A Diverse Family of Inositol 5-Phosphatases Playing a Role in Growth and Development in *Dictyostelium discoideum*[^s]

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Inositol phosphate-containing molecules play an important role in a broad range of cellular processes. Inositol 5-phosphatases participate in the regulation of these signaling molecules. We have identified four inositol 5-phosphatases in *Dictyostelium discoideum*, Dd5P1-4, showing a high diversity in domain composition. Dd5P1 possesses only an inositol 5-phosphatase catalytic domain. An unique domain composition is present in Dd5P2 containing a RCC1-like domain. RCC1 has a seven-bladed propeller structure and interacts with G-proteins. Dd5P3 and Dd5P4 have a domain composition similar to human Synaptojanin with a Sac1 domain and OCRL with a RhoGAP domain, respectively. We have expressed the catalytic domains and show that these inositol 5-phosphatases have different substrate preferences. Single and double gene inactivation suggest a functional redundancy for Dd5P1, Dd5P2, and Dd5P3. Inactivation of the gene coding for Dd5P4 leads to defects in growth and development. These defects are restored by the expression of the complete protein but not by the 5-phosphatase catalytic domain.

Inositol phosphates play a role in a variety of eukaryotic cellular processes, including chemotaxis and membrane trafficking. They are regulated by a number of enzymes. The group of phosphatidylinositol 3-kinases (PI3K) phosphorylates the lipid substrates PI, PI(4)P, and PI(4,5)P₂ at the 3-position of the inositol ring (1). The lipid product PI(3,4,5)P₃ has been shown to transiently localize at the leading edge of cells moving in a chemotactic gradient (13–15). As these PH domains bind to different members of a number of proteins involved in chemotaxis, including CRAC, Akt/PKB, and PhdA, have been shown to transiently localize at the leading edge of cells moving in a chemotactic gradient (13–15). As these PH domains bind to Dd5P1/2-null cells, a strain with two putative PI3Ks inactivated (16), the transient localization of PhdA can no longer be observed and cells show reduced chemotaxis (15). On the other hand, in PTEN-null cells, a strain in which a putative 5-phosphatase is inactivated, the localization of PH-domains is prolonged and broadened and chemotaxis is also reduced (17, 18). Inositol 5-phosphatases may play an important role in the regulation of the phosphoinositides. As this group of enzymes leads to the degradation of PI(3,4,5)P₃ and at the same time formation of PI(3,4)P₂, another PH-binding molecule, they can be central players in the metabolic route of these signaling molecules.

Phosphoinositides have also been implicated in endocytosis in *D. discoideum*. Dd5K1/2-null cells are affected with respect to pinocytosis (19), suggesting a role for PI(3,4,5)P₃ in this process. The inhibitors of phospholipase C, an enzyme converting PI(4,5)P₂ into Ins(1,4,5)P₃ and diacylglycerol, reduce the rate of phagocytosis (20, 21). Because inositol 5-phosphatases can act on PI(4,5)P₂, PI(3,4,5)P₃, and Ins(1,4,5)P₃, they are probably important in the endocytic pathway.

