DdPDE4, a Novel cAMP-specific Phosphodiesterase at the Surface of Dictyostelium Cells*

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Dictyostelium discoideum cells possess multiple cyclic nucleotide phosphodiesterases that belong either to class I enzymes that are present in all eukaryotes or to the rare β-lactamase class II. We describe here the identification and characterization of DdPDE4, the third class I enzyme of Dictyostelium. The deduced amino acid sequence predicts that DdPDE4 has a leader sequence, two transmembrane segments, and an extracellular catalytic domain that exhibits a high degree of homology with human cAMP-specific PDE8. Expression of the catalytic domain of DdPDE4 shows that the enzyme is a cAMP-specific phosphodiesterase with a K_m of 10 μM; cGMP is hydrolyzed at least 100-fold more slowly. The full-length protein is shown to be membrane-bound with catalytic activity exposed to the extracellular medium. Northern blots and activity measurements reveal that expression of DdPDE4 is low during single cell stages and increases at 9 h of starvation, corresponding with mound stage. A function during multicellular development is confirmed by the phenotype of ddpde4− knock-out strains, showing normal aggregation but impaired development from the mound stage on. These results demonstrate that DdPDE4 is a unique membrane-bound phosphodiesterase with an extracellular catalytic domain regulating intercellular cAMP during multicellular development.

During different developmental stages of Dictyostelium discoideum the cyclic nucleotides cAMP and cGMP play a central role in diverse signal transduction processes. cAMP mediates chemotaxis during cell aggregation and controls gene expression during development. cGMP regulates cytoskeletal organization affecting shape, stability, and motility of single cells. The intracellular concentration of the second messengers cAMP and cGMP is determined by the combined action of production and removal. Production directly depends on the enzymatic activity of the adenylyl cyclases and guanylyl cyclases to form cAMP and cGMP, respectively. Removal of intracellular cAMP or cGMP depends on the activity of phosphodiesterases (PDEs)3 that hydrolyze cAMP to form 5′-AMP and cGMP to form 5′-GMP and on the ability of D. discoideum cells to secrete cAMP. This extrusion mechanism is pivotal in the formation of extracellular cAMP waves that mediate chemotaxis during aggregation, mound formation, and slug movement.

In D. discoideum three different adenylyl cyclases and two guanylyl cyclases have been identified (see Refs. 1 and 2). In addition, five different phosphodiesterases have been reported and characterized in D. discoideum. These PDEs belong to two classes that exhibit distinct differences in the amino acid sequence of the putative catalytic domains, namely class I, which is ubiquitous in eukaryotes, and class II, which predominantly occurs in lower eukaryotes.

PdsA (or DdPDE1) is a class II dual specificity PDE that degrades cAMP as well as cGMP and is exposed on the cell surface or secreted in the medium. It is the main PDE that degrades extracellular cAMP and thereby essential for shaping cAMP waves (3–13). RegA (or DdPDE2) encodes a cAMP-specific class I PDE localized in the cytosol of the cell where it degrades intracellular cAMP. The knock-out cell strain regA− develops very small aggregates and shows defects in spore formation. Additionally RegA has been implicated in suppression of pseudopodium formation (14–22). DdPDE3 is a cGMP-specific class I PDE that consists of a catalytic domain without regulatory domains and is constitutively active. The pde3− null cell strain shows a moderate phenotype with increased basal levels of cGMP, revealing that the enzyme accounts for about 20% of total cGMP-PDE activity in unstimulated cells (23). DdPDE5 (also named GbpA or PDED) is characterized as a cGMP-stimulated cGMP-specific class II PDE. The pde5− cell strain exhibits a phenotype with strongly elevated levels of cGMP implying that it is the main intracellular cGMP PDE accounting for about 75% of the cGMP PDE activity. The biochemical phenotype of this pde5− null cell is similar to what is observed in the mutant NP368, a Streamer F cell strain (24–26). Finally, DdPDE6 (GbpB or PDEE) is a dual specificity class II PDE that degrades intracellular cAMP and cGMP and accounts for only 5% of the total cGMP PDE activity (24, 26–28).

