Fission yeast orb6, a ser/thr protein kinase related to mammalian rho kinase and myotonic dystrophy kinase, is required for maintenance of cell polarity and coordinates cell morphogenesis with the cell cycle

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ABSTRACT The molecular mechanisms that coordinate cell morphogenesis with the cell cycle remain largely unknown. We have investigated this process in fission yeast where changes in polarized cell growth are coupled with cell cycle progression. The orb6 gene is required during interphase to maintain cell polarity and encodes a serine/threonine protein kinase, belonging to the myotonic dystrophy kinase/col1/warts family. A decrease in Orb6 protein levels leads to loss of polarized cell shape and to mitotic advance, whereas an increase in Orb6 levels maintains polarized growth and delays mitosis by affecting the p34cdc2 mitotic kinase. Thus the Orb6 increase in Orb6 levels maintains polarized growth and delays loss of polarized cell shape and to mitotic advance, whereas an

Eukaryotic cells establish and maintain polarized cellular domains that are essential for intracellular transport, cell differentiation, cell shape, and cell locomotion (1). Changes in cell shape and cell polarity occur during the cell cycle and in response to exogenous signals, but little is known about how cell morphogenesis is regulated or how polarized cell growth is integrated with the cell cycle. Fission yeast Schizosaccharomyces pombe cells grow in a polarized fashion that undergoes distinct changes during the cell cycle. Cells grow from one tip during G1 and S-phase, and activate the second tip during early G2, becoming bipolar in growth (2). Actin dots are located at one end of the cell when cells are growing with one tip and are found at both ends when cells grow in a bipolar manner (3). At the onset of mitosis, polarized cell growth ceases, and actin relocalizes from the cell tips to the middle of the cell where an actin ring forms. Both activation of bipolar growth and onset of mitosis require the attainment of a minimal cell size (2, 4).

In fission yeast, cell polarity and morphology is thought to be regulated by the product of the ras homologue, ras1, acting upstream of the Cdc42 GTPase (5, 6). Ras1 and Cdc42 are part of a complex including Scr1, the putative Cdc42 guanine nucleotide exchange factor, and Scd2, a Sra homologue (3SH3) domain-containing protein (6). Cdc42 also has been shown to be associated with the serine/threonine kinase Pak1/SHK1, a homologue of mammalian PAKs kinases and the budding yeast Ste20 protein kinase (7). In fission yeast, pak1/shk1 has been shown to be essential for cell viability and to participate in the Ras1-dependent mating response pathway (8, 9). Although overexpression studies suggest a role for the Pak1/SHK1 kinase in the control of cell morphology (9), the molecular details of Pak1/SHK1-dependent regulation of the cytoskeleton have yet to be fully clarified.

We previously identified 19 genes that are required for various aspects of cell morphogenesis (10). The orb6 gene is required to maintain cell polarity throughout the interphase period of the cell cycle. Mutants in orb6 lose growth polarity and become spherical with disorganized microtubule arrays and delocalized actin dots (10). In this paper, we report the cloning of Orb6 and its identification as a fission yeast kinase closely related to a number of higher eukaryotic kinases, including Drosophila warts kinase, mammalian Rho-associated kinase, and the human myotonic dystrophy kinase (DMPK). We show that Orb6 is required for maintenance of cell polarity during interphase and to promote actin reorganization during morphological transitions. Moreover, we demonstrate that Orb6 also has another role during the cell cycle, specifically to delay onset of mitosis, suggesting that it functions to coordinate cell morphogenesis with the cell cycle. Finally, we show that orb6 interacts genetically with Pak1/SHK1 protein kinase and that pak1/shk1 is required for proper Orb6 intracellular localization. We propose that the Orb6 kinase acts downstream of a morphogenetic control pathway involving Cdc42 and Pak1/Shk1, which maintains the cell in a polarized state during interphase while simultaneously delaying the onset of mitosis.

MATERIALS AND METHODS

Strains and Growth Conditions. The strains used in this paper were orb6–25 ade6-M210 leu1–32h– orb2–34 ade6-M210 leu1–32h– wee1–50 leu1–32h– and leu1–32h–. Cells were cultivated in rich medium (yeast extract liquid or agar), or Edinburgh minimal medium liquid with the appropriate supplements (11), as indicated. Cells in liquid cultures had been growing exponentially for at least eight generations, at densities below 107 cells/ml, before observation.