To investigate the role of inositol 5-phosphatases in chemotaxis and endocytosis, we cloned and characterized four *D. discoideum* inositol 5-phosphatases. Catalytic activity was determined, indicating that they act as inositol 5-phosphatases. Single and double gene disruptants were obtained and growth, chemotaxis, and development were studied in these knock-out strains.
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Identification and Sequence Analysis—The first putative inositol 5-phosphatase sequence was obtained using degenerated primers complementary to the conserved motifs I and II found in inositol 5-phosphatases (see "Results"), and the PCR product was used to screen a cDNA library kindly provided by Dr. R. A. Firtel. The D. discoideum genomic (www.sdsc.edu/mpr/dicty) and cDNA databases (www.csm.biol.tsukuba.ac.jp) were screened for other putative inositol 5-phosphatases using either the conserved motif I or II. Using Sequenac from Limerick, Ireland, contigs were formed. Additional sequences were obtained by screening a cDNA library kindly provided by Dr. R. H. Gomer, with a PCR fragment containing part of the catalytic domain (used primers: Dd5P2: 5'PS21 + 5'P2R1; Dd5P3: 5'PS1 + 5'PS1; and Dd5P4: 5'PS1 + 5'P4R1, see "Appendix A"). The longest clones obtained for Dd5P3 (2421 bp) and Dd5P4 (2795 bp) encode the complete open reading frame of 1377 and 787 amino acids, respectively. For Dd5P2, the longest clone only encoded for the amino acid 1–599. In combination with data from the genomic data base, a complete open reading frame of 1794 amino acids was constructed. The longest clone obtained for Dd5P1 encodes for amino acids 118–678; the missing part of the 5’-open reading frame was obtained from the data base. A comparison of the sequence obtained from the cDNA clones with the genomic data base sequences revealed the presence of one intron in each inositol 5-phosphatase gene (Dd5P1: nucleotides 397–499; Dd5P2: nucleotides 53–187; Dd5P3: nucleotides 241–384; and Dd5P4: nucleotides 1357–1622) (GenBank™ accession numbers AY184992, AY184993, AY184994, and AY184995, respectively). BLAST, Smart, Pfam, and Expasy programs were used to analyze the obtained sequences. Alignments were made using Clustal V (19) and GeneDoc followed by optimizing by eye.

Strains and Growth Conditions—The D. discoideum strains AX3 (wild type), DH1 (ura3-52, trp1-289, his4-200, met14-112, hyp2-10, and pyr2-1), and DH6 (Dd5P1::M13) were used. Growth on bacterial lawns was studied using EN3Hance spray (PerkinElmer Life Sciences) and scraped from the surface of the bacterial lawn using 25-mm diameter forceps. The first potential inositol 5-phosphatase in D. discoideum, the domain was excised from the pMB74 construct. The inositol 5-phosphatase catalytic domain, the domain was excised from the pMB74 construct (see above) with BamHI and BglII and cloned into the BamHI site of the pGemBlgE vector. The insert was sequenced and cloned into the pGemBlgE site of pRSETB. The insert was sequenced and cloned into the BlgII site of pRSETB.

Activity Measurements—Activity toward Ins(1,4,5)P3 and Ins(1,3,4,5)P4 was determined as described previously (23). 5 μl (diluted) of enzyme was added to the reaction mixture (50 μl HEPES, pH 7.4, 2 mM MgCl2, 48 mM β-mercaptoethanol, 1 mg/ml bovine serum albumin), containing either 100 μM Ins(1,4,5)P3 or 50 μM Ins(1,3,4,5)P4. After 15 min of incubation the reaction was stopped and the products were separated on Dowex columns.

RESULTS

Identification of Four Inositol 5-Phosphatase in D. discoideum—The first potential inositol 5-phosphatase in D. discoideum was identified by performing a PCR with degenerated primers. The obtained PCR product was used as probe for cDNA library screening. The gene found using this method was called Dd5P1 (D. discoideum 5-phosphatase 1) and codes for a protein of 678 amino acids (see "Appendix B"). To identify other putative inositol 5-phosphatases in D. discoideum, we sequenced the D. discoideum database with probes for sequences showing homology to the conserved motifs I and II, WXXGDXXN/Y/F/R and PA/S/W(C/T)/DR/I/V/L, respectively, which are characteristic for inositol 5-phosphatases (6). Using the partial sequence obtained from the data base, complete sequences were obtained from cDNA library screens. Three putative inositol 5-phosphatases were identified (see "Appendix B").
The other two inositol 5-phosphatases identified in *D. discoideum* are homologous to human inositol 5-phosphatases. Dd5P3 resembles the synaptojanin-like proteins found in both human and yeast with the highest BLAST score of the catalytic domain with that of INP55b from fission yeast (37% identity, 59% similarity) (34). Similar to human Synaptojanin and yeast INP52p, Dd5P3 has a Sac1-like domain including the conserved RXCXDCLRTN motif (35) in front of the inositol 5-phosphatase domain (see “Appendix E”). The Sac1 domains of Synaptojanin and INP52p have been shown to remove the phosphate group of PI(4)P, PI(3)P, and at a low rate, both phosphates of PI(3,5)P2 (36). The long C-terminal part of Dd5P3 does not have any homology with known domains and consists of poly(Q) and poly(N) repeats.