In searches of the then ongoing Dictyostelium genome sequencing project we identified a putative PDE that was named DdPDE4 for its order of initial recognition. DdPDE4 appears to be a class I phosphodiesterase with unusual properties. DdPDE4 is a transmembrane phosphodiesterase with its catalytic domain exposed to the extracellular medium. It degrades extracellular cAMP together with the class II enzyme DdPDE1. Cells lacking DdPDE4 are defective in development at the mound stage, which is also the stage of maximal expression of ddpde4.

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3 The abbreviations used are: PDE, phosphodiesterase; DTT, dithiothreitol; IBMX, isobutylmethylxanthine.
**EXPERIMENTAL PROCEDURES**

**Identification and Reconstruction of the ddpde4 Gene**—The Dictyostelium genome data base was screened for sequences potentially encoding type I phosphodiesterases. At the initial phase of the sequencing project we identified clone JAX4b25f06. The encoding DNA fragment was obtained by PCR with primer 99B245 (ATGCGAATTCGGAGAACATAGCCATTG) and primer 99B301 (ATGCGAATTCGGGTACCCGCAACTGATG) on genomic DNA. With this fragment as a probe a cDNA library (kindly provided by Dr. R. H. Gomer) was screened. Clone pdek9 had the largest insert of 2150 base pairs but lacked the start of the open reading frame. The Dictyostelium data bases were searched with the pdek9 insert revealing that 400 base pairs of coding sequence were missing. The first 100 base pairs were obtained by PCR with primers 00B369 and 00B345 to create the 400-base pair fragment. This way a BglII site was obtained by PCR with primers 00B367 (GTGGTATACCAGACCTAAG) and 00B345 (CTTAGGTCTGGTATACCAC) and the subsequent 300 base fragments with primers 00B368 (TCCGGTACCCGCAACTGATG) on genomic DNA. With the pdek9 insert revealing that 400 base pairs of coding sequence were missing. The first 100 base pairs were obtained by PCR with primer 99B245 (CATGCGAATTCGGGAAAC-)

**Construction of Knock-outs**—pdek9 was cloned into pBlue-script SK+ with BamHI and XhoI. The Bsr gene (32) was cloned into the HindIII site. The knock-out fragment was amplified by PCR using primer 00B254 (ATCATGGATCCAAAATGCCAGAGATAACAGATCAGG) and primer 00B260 (CACTTGTCTATTTGACC). The PCR product was purified with the QIAquick PCR purification kit by Qiagen (Westburg), and Bsr gene with expected size on Southern blots. ddpde4, and the radioactivity in 200 μl of lysate was determined.

**Construction of Overexpressors**—A genomic DNA fragment of 1450 base pairs comprising the catalytic domain of DdPDE4 was amplified by PCR using the primers PDE4catF (ATCATGGATCCAAAATGAGATAACAGATCAAGG) and PDE4catR (CATCTGAAACACATATATTAAAAATTTGATTG). Subsequently, the BamHI-Xbal fragment was cloned into the BglII-SpeI site of the vector MB74-GFP (31), resulting in the plasmid MB74-PDE4cat-GFP. Finally, AX2 Dictyostelium cells were transfected with 5 μg of plasmid yielding cells overexpressing the full-length PDE4 protein. We will refer to this overexpressor as DdPDE4OE.

**Phosphodiesterase Assays**—Dictyostelium cells were washed with PDE lysis buffer (40 mM HEPES/NaOH, pH 7.0, 1 mM EGTA) and resuspended to a density of 10⁶ cells/ml in PDE lysis buffer supplemented with 0.25 mM sucrose. The cells were lysed at 4 °C by passage through a 0.45-μm Nucleopore filter. The lysate was centrifuged for 2 min at 14,000 × g, and the supernatant was used; the pellet was washed once with lysis buffer, resuspended in the original volume of lysis buffer, and used in the assays. PDE activity at the surface of intact cells was assayed using freshly washed cells resuspended in lysis buffer.