Libraries and Transformation Procedures. The libraries used for cloning orb6 and orb2 were a cDNA library cloned in the plasmid Rep3X (29) and a genomic library in plasmid pDB248 (30). Cells were transformed by the lithium acetate procedure (11).

This paper was submitted directly (Track II) to the Proceedings office. Abbreviations: HA, hemagglutinin; DMPK, myotonic dystrophy kinase.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. AF009512).

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Cloning of orb+*. orb6 mutants were transformed with a cDNA library controlled by the thiamine-repressible nmt1 promoter (29); 145,000 colonies were screened for transformants showing reversion of lethality and morphological phenotype. The screen yielded 14 rescuing plasmids. Nine plasmids (pFV10, 44, 39, 6, 75, 42, 23, 271, and 622) rescued both lethality and shape defects under low level of expression (in thiamine-containing medium). They all identified the same gene sequence. One plasmid, pFV10, was chosen and used for all successive experiments. The coding sequence was cloned in a REP6X plasmid (containing the sup3–5 sequence) and integrated in an orb6–25 ade–6–704 strain. Thirty-four integrants were isolated and crossed to a wild-type strain: on average one mutant colony appeared for 500 colonies screened, indicating integration at the orb6 locus and consistent with a 0.5–1 cm distance between the mutant gene and its wild-type integrated copy. As a control, one of these integrants (number 27) was crossed to an orb6–25 ade–6–704 strain: orb6+ colonies were found to be adenine prototrophs, indicating that the rescuing sequence was linked to the sup3–5 marker and excluding the possibility of reversion. Plasmid pFV10 contained a single continuous ORF of 1,410 bp.

Deletion of orb+. We identified a genomic clone containing the orb6 sequence again by complementation of orb6 mutants. The genomic sequence, ~10 kilobases long, was shown by PCR and Southern blotting analysis to contain the orb6+ gene, with a 267-bp intron. The deletion construct, substituting the whole catalytic domain of orb6 with the ura4+ gene sequence, was built leaving a 2.9-kilobase 5' flanking sequence and 0.530-kilobase 3' flanking sequence.

Cloning of orb2+. To clone the orb2 gene, we identified a 12-kilobase suppressing genomic fragment that was shown to integrate very closely to the orb2–34 mutation. We identified the suppressing gene by transposon knockout (12) and sequenced outwardly from the transposon insertion site. The orb2–34 mutation also was suppressed by transformation with pak1/shk1 cDNA, which was a kind gift of J. Chernoff (Fox Chase Cancer Center, Philadelphia) (9).

Cytology. Cells were grown exponentially at 25°C for at least eight generations, then incubated at the indicated temperature for the indicated time. Immunofluorescence was performed as described (13). Cells were fixed in methanol and stained with mouse anti-actin antibody (Sigma) or anti-hemaggutatin (HA) primary antibody (Babco, Richmond, CA) and a CY3-conjugated anti-mouse secondary antibody. Cells then were immobilized on coverslips and observed using a Zeiss Axioskop microscope. Cells were photographed using a Bio-Rad 600 confocal microscope, with a 0.2-μm interval between successive pictures and analyzed using Bio-Rad software. For quantification of actin delocalization, the two-dimensional projections of the confocal microscope pictures were analyzed by using National Institutes of Health IMAGE software, measuring the intensity of immunofluorescence signal in different areas of the cell (% of intensity throughout the cell and at the cell tips). Ten cells were measured for each time point. Finally, the penetrance of the corresponding phenotype was quantified in the original sample, by counting 200 cells for each time point.