Dd5P4 is homologous to human OCRL (10) and INPP5b (37, 38), consisting of a inositol 5-phosphatase domain followed by a RhoGAP domain (see “Appendix F”). The catalytic domain has the highest BLAST score with the catalytic domain of human INP55b (44% identity, 60% similarity). RhoGAP domains are known to catalyze the GTPase activity of Rho proteins. The crystal structure of human RhoGAP has been solved indicating a role for two conserved amino acids, Arg-85p50RhoGAP and Asn-194p50RhoGAP (39) in GAP-activated GTP hydrolysis (39). The role of these amino acids is supported by mutational analysis (39, 40). Mutational analysis has also shown that the conserved Arg is not predominantly involved in the binding of Rho proteins (40). In Dd5P4, the Arg and the Asn are substituted by an Ile and Gln, respectively. This observation may suggest that Dd5P4 does not exhibit high RhoGAP activity, but may still bind Rho proteins.

**Different Transcription Levels during Development**—To determine the transcription levels of the *D. discoideum* inositol 5-phosphatases, Northern blot analysis was performed. Very low transcription levels were observed for the four inositol 5-phosphatases in all stages of the *D. discoideum* life cycle (Fig. 3). Dd5P1 and Dd5P3 were equally transcribed in all stages with the exception of the even lower transcription of Dd5P3 in the vegetative stage. The levels of transcription of Dd5P2 were higher during aggregation than during growth and multicellular development. The transcription of Dd5P4 was relatively high during growth, decreased during aggregation, and returned to almost vegetative levels in the multicellular stages. Furthermore, a smaller transcript of 2.5 kilobases was observed in the multicellular stages.

**Activity toward Soluble and Lipid Substrates of *D. discoideum* Inositol 5-Phosphatases**—To determine whether the four putative inositol 5-phosphatases identified in *D. discoideum* can function as inositol 5-phosphatases, the catalytic activity and specificity of the inositol 5-phosphatase domains were studied in *vitro*. The catalytic domains were expressed as His tag fusion proteins in *E. coli* and purified. The protein of the expected size could be detected by Western blot analysis for Dd5P1–3 (Fig. 4). The Western blot analysis performed for Dd5P4 showed a band at a higher position than expected (62 instead of 55 kDa), but the purified protein did show inositol 5-phosphatase activity. Unfortunately, because of the very low expression levels obtained for the catalytic domain of Dd5P1, no activity could be determined for this protein.

The purified catalytic domains of Dd5P2, Dd5P3, and Dd5P4 were incubated with the phospholipids PI(4,5)P2 and PI(3,4,5)P3 and the water-soluble inositol phosphates Ins(1,4,5)P3 and Ins(1,3,4,5)P4. Substrate degradation was quantified using thin-layer chromatography for the phospholipids and ion-exchange chromatography for the water-soluble inositol phosphates. The results are summarized in Fig. 5 demonstrating good degradation of PI(4,5)P2 by all inositol 5-phosphatases,

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**Fig. 1. Domain composition of the *D. discoideum* inositol 5-phosphatases Dd5P1–4 and the human inositol 5-phosphatases Synaptojanin (Syj), OCRL, and SHIP1.** The black region represents the inositol 5-phosphatase catalytic domain. RCC1, Sac1, RhoGAP (GAP), and SH2-like domains are shown in gray. Inverted triangles indicate the position of the introns, whereas asterisks indicate the place of gene disruption.

1800, 1377, and 787 amino acids, respectively. A comparison of the sequence obtained from cDNA clones with the genomic data base sequences revealed the presence of one intron in each inositol 5-phosphatase gene. The position of the introns is indicated by a triangle in Fig. 1.