**RESULTS**

**DNA Sequence and Protein Topology**—The Dictyostelium DNA data base was searched for sequences similar to two stretches of amino acids that are conserved in the catalytic domains of mammalian phosphodiesterases (23). These

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FIGURE 1. Predicted topology of DdPDE4. A, predicted domain composition of DdPDE4 and HsPDE8. The DdPDE4 sequence from amino acids 1–29 depicts a signal sequence. Amino acids 42–62 and amino acids 88–108 are transmembrane regions. Amino acids 117–145 are predicted by SMART to be a coiled coil region. Amino acids 146–255, indicated as precatalytic region, form a potential regulatory domain also found in human PDE8. The catalytic domain lies between amino acids 256 and 688 (with a stretch of repetitive sequence from amino acids 471 to 580). The topology of HsPDE8A is depicted below that of DdPDE4. It has a PAS domain, a precatalytic region, and a catalytic domain. B, proposed topology of DdPDE4. The signal sequence leads the DdPDE4 protein through the membrane and is supposed to be cleaved off. From this it follows that the two transmembrane regions enter the membrane in the depicted fashion, and consequently the remaining part of the protein resides at the extracellular side of the membrane, as demonstrated in Fig. 6.

sequences are HDyDHpGTtNqFIVntKSeLALYndESVMEnHH and DLsnpTKplpyRrwAELImeEEFxQGDkEKeMG. Capital letters represent amino acids that are identical in (almost) all phosphodiesterases, and lowercase letters represent amino acids that are conserved in the major part of the mammalian phosphodiesterases. With this method we identified a sequenced clone JAX4b25f06. The 665-base pair insert coded for part of a putative phosphodiesterase catalytic domain. This sequence was used to screen a cDNA library and to search further in the Dictyostelium genomics database. Eventually we identified and assembled by reverse transcription-PCR the entire open reading frame of 2.6 kb (for detailed information, see “Experimental Procedures”). The start ATG is preceded by a long AT stretch. The DdPDE4 amino acid sequence and a leucine for a tryptophan at position 679 (∪ in Fig. 2). The two metal binding sites depicted in Fig. 2 are also conserved in DdPDE4 (+ and + in Fig. 2). We identified previously two amino acids, an aspartic acid and an arginine, that are conserved in all cAMP-specific PDEs but not in cGMP-specific enzymes or enzymes with dual specificity (23). DdPDE4 contains this aspartic acid and arginine (bracket symbol in Fig. 2), suggesting that DdPDE4 belongs to the group of cAMP-specific enzymes, which is confirmed below in biochemical experiments.

The DdPDE4 amino acid sequence directly N-terminal to the catalytic domain (named the precatalytic region; amino acids 146–255, Fig. 1A) shows homology to an amino acid sequence preceding the catalytic domain of PDE8 isoforms found in vertebrates (Fig. 3). Members of this PDE subfamily with a precatalytic region were also found in the insects Drosophila melanogaster and Apis mellifera, and the worm Caenorhabditis elegans (Fig. 3). In vertebrate and insect PDE8 this precatalytic region is preceded by a PAS domain (43) that is not present in worms and Dictyostelium DdPDE4.

To further explore the relationship of DdPDE4 with other class I enzymes, we performed a cluster analysis of the catalytic domains as defined in Fig. 2 of DdPDE4, one member of each of the 11 subfamilies of mammalian enzymes and the three PDE8 isoforms of insects and worms. Two groups of phosphodiesterases can be distinguished based on the bootstrap values (Fig. 4), similar to what has been reported previously (47). One group harbors PDEs that are cGMP-specific or have a cAMP/cGMP dual specificity (HsPDE2A, HsPDE5A, BtPDE6A, HsPDE10A, and HsPDE11). The second group consists of cAMP-specific and dual specificity enzymes (HsPDE1A, HsPDE3A, HsPDE4A, HsPDE7A, HsPDE8A, and HsPDE1A). DdPDE4 belongs to this second group. The catalytic domains of PDEs from insects and worms are clearly PDE8 isoforms. Dictyostelium DdPDE4 is placed relatively close to this PDE8 subfamily. However, bootstrap values show that the node of DdPDE4 is not very reliable.

The other Dictyostelium phosphodiesterases (DdPDE2 and DdPDE3) and HsPDE9A do not fall into one of these groups.

ments, DdPDE4 has a coiled coil region (117–145) and a region in front of the catalytic domain (146–255) that is homologous to a region found in mammalian HsPDE8.