RESULTS

**orb6 Encodes a Serine Threonine Protein Kinase.** Mutant orb6 cells are round at restrictive temperature (Fig. 1b). Moreover, the number of cells undergoing the process of cell division increases from 14% to 60% after 5 hr at 36°C: 15% of these septa are aberrant (see Fig. 5b). The orb6 gene was cloned by complementation. orb6 mutants were transformed with a cDNA library controlled by the thiamine-repressible nmt1 promoter (29); 145,000 colonies were screened for transformants showing a rescue of the morphological phenotype. The screen yielded 14 rescuing plasmids. Nine plasmids contained the same gene sequence that was shown by inte-
construct deleted all 11 kinase domains in the orb6 gene, leaving 106 bp at the 5’ end and 224 bp at the 3’ end. The ura4+ gene was substituted for the whole orb6 kinase domain by the one-step gene replacement method in diploids cells using this deletion construct (11). Deletion of the orb6 gene led to cell lethality, establishing that orb6 is essential. Spores deleted for the orb6 gene germinate to form spherical cells that undergo 3–4 cell divisions before lysing (Fig. 2b). Lysis is not prevented by addition of 1 M sorbitol as an osmotic stabilizer.

orb6 Is Necessary for Actin Reorganization After Mitosis and During Activation of Bipolar Growth. To investigate Orb6 function more fully we constructed a triple HA-tagged orb6 gene under control of the thiamine repressible nmt1 promoter. This construct was integrated into the genome of a strain in which the endogenous orb6+ gene was replaced by the ura4+ marker (orb6::ura4+). When orb6-HA was expressed in the absence of thiamine, this strain was perfectly viable, showing a generation time of 2 hr and 50 min (at 32°C) as compared with the generation time of 2 hr and 40 min of the ade6-M210 control (determined by monitoring the optical density and the cell number increase of the cell culture). Cells displayed a normal cell morphology with a correctly localized actin cytoskeleton (Fig. 3a and d). In contrast, when thiamine was added to repress orb6-HA expression, we observed changes in cell morphology and a reorganization of the actin cytoskeleton. Early in the time course, between 4 and 8 hr after thiamine addition, cell length shortened (Fig. 3b) and actin became less localized at the cell tips. Delocalization of 61.5% of actin was found in 67% of cells, as compared with time zero where 91% of actin was found localized at the cell tips (see Materials and Methods) (Fig. 3e). Most cells showed a monopolar pattern of growth (see below). Actin dots also were found in a ring pattern at the new end after septation in 10% of cells, suggesting a delay in actin reorganization after mitosis (Fig. 3e, arrow). Later in the time course, 12 hr after thiamine addition, cells became spherical and actin was found dispersed throughout the cell cortex in 81% of cells (Fig. 3c and f), with the exception of actin clusters, which persisted in the area where

Table 1. orb6 gene dosage effects

<table>
<thead>
<tr>
<th>Strain</th>
<th>Plasmid</th>
<th>Cell length at division</th>
<th>% wt length</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) leu1-32</td>
<td>Multicopy plasmid (Rep3X)</td>
<td>14.6 ± 1.0</td>
<td>100%</td>
</tr>
<tr>
<td>(1) leu1-32</td>
<td>Integrant nmt1 orb6HA</td>
<td>17.8 ± 1.9</td>
<td>121%</td>
</tr>
<tr>
<td>(1) leu1-32</td>
<td>Multicopy nmt1 orb6HA</td>
<td>26.6 ± 4.9</td>
<td>181%</td>
</tr>
<tr>
<td>(1) leu1-32</td>
<td>Multicopy nmt1 orb6HA ala122</td>
<td>13.7 ± 2.7</td>
<td>93%</td>
</tr>
<tr>
<td>(2) leu1-32</td>
<td>Multicopy plasmid (Rep3X)</td>
<td>14.2 ± 1.0</td>
<td>100%</td>
</tr>
<tr>
<td>(2) leu1-32</td>
<td>Multicopy nmt1 orb6HA</td>
<td>28.9 ± 5.0</td>
<td>203%</td>
</tr>
<tr>
<td>△ orb6</td>
<td>Integrant nmt1 orb6HA</td>
<td>18.0 ± 1.3</td>
<td>122%</td>
</tr>
<tr>
<td>△ orb6</td>
<td>Integrant nmt1 orb6HA (+th., 5h.)</td>
<td>12.4 ± 0.7</td>
<td>84%</td>
</tr>
<tr>
<td>leu1-32</td>
<td>Multicopy plasmid (Rep3X)</td>
<td>9.5 ± 0.6</td>
<td>65%</td>
</tr>
<tr>
<td>leu1-32</td>
<td>Multicopy nmt1 orb6HA (+th., 7.5h.)</td>
<td>20.0 ± 5.6</td>
<td>137%</td>
</tr>
<tr>
<td>wee1-50 leu1-32</td>
<td>Multicopy plasmid (Rep3X)</td>
<td>7.4 ± 0.9</td>
<td>50%</td>
</tr>
<tr>
<td>wee1-50 leu1-32</td>
<td>Multicopy nmt1 orb6HA</td>
<td>7.5 ± 0.9</td>
<td>51%</td>
</tr>
</tbody>
</table>