**High Diversity in Inositol 5-Phosphatase Domain Composition Present in *D. discoideum*—**Alignment of the catalytic domain of the four *D. discoideum* inositol 5-phosphatases with other inositol 5-phosphatase catalytic domains shows a high degree of identity between the amino acid sequence of the four proteins and type II inositol 5-phosphatases (Fig. 2) (see “Appendix C”). All of the four proteins contain the conserved amino acids present in motifs I and II that are important for inositol 5-phosphatase activity (26–28). Also, the amino acids strongly conserved in other regions of the inositol 5-phosphatase domains are present in all four *D. discoideum* inositol 5-phosphatases, suggesting that they are active inositol 5-phosphatases.

The four inositol 5-phosphatases differ with respect to their domain composition (Fig. 1). Dd5P1 shows the least complex composition containing only the inositol 5-phosphatase domain. A BLAST analysis with the catalytic domain shows the highest score (Expect value = 2e−54) with the catalytic domain of human SHIP2 (30% identity, 44% similarity) (29). This score is clearly higher than scores for non-SHIP-like proteins with the highest score for inositol polyphosphate 5-phosphatase II of *Mus musculus* (Expect value = 7e−57). The homology with SHIP2 does not expand to the N-terminal part of the protein, because the SH2 domain present in SHIP2 is not present in Dd5P1.

Besides the inositol 5-phosphatase domain, Dd5P2 contains a region homologous to RCC1 (“Appendix D”) (30). The structure of RCC1 has been solved, revealing a seven-bladed propeller with each blade comprising by four β-sheets (31). RCC1 is known to bind Ran, a small G-protein present at high concentrations in the nucleus. The binding of RCC1 to Ran leads to an increased rate of exchange of GDP for GTP, thus acting as a guanine exchange factor for Ran (32). The highest BLAST score of the catalytic domain of Dd5P2 is shown with the catalytic domain of mouse inositol polyphosphate 5-phosphatase b (INPP5b), a RhoGAP domain containing protein (41% identity, 59% similarity) (33). The long C terminus of Dd5P2 (~1000 amino acids) does not represent a known domain structure. Three poly(N) stretches and a poly(D/E) stretch are present in this C-terminal part of the protein. These kind of stretches are not unusual in *D. discoideum* proteins.
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The catalytic domains were expressed in E. coli, purified by binding to nickel-nitriilotriacetic acid, and eluted with 3 ml of imidazole-containing binding buffer. The proteins were visualized using Coomassie Brilliant Blue staining (A) or using a His-tag antibody followed by chemiluminescence (B). The amount of elute loaded onto the gel was 10 μl of each sample (A) or 7.5, 1.5, 1.5, 0.05, and 7.5 μl for Dd5P1, Dd5P2, Dd5P3, Dd5P4, and empty pRSETB vector, respectively (B). Sizes of standards (in kilodaltons) are shown on the left. The arrows indicate a band at the expected size for Dd5P1.

whereas PI(3,4,5)P₃ is degraded predominantly by Dd5P2 and also by Dd5P4 but not by Dd5P3. Good degradation of the water-soluble inositol phosphates Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ is observed for Dd5P2, Dd5P3, and Dd5P4, showing the best degradation of Ins(1,4,5)P₃ by Dd5P3, whereas Ins(1,3,4,5)P₄ is best degraded by Dd5P2 (Fig. 5C). Comparing the activity of the four enzymes for each substrate indicates the relatively preferred substrates (Table I). The protein with the highest activity toward Ins(1,4,5)P₃ is the catalytic domain of Dd5P2. This protein, compared with the other proteins, is poor in dephosphorylation of lipid-soluble substrates. This substrate specificity is almost opposite to the specificity of the homologue INP51p, which degrades PI(4,5)P₂ but does not hydrolyze Ins(1,4,5)P₃. Dd5P2, in contrast, is a very good PI(3,4,5)P₃- and Ins(1,3,4,5)P₄-metabolizing enzyme.