Amino Acid Sequence and Cluster Analysis of the Catalytic Domain—Eleven subfamilies of mammalian phosphodiesterases have been identified. Each subfamily has a variety of phosphodiesterase genes (A, B, C, etc.) that often display different splice variants. The sequence identity of the catalytic domains within members of a subfamily is ~90%, whereas the sequence identity between subfamilies is 45%. We have used one member of each subfamily in the sequence analysis of DdPDE4. Fig. 2 shows an alignment of the catalytic domain of DdPDE4 with Dictyostelium RegA (or DdPDE2) (19, 33), DdPDE3 (23), and the 11 mammalian phosphodiesterases (34–46). DdPDE4 shows high sequence identity with the other phosphodiesterases. Of the 40 amino acids that are identical or conserved in all the other depicted phosphodiesterases, DdPDE4 has 38 amino acids that are identical or conserved. The exceptions are substitutions of a tyrosine for a histidine at position 412 of the DdPDE4 amino acid sequence and a leucine for a tryptophan at position 679 (∪ in Fig. 2). The two metal binding sites depicted in Fig. 2 are also conserved in DdPDE4 (+ and + in Fig. 2). We identified previously two amino acids, an aspartic acid and an arginine, that are conserved in all cAMP-specific PDEs but not in cGMP-specific enzymes or enzymes with dual specificity (23). DdPDE4 contains this aspartic acid and arginine (bracket symbol in Fig. 2), suggesting that DdPDE4 belongs to the group of cAMP-specific enzymes, which is confirmed below in biochemical experiments.

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Catalytic Activity and Localization of DdPDE4—The DdPDE4 catalytic domain (amino acids 264–771) was expressed in vegetative AX2 cells. We measured PDE activity in the cytosolic fraction of a cell lysate of these DdPDE4catOE cells and used PDE activity in lysates from the wild type AX2 cell strain as a control. The results in Fig. 5 show that the overex-

FIGURE 2. Alignment of the catalytic domain of the class I PDEs. Multiple sequence alignments were made by using the CLUSTAL W program with improvements made by hand. All sequences are from published reports (GenBank accession numbers AY162269, U60170, U40370, U67733, M91667, U68532, AF043731, M27541, U67932, O60658, AF048837, AB020593, and AB036704). To improve the alignment, insertions of 44 and 47 residues in HsPDE3A, 60 amino acids in HsPDE1A, and 95 residues in DdPDE4 relative to other PDEs have been deleted and are indicated by [44], [47], [60], and [95], respectively. * and /H11001 indicate two metal binding sites; [ refers to two amino acids that are conserved in all cAMP-specific enzymes including DdPDE4 but different in cGMP-specific or dual specificity enzymes. Black boxes represent positions where all 14 sequences are identical or conserved. Positions where 10–13 amino acids are identical or conserved are represented by gray boxes. Conserved amino acids are EQDN, KRH, FYW, MVLI, and GASTP (single letter codes).

FIGURE 3. Alignment of the precatalytic region of Dictyostelium DdPDE4 and members of the PDE8 subfamily. Multiple sequence alignments were made by using the CLUSTAL W program with improvements made by hand. All sequences are from GenBank accession numbers: O60658 (HsPDE8A), NP003710 (HsPDE8B), AAR96128 (DmPDEA), XP392234 (AmPDE), and AAF60898 (CePDE6). Species abbreviations are as follows: Hs, Homo sapiens; Dm, Drosophila melanogaster; Am, Apis mellifera; Ce, Caenorhabditis elegans. Black boxes represent positions where all six sequences are identical or conserved. Positions where 4 or 5 amino acids are identical or conserved are represented by gray boxes. Conserved amino acids are EQDN, KRH, FYW, MVLI, and GASTP (single letter codes).
pressor DdPDE4catOE provides a 3-fold increase of cAMP phosphodiesterase activity compared with the control. There was no difference in cGMP PDE activity between DdPDE4catOE and the control, suggesting that DdPDE4 may be cAMP-specific. The increased cAMP hydrolysis of DdPDE4catOE cells compared with AX2 cells was observed only in the soluble fraction and not in the particulate fraction of a cell lysate (data not shown). Subsequently, the hydrolysis of \[^3H\]cAMP in the presence of increasing concentrations of unlabeled cAMP or cGMP was measured for both cell lines. We calculated the hydrolysis of \[^3H\]cAMP in lysates form DdPDE4catOE cells after subtracting \[^3H\]cAMP hydrolysis in lysates from AX2 cells, providing direct characterization of DdPDE4. The results in Fig. 5B show half-maximal inhibition of \[^3H\]cAMP hydrolysis at 3–10 μM cAMP, whereas no inhibition was observed at 100 μM cGMP, indicating that DdPDE4 is at least 100-fold more specific for cAMP than for cGMP. The kinetic data for DdPDE4 were obtained from an Eadie–Hofstee plot (Fig. 5C); linear regression analysis yields a slope of \(K_m = 9.6 \mu M\) and an intercept at \(V_{max} = 4200 \text{ pmol/min/mg}\).