*Thirty cells at division were measured for each sample. The mean values ± SD are shown.
caused by a reduction in cell growth rate, which was unchanged until 8.5 hr after thiamine addition (data not shown).

**Orb6 Acts as a Dose-Dependent Inhibitor of Mitosis.** To confirm the function of Orb6 in the regulation of mitosis, we overexpressed orb6-HA in wild-type cells (Fig. 4 and Table 1). Expressing orb6-HA from a single-copy integrant caused cells to divide at a larger cell size (Fig. 4b). Expression of orb6-HA on a multicopy plasmid using the nmt1 promoter led to an increase of cell length at division, indicating that onset of mitosis was substantially delayed (Fig. 4c). FACS analysis established that these cells were delayed in the G2 phase of the cell cycle (data not shown). This delay of mitotic onset required Orb6 protein kinase activity because an orb6 kinase inactive mutant (K122A) did not induce cell elongation when overexpressed (Table 1). orb6-HA overexpression in a wee1–50 strain, which is defective in the Wee1 tyrosine protein kinase that phosphorylates and inhibits p34cdc2, failed to increase cell length at division (Fig. 4d-f and Table 1). This experiment demonstrated that the Orb6-dependent delay over mitosis acts through the p34cdc2 mitotic kinase. Overexpression of wee1t in orb6–25 mutant cells did not suppress their morphological defects (Fig. 5a–d). Moreover, wee1–50 and orb6–25 mutants show a different morphological phenotype at restrictive temperature; orb6–25 cells are round and their actin cytoskeleton is dispersed (Fig. 5e, h) whereas wee1–50 cells are very short, but still grow in a polarized fashion (Fig. 5e) and show a polarized actin cytoskeleton (Fig. 5h). These experiments indicate that Orb6 kinase has a specific function in the control of cell morphology, maintaining a polarized actin cytoskeleton and promoting polarized cell growth, while delaying the onset of mitosis.

**Orb6 Localizes to Sites of Cell Growth.** The intracellular location of Orb6-HA protein was determined by using the Orb6-HA integrant strain (Fig. 6). Immunofluorescence showed that Orb6-HA protein is enriched at the cell tips during interphase (Fig. 6a and e). During mitosis and cytokinesis Orb6-HA protein disappears from the tips and is found in the region of the developing septum (Fig. 6b and f), indicating that Orb6-HA protein localizes to areas of cellular growth. This observation was confirmed by staining Orb6-HA in cdc25Δ and orb2ts mutant cells. Both tips were stained in the cdc25–22ts mutant cells, which were arrested in G1 with both ends growing (Fig. 6c), whereas in the orb2–34 mutant, which cannot activate bipolar growth and only grows at one end (10) (Fig. 6h), the Orb6-HA protein was found only at one growing tip (Fig. 6d).

**Fig. 5.** Overexpression of Wee1 in orb6 mutants and actin localization in orb6–25 and wee1–50 mutants. (a–d) Overexpression of Wee1 in orb6 mutants. (a) leu1–32Δ2 hr Rep3Δx. (b) orb6–25 leu1–32Δ2 hr Rep3Δx. (c) leu1–32Δ2 hr Rep3Δx wee1+. (d) orb6–25 leu1–32Δ2 hr Rep3Δx wee1+. (e) wee1–50Δ2 hr. Cells were grown exponentially at 25°C in the presence of thiamine for at least eight generations; thiamine was washed away, and cells were grown in the absence of thiamine at 25°C for 16 hr, then shifted at 36°C for 5 hr. (f–h) Actin localization in orb6–25 and wee1–50 mutants. (f) leu1–32Δ2 hr. (g) orb6–25 leu1–32Δ2 hr. (h) wee1–50Δ2 hr. (Bar = 5 μm.)

