Eco*RI fragment. When the transformants were developed on filters, the fusion genes were also correctly regulated during development (data not shown).

DISCUSSION

We have isolated and characterized the DG17 gene, a developmentally regulated gene of *D. discoideum*. The translational open reading frame of the DG17 gene encodes a 458-amino-acid polypeptide of unknown function which is rich in cysteine and lysine residues. In two and possibly three regions, these residues are arranged in a manner characteristic of the zinc-binding domains found in proteins which interact with nucleic acids (3). The homology is particularly good for region 1, the element most proximal to the amino terminus of the protein (Fig. 5). Although this is intriguing, we have no direct evidence that the protein interacts with DNA or RNA. The pDG17 polypeptide does not show any significant homology to any of the protein sequences currently available in the databases.

The DG17 gene is 910 bp upstream of the CP1 gene, which encodes a cysteine proteinase. The two genes are divergently transcribed, and their mRNAs are coordinately induced late in aggregation. The addition of exogenous cAMP activates the transcription of both the DG17 and CP1 genes, which suggests that the rise in extracellular cAMP that occurs during aggregation is responsible for the activation of these genes during normal development. The lag period between the addition of cAMP and activation of gene expression depended on the time at which cAMP was added. The results in Fig. 3 suggest that cells become competent to activate the transcription of the DG17 and CP1 genes sometime between 1.5 and 3 h of development. Recent studies have shown that the effects of extracellular cAMP on gene

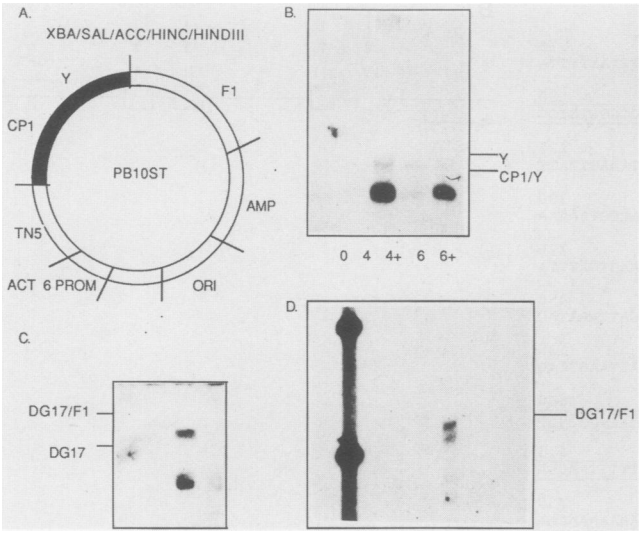

FIG. 7. Analysis of the expression of mRNAs in stable transformants. The transformation vector pB10ST is a derivative of the B10 vector described by Nellen et al. (30) which contains the bacterial *neo* gene under the control of the actin 6 promoter (ACT 6 PROM). (A) Restriction map of the pB10ST vector (Early and Williams, in press). To provide *Dictyostelium* termination signals, a *Sau*3A fragment containing the 3′ end of the CP1 gene, indicated by the solid line, was fused to the *neo* gene. This fragment also contains 650 nucleotides from the 3′ end of a constitutively expressed gene, Y, which is convergently transcribed towards CP1 (Early and Williams, in press). The pB10ST vector also contains the M13 sequencing primer site, polylinker, and F1 region of pEMBL. For *Dictyostelium* transformation studies, the 1.35-kb *Eco*RI fragment of the pDG17 clone (Fig. 1) was inserted into the *Sal*I site of the pB10ST polylinker by blunt-end ligation. This fragment contains the start sites of transcription of the DG17 and CP1 genes and the intergenic region. This created two gene fusions, with the 5′ end of the CP1 gene fused to the 3′ end of the Y gene, and the 5′ end of the DG17 gene fused to the F1 region of the vector. The pB10ST vector containing the 1.35-kb *Eco*RI fragment was transformed into AX-2 cells, and stable transformants were selected in G418 as described (29, 30; Early and Williams, in press). Pooled populations of stable transformants were developed in shaking suspension with 1 mM cAMP added at 1.5 h (44), and cells were harvested during development at the indicated times. Total cellular RNA was isolated and analyzed by Northern transfer as described (16, 45). (B) Filters were hybridized with the Y gene as a probe. (C) RNAs were hybridized to a 0.4-kb fragment from the 5′ end of the DG17 gene (Fig. 1). (D) RNAs were also analyzed by primer extension with the M13 17-mer sequencing primer as the primer, as described in Materials and Methods.