DdPDE4 has a putative signal sequence and two transmembrane segments, predicting it to be a plasma membrane protein with the catalytic domain exposed to the outside of the cell. The topology of DdPDE4 was investigated by overexpressing the full-length protein in wild type cells, yielding strain DdPDE4OE. To determine the location of the catalytic domain of DdPDE4 at the outside or inside of the plasma membrane, we measured \[^3H\]cAMP hydrolysis using intact cells and the particulate fraction of a cell lysate (Fig. 6). Wild-type cells contain on
their surface the very active DdPDE1 (3000 fmol/min/10^7 cells) whose activity can be inhibited strongly with dithiothreitol (DTT) (48). Wild type cells possess a small residual activity in the presence of 5 mM DTT (29 fmol/min/10^7 cells). DdPDE4^OE cells contain approximately the same amount of total PDE activity compared with control cells (2900 fmol/min/10^7 cells) but about 4-fold higher DTT-resistant [3H]cAMP hydrolysis activity than control cells. When the activity was measured with [3H]cGMP as substrate, very little activity was observed in control cells with DTT (18.1 ± 1.8 fmol/min/10^7 cells), which was not elevated in DdPDE4^OE cells (17.7 ± 1.1 fmol/min/10^7 cells). This result confirms the cAMP-specificity of DdPDE4 and also indicates that cells are not leaky for the very active cytosolic cGMP-specific enzymes. Membranes isolated from wild type cells contain slightly less [3H]cAMP hydrolysis activity than intact cells, suggesting that little or no phosphodiesterase activity is exposed to the cytoplasmic face of the plasma membrane. Similarly, PDE activity in membranes isolated from DdPDE4^OE cells is slightly less than the PDE activity of intact DdPDE4^OE cells. The results strongly suggest that the catalytic domain of DdPDE4 is exposed to the extracellular medium, confirming the topology programs predicting a signal sequence, two transmembrane regions, and extracellular N and C termini. Programs predict the cleavage of the signal sequence, for which we have no experimental evidence.

Expression of mRNA—To investigate the expression level of ddpde4 at different time intervals during development, we isolated mRNA at 0–21 h after starvation on non-nutrient agar. A Northern blot was made with the isolated mRNAs, and it was probed with part of the catalytic domain of ddpde4 (see M&M). Fig. 7A shows a mRNA with an approximate size of 3 kb that is present in the wild type Dictyostelium strain AX3 predominantly at 9 h of starvation, during the mound stage. Expression is very low during development and cell aggregation (up to 6 h), maximal during the mound stage, but ddpde4 remains expressed at significant levels during the other stages of multicellular development.

To assay for DdPDE4 activity at different stages of development, we made use of the localization of DdPDE4 at the cell surface and its resistance to DTT and inhibition by IBMX. We define DdPDE4 activity as the IBMX-mediated decrease of phosphodiesterase activity measured with intact cells in the presence of 10 mM DTT. The results of figure 7B demonstrate very low DdPDE4 activity in single cells starved for 1 and 5 h. The activity starts to increase at 7 h of development when cells are in loose aggregates. A substantial increase of activity is observed between 7 and 9 h of development when tight aggregates are formed. DdPDE4 activity increases slightly in slugs at 12 h of development. The biochemical assays confirm the Northern blot, showing that DdPDE4 is active predominantly in the multicellular stage. It should be mentioned that DdPDE4 is only a small fraction of total PDE activity on intact cells (note the differences in axes for total PDE and DdPDE4). Even in slugs, DdPDE4 is less than 20% of total cell surface PDE activity.
Knock-out—Two independent knock-out strains with a deletion of the *ddpde4* gene were created. Phosphodiesterase assays show a reduction of PDE activity on the cell surface from 29 fmol/min/10^7 cells in control AX2 cells to 9 fmol/min/10^7 cells in *ddpde4*−/−null cells (Fig. 6). Assays showed a slower development of the knock-outs in comparison to AX3. Aggregation speed was similar, but from the mound stage until fruiting body formation the knock out strains were about 5 to 10 h slower than wild type AX3 (Fig. 8). Cell aggregation was nearly completed at 10 h after the onset of starvation in both AX3 and *ddpde4*−/−null cells. These aggregates developed into slugs at 15 h in AX3 cells but largely remained as mounds in *ddpde4*−/−null cells. At 20 h after the onset of starvation, the knock-outs are still in early mound stage, while the wild type strain AX3 starts to culminate. At 27 h of starvation fruiting body formation was completed in AX3, whereas many mounds were still present in *ddpde4*−/−null cell (Fig. 8). Thus, especially slug formation and culmination appear to be affected in *ddpde4*−/−null cells; these processes were not only slower but also less slugs and fruiting bodies were formed. The expression of full-length DdPDE4OE in *ddpde4*−/−null cell restored the phenotypic defects of the null cell line, and no deteriorating effects on development were observed (Fig. 8).