Gene Inactivation Leads to Defects in Growth and Development—Upon starvation, D. discoideum wild-type cells show chemotaxis toward the cAMP secreted by other starving cells. The formed aggregate develops into a migrating slug or fruiting body. The spores of the fruiting body are resistant to severe conditions and mature into single amoeba under better conditions. To get an indication of the function of inositol 5-phosphatases in D. discoideum, the four identified inositol 5-phosphatase genes have been knocked out. The effect of gene inactivation on chemotaxis has been investigated by determining the response of cells toward different cAMP concentrations using the small-droplet chemotaxis assay. Single gene inactivation of either Dd5P3 or Dd5P4 or double gene inactivation of Dd5P2 and Dd5P3 (Dd5P2/Dd5P3) did not negatively affect chemotaxis, whereas single disruption of Dd5P1 or Dd5P2 slightly improved chemotaxis (Fig. 6). Also, the double disruption of either Dd5P1 and Dd5P2 (Dd5P1/Dd5P2) or Dd5P1 and Dd5P3 (Dd5P1/Dd5P3) resulted in slightly improved chemotaxis. Growth and development of the cells were also studied. The single gene inactivation of Dd5P1, Dd5P2, or Dd5P3 did not result in any observable difference in growth rate in axenic medium or on bacterial lawns (data not shown). The single disruptants Dd5P1*, Dd5P2*, and Dd5P3* were deposited on non-nutrient agar plates or grown on bacterial lawns to study the development of the cells. Aggregation of the cells proceeded at a rate...
Inositol 5-Phosphatases in D. discoideum

The implicated role of phosphoinositide molecules in signal localization has led to an increased interest in metabolizing enzymes such as inositol 5-phosphatases. The amount of data on mammalian inositol 5-phosphatases has expanded rapidly over the last few years, showing important functions for inositol 5-phosphatases in several processes (6).

Human inositol 5-phosphatases are divided into two groups. Type I inositol 5-phosphatases only convert the water-soluble substrates Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄. They do not convert lipid substrates. Type II inositol 5-phosphatases do convert phosphoinositides (e.g. PI(4,5)P₂ and PI(3,4,5)P₃) and in most cases, they also convert water-soluble substrates. The insertions present in Type I enzymes may prevent the enzymes from binding to the membrane surface, which may explain the differences in substrate specificity between Types I and II inositol phosphatases.

**DISCUSSION**

7). Whereas the doubling time of wild-type cells is ~15 h in shaking culture, the doubling time of Dd5P4-deficient cells is 41 h. The growth rate on bacterial lawns was affected as well for Dd5P4⁻ cells. Amoeba were deposited on a bacterial lawn and incubated at 22 °C. Approximately 90% wild-type amoeba formed visible plaques within 4 days, whereas <1% Dd5P4⁻ cells formed visible plaques after 9 days. The development of the Dd5P4 cells when grown on bacterial lawns was affected as well. The inactivation of Dd5P4 lead to the formation of multiple-tipped aggregates (Fig. 8).

**Overexpression of Full-length Dd5P4 but Not the Separate Domains Rescues Dd5P4⁻ Cells**—The overexpression of the full-length Dd5P4 restored the defects of Dd5P4⁻ cells in growth and development, confirming that the disruption of Dd5P4 was indeed responsible for the observed defects. Growth rate in axenic medium was comparable to the growth rate of wild-type cells (AX3) with a doubling time of 15 h (Fig. 7). When grown on bacterial plates, ~90% of the amoeba formed visible plaques within 4 days, and the multiple-tipped phenotype observed for the Dd5P4⁻ cells could not be observed anymore (Fig. 8). Overexpression of the inositol 5-phosphatase catalytic domain or the RhoGAP domain of Dd5P4 in the Dd5P4 knockout strains (Dd5P4⁻/cat5P4 and Dd5P4⁻/GAP5P4, respectively) did not restore any of the defects. As can be seen in Fig. 7, growth rate in axenic medium was not enhanced by overexpression of either one of the domains. Also, growth rate and development on bacterial lawns were not restored (data not shown).