expression are mediated by the cell surface cAMP receptor (11, 23, 29). It seems likely that the lag reflects a requirement for the synthesis of some component involved in the signal transduction pathway.

Although the CP1 and DG17 mRNAs both reached a peak concentration at the time of tip formation, the CP1 mRNA was expressed at high levels throughout subsequent development, whereas the concentration of the DG17 mRNA declined within 2 h. Whether this decline is mediated by transcriptional or posttranscriptional mechanisms is not known. If cessation of transcription is solely responsible for the decline in the concentration of DG17 mRNA, then the mRNA must have a relatively short half-life of approximately 2 h.

The sequence between the DG17 and CP1 genes is typical of *Dictyostelium* intergenic DNA in being extremely A+T rich, except for four G+C rich regions. From restriction enzyme mapping, the two tandem 80-nucleotide direct repeats upstream of the CP1 gene appear to be present in a number of *Dictyostelium* strains, which suggests that the duplication is not a very recent event. The two repeats are identical in the gene cloned from AX-2 cells, except for a single mismatch in the G-rich core. This high degree of homology suggests that there may be strong constraints on the potential divergence of the sequence. Whether this nucleotide difference occurs in other *Dictyostelium* strains and is of functional importance is not known. A copy of the G-rich core of the repeat is present in box 4, 150 nucleotides upstream of the DG17 gene. The G-rich region in box 3, 250 nucleotides upstream of the DG17 gene, contains several short stretches of alternating purine and pyrimidine residues, which would have the potential to form Z-DNA. It has been suggested that transitions from B- to Z-DNA may be important in gene regulation (36).

Using DNA-mediated transformation, we showed that a 1.35-kb *Eco*RI fragment which contained the intergenic region could direct bidirectional transcription which was developmentally regulated and cAMP-inducible. It seems likely that the sequences required for the expression of the DG17 and CP1 genes lie within the G-rich regions, given their unusual sequence, position, and hypersensitivity to nucleases during development (32). Short G+C rich regions are found upstream of a number of other cAMP-inducible genes in *Dictyostelium* spp. (2, 7, 13, 33), but comparison of these sequences with those upstream of the DG17 and CP1 genes reveals no strict consensus sequence. In the case of the CP2 gene, a 30-nucleotide G-rich region, containing two 9-nucleotide direct repeats, is found 250 bp upstream of the start site, and deletion or mutation of this region drastically reduces the cAMP inducibility of the CP2 gene (7, 33). However, it is not known whether this G-rich element functions as a promotor, upstream activating sequence, or enhancer.

The DG17 and CP1 genes are the first example in *D. discoideum* of a pair of divergently transcribed genes which are coordinately expressed during development. Although it is not surprising to find closely linked genes given the small genome size, the only other examples in *Dictyostelium* spp. are the M4 genes, which are divergently transcribed but not coregulated (19), and the discoidin 1B and 1C genes, which are coordinately expressed but transcribed in the same direction (20). The DG17/CP1 gene complex is of particular interest because all of the 5′-flanking sequences required for developmentally regulated expression must be located within the 910-bp intergenic region, which raises the possibility that the two genes share regulatory elements. Analysis of the divergently transcribed *GAL1* and *GAL10* genes in *Saccharomyces cerevisiae* has identified the upstream activating sequence between the two genes that is responsible for their coordinate induction by galactose (11, 18).

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