**DISCUSSION**

The completed genome sequence of *D. discoideum* (49, 50) uncovers several PDEs, of which five enzymes have been characterized in detail. In this paper we described the sixth member, DdPDE4, with unusual properties. The predicted topology of this PDE strongly suggests that DdPDE4 is a membrane bound phosphodiesterase with an extracellular catalytic domain. Biochemical experiments of the expressed protein confirm this topology. Since in wild type cells DdPDE4 is expressed during the onset of multicellular development and has cAMP phosphodiesterase activity, we suggest that it is involved in regulating cell surface cAMP phosphodiesterase activity, we suggest that it is involved in regulating cell surface cAMP phosphodiesterase activity.
inter-cellular cAMP levels in the mound and slug stage. This is also indicated by the retarded multicellular development of the knock-out cell line of ddPDE4.

Dictyostelium cells contain three pools of cyclic nucleotides with different functions: 1) extracellular cAMP as signal molecule for chemotaxis and morphogenesis, 2) intracellular cAMP as source for cAMP secretion and inducing development, and 3) intracellular cGMP as mediator of chemotaxis (Table 1). The characterized PDEs of Dictyostelium belong to two different classes (I and II) with three members in each class. It is striking that each cyclic nucleotide pool is regulated by a combination of a class I and a class II phosphodiesterase. Intracellular cGMP is degraded by cGMP-specific class I DdPDE3 (23) and class II DdPDE5 (and to a lesser extent by the dual specificity DdPDE6) (22, 24, 26–28). Intracellular cAMP is degraded by the cAMP-specific class I DdPDE2 (RegA) and by the dual specificity class II DdPDE6 (14–22). Extracellular cAMP is degraded by the class II DdPDE1 (PdsA) (3, 4, 6–11) and class I DdPDE4. It is of further interest that each cyclic nucleotide pool is degraded by at least two enzymes with different kinetic properties: one enzyme has a high affinity for the substrate and a relatively low capacity, while the other enzyme has a lower affinity and higher capacity. These properties allow the degradation of the substrate over a wide range of concentrations to occur with approximately first order kinetics. At low substrate concentrations degradation mainly takes place by the high affinity enzyme. At intermediate substrate concentration the high affinity enzyme becomes saturated, and the low affinity enzyme takes over. Due to the high capacity of this low affinity enzyme, substantial degradation of substrate is possible even at very high concentrations.

Northern blot indicate that DdPDE4 is expressed mainly in the multicellular stage, while expression in the single cell stages is undetectable, confirming biochemical experiments. The phenotype of ddpde4-null cells also suggests that DdPDE4 does not significantly contribute to the degradation of extracellular cAMP during the single cell stage, because cell aggregation is not affected. The ddpde4-null mutant exhibits defective development from the mound stage until culmination, which is the developmental stage in which wild type cells show high expression of DdPDE4. In ddpde4-null cells the normal shaped mounds develop more slowly to slugs and fruiting bodies, which are much smaller than in control cells. The apparent $K_m$ of 0.8 μM cAMP for DdPDE1 (8) matches the dynamic range of the cAMP concentration during aggregation where cAMP waves reach concentrations up to ~1 μM (51). During mound and slug stage intercellular cAMP is expected to reach significantly higher levels due to the small intercellular space (1). At such high concentrations DdPDE1 will be saturated with cAMP. DdPDE4 with a $K_m$ of 10 μM may be in a better position to match the dynamic range of cAMP in the multicellular stage.

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