**TABLE I**

<table>
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<tr>
<th>Enzymes</th>
<th>Relative activity</th>
<th>( \text{Ins}(1,4,5)\text{P}_3 )</th>
<th>( \text{Ins}(1,3,4,5)\text{P}_4 )</th>
<th>( \text{PI}(4,5)\text{P}_2 )</th>
<th>( \text{PI}(3,4,5)\text{P}_3 )</th>
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<td>Dd5P1</td>
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<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
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<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Dd5P3</td>
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<td>+++</td>
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<tr>
<td>Dd5P4</td>
<td>+</td>
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**Fig. 5.** Activity of the catalytic domains of inositol 5-phosphatase Dd5P1–4. A, representative TLC analysis of the reaction toward \( \text{PI}(3,4,5)\text{P}_3 \) using 60 \( \mu \text{l} \) of elute for Dd5P3 and Dd5P4. Results for Dd5P1 were similar to results obtained for the negative control (empty pRSETB vector) (−). Results obtained for Dd5P2 were similar to results obtained for Dd5P4. \( \text{PI}(3,4,5)\text{P}_3 \) and \( \text{PI}(4,5)\text{P}_2 \) were used as standards. B, representative TLC analysis of the reaction toward \( \text{PI}(4,5)\text{P}_2 \) using 60 \( \mu \text{l} \) of elute for Dd5P2 and Dd5P3 and 30 \( \mu \text{l} \) of elute for Dd5P4. Results for Dd5P1 were similar to results obtained for the negative control (−). C, activity of Dd5P1–4 is expressed in either nanomoles of \( \text{Ins}(1,4,5)\text{P}_3 \) or picnolos of \( \text{Ins}(1,3,4,5)\text{P}_4 \) per min per microgram of protein or in percentage of degraded \( \text{PI}(4,5)\text{P}_2 \) or \( \text{PI}(3,4,5)\text{P}_3 \) per min per microgram of protein.

**Fig. 6.** Small droplet chemotaxis assay. Chemotaxis toward different concentrations of cAMP (10⁻³, 10⁻⁶, 10⁻⁹, and 10⁻⁶ M cAMP) was determined for wild-type AX3 and knock-out cell lines using the small droplet chemotaxis assay. Asterisks, significant differences from wild-type according to the Student’s t test (\( p < 0.001 \)) using data of all four cAMP concentrations; NS, no significant difference from wild type.
The four inositol 5-phosphatases in *D. discoideum* show a high diversity in domain composition compared with the evolutionary position of this organism between plants and yeast. A homologue of OCR1 (*Dd5P4*) and a homologue of Synaptojanin (*Dd5P3*) were identified. Also, a unique combination of a RCC1-like domain in front of the inositol 5-phosphatase domain was identified (*Dd5P2*). This combination of domains has not been found in any other protein reported or present in the GenBank™ so far.

Knock-out strains with one or a combination of two inactive genes for *Dd5P1*, *Dd5P2*, and *Dd5P3* show no defects in growth and development, suggesting a redundancy among these proteins. Chemotaxis toward cAMP is similar or slightly improved in all of the inositol 5-phosphatase knock-out cell lines compared with wild type. Recently, it has been shown that the 3-phosphatase PTEN is the major PI(3,4,5)P3/PI(3,4)P2-degrading enzyme. It has been suggested that inositol 5-phosphatases add an addition layer of regulation of these molecules, fine-tuning the chemotactic signal (42). Our results support the role of the group of inositol 5-phosphatases as a minor negative regulator of chemotaxis in *D. discoideum*. It will be interesting to study the effects of either overexpression or inactivation of the inositol 5-phosphatases in a PTEN-null background.

Inactivation of *Dd5P4* resulted in defects in growth and development. The reduced growth rate, either in axenic culture or grown on bacterial plates, suggests a role for *Dd5P4* in endocytosis. Development is also affected as the cells form multiple-tipped aggregates. The relative high transcription levels of *Dd5P4* at vegetative and multicellular stages support the role of *Dd5P4* in growth and development. Knock-out strains of PI3K1 + 2 are defective in chemotaxis, growth, and development (15, 16). The cells grow slowly on bacterial lawns and in axenic medium and form multiple-tipped aggregates resembling the phenotype of *Dd5P4*. No enzyme activity measurements have been reported for the PI3K in *D. discoideum*, but they have been suggested to catalyze the formation of PI(3)P, PI(3,4)P2, and PI(3,4,5)P3 on basis of sequence homology. Our results support a role for PI(3,4,5)P3 and/or PI(3,4)P2 in growth and development; *Dd5P4* regulates the levels of these phospholipids by degradation of PI(3,4,5)P3 and production of PI(3,4)P2. On the other hand, the effect of inactivation of *Dd5P4* could also be assigned to its action on either PI(4,5)P2 or Ins(1,4,5)P3, two signaling molecules also implicated in endocytosis (20, 21). In addition, the RhoGAP domain could be responsible for the function of *Dd5P4* in endocytosis. Rho proteins have been shown to play a role in both development and growth. The defects in growth and development of *Dd5P4* cells can be rescued by overexpression of the full-length protein. These defects can not be restored by transfection of *D. discoideum* cells with an expression vector containing either the inositol 5-phosphatase catalytic domain or the RhoGAP domain. Although we can not exclude that the domains are not properly expressed or folded, the fact that the inositol 5-phosphatase catalytic domain expressed in *E. coli* is catalytically active would suggest that inositol 5-phosphatase activity is not sufficient to restore the defects of *Dd5P4* cells. It is possible that the RhoGAP and inositol 5-phosphatase catalytic domain act together to perform its function in growth. The binding of a Rho protein to the RhoGAP domain could affect the inositol 5-phosphatase activity. This would lead to a direct interaction between the Rho and phosphoinositide pathways. It would be interesting to see if and which Rho protein binds to the GAP domain of *Dd5P4*.

**Acknowledgments**—We are indebted to all of the teams involved in the *Dictyostelium* sequencing projects. We thank L. Drayer and H. Otsuka for their contribution in cloning and analyzing *Dd5P1*.  

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**Fig. 7. Growth rate of wild type and mutant strains.** Doubling times were 15 h for wild-type AX3, 41 h for *Dd5P4*<sup>−</sup>, 15 h for *Dd5P4*/<sup>−</sup>*Dd5P4*, 47 h for *Dd5P4*/<sup>−</sup>*cat5P4*, and 48 h for *Dd5P4*/<sup>−</sup>*GAP5P4*.

**Fig. 8. Development of wild-type AX3, *Dd5P4*<sup>−</sup>, and *Dd5P4*/<sup>−</sup>*Dd5P4* on *K. aerogens*.** Pictures were taken of tipped aggregates using ×40 magnification.

5-phosphatases (28). The insertions present in Type I inositol 5-phosphatases are not present in either one of the *D. discoideum* inositol 5-phosphatases. As expected by this sequence analysis, the *D. discoideum* 5-phosphatases *Dd5P2*, *Dd5P3*, and *Dd5P4* catalyze the dephosphorylation of both water-soluble and lipid substrates, which classify them as Type II inositol 5-phosphatases. The *D. discoideum* genome has been sequenced to near completion, making it likely that no inositol 5-phosphatases containing the motifs WXGDXN/Y/F/R and PA/S/W/C/T/DR/I/V/L are present. Therefore, probably no Type I and only four Type II inositol 5-phosphatases are present in *D. discoideum*.

Type II inositol 5-phosphatases can be divided in three subgroups on the basis of domain composition. SHIP1 represents the group of SH2 domain containing inositol 5-phosphatases Synaptotagmin, the Sac1 domain containing enzymes, and OCRL, the RhoGAP domain containing inositol 5-phosphatases (Fig. 1). Mammals contain all three groups of proteins. Six non-mammalian organisms containing putative inositol 5-phosphatases have been sequenced completely so far. The metazoa *C. elegans* and *D. melanogaster* lack inositol 5-phosphatases containing a SH2 domain, but both contain one Synaptotagmin-like protein (41) and Q9W296, respectively) and one OCRL-like protein (O17590 and O46049, respectively). The diversity in the plant *Arabidopsis thaliana*, the yeast strains *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and the microsporidia *Encephalitozoon cuniculi* is even less. The inositol 5-phosphatases of *A. thaliana* contain only WD40 repeats as additional domains (e.g. Q8SKB7 and O80560), whereas in yeast only Synaptotagmin-like proteins are present (28, 34). The one inositol 5-phosphatase of *E. cuniculi* (CAD25856) only contains the inositol 5-phosphatase catalytic domain. In prokaryotes, no inositol 5-phosphatases have been identified.