The actin ring had formed during the previous cell division (in 12% of cells) (Fig. 5f, arrow). These observations indicate that when Orb6 levels decrease, the actin cytoskeleton becomes disorganized. Moreover, early in the time course, the reorganization of actin during the cell cycle transitions between monopolar and bipolar growth and between mitosis and interphase is defective, as indicated by an increase in the number of cells growing with one tip only (68% compared with 28% in orb6+ cells) or undergoing the process of cell division (24% compared with 14% in orb6+ cells) (Fig. 3b). We conclude that the Orb6 protein kinase is required to maintain polarity of the actin cytoskeleton during interphase and to promote actin reorganization both after mitosis and during activation of bipolar growth.

This experiment also revealed that Orb6 delays the onset of mitosis (Table 1). Five hours after switching off the orb6 gene, cell length was reduced to 84% of orb6+ cells, and at 7.5 hr cell length was reduced to 65%. This reduction in average cell length was found to be highly significant (P < 0.05). Cell width was unchanged, indicating that these cells were advanced into mitosis at a reduced cell size. Shortening of cell length was not caused by a reduction in cell growth rate, which was unchanged until 8.5 hr after thiamine addition (data not shown).
**orb6** Interacts Genetically with **orb2**. **orb6** mutants were found to be synthetically lethal with **orb2** mutants (10). Synthetic lethality interactions were determined by tetrad analysis, and phenotypes were observed at 25°C, 32°C, and 36°C. At 32°C mutants in either **orb6** or **orb2** are viable, but the double-mutant **orb6–25 orb2–34** is lethal, with the cells rapidly undergoing lysis (Fig. 7a). **orb6** mutants are unable to activate bipolar growth and grow with only one tip (10). Analysis of the actin cytoskeleton in **orb2** mutants revealed that actin is localized only at one pole of growth (Fig. 6h) and that its reorganization after mitosis is delayed (data not shown). This phenotype is reminiscent of the one observed when expression levels of **Orb6** decrease, suggesting that **Orb2** and **Orb6** might fulfill a similar function.

**orb2** Encodes the Protein Kinase **Pak1/Shk1**. Given this interaction with **orb6**, **orb2** was cloned by complementation (Fig. 7c and d), and its sequence confirmed that **orb2** is identical to the previously identified **pakt1**/**shk1** gene (8, 9). The **orb2–34** mutant was also suppressed by **pakt1**/**shk1** cDNA (Fig. 7e). The **pakt1**/**shk1** gene encodes a protein kinase that interacts with **Cdc42** and is similar to the CDC42 and RAC1-interacting **PAK** protein kinase identified in mammalian cells and STE20 found in budding yeast cells (7). Overexpression of **orb6** partially suppressed the **pakt1**/**shk1**/**orb2** mutant shape defect (Fig. 7f). Overexpression of **pakt1**/**shk1** did not rescue the **orb6** mutant phenotype (data not shown), but did induce cell elongation in wild-type cells (data not shown). These results suggest that the **Orb6** kinase might function downstream of the **Pak1/Shk1** protein kinase. This finding also is supported by the observation that in **pakt1**/**shk1**/* orb2** mutants, which are defective in the activation of the second tip, the **Orb6**-HA protein was found only at the one growing tip, suggesting that **pakt1**/**shk1** is required to bring about bipolar growth and to localize **Orb6** protein correctly (Fig. 6d).

**DISCUSSION**

**Orb6 Belongs to a Family of Kinases Essential for Morphological Control.** In the present study, we report the cloning and characterization of a fission yeast gene, **orb6**, which is required for maintenance of cell polarity during interphase and to promote actin reorganization during morphological transi-

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**Fig. 7.** Cloning of the **orb2** gene. (a) top, **ade6-M210 leu1–32h**; left, **orb6–25 ade6-M210 leu1–32h**; bottom, **orb2–34 ade6-M210 leu1–32h**; right, **orb2–34 orb6–25 ade6-M210 leu1–32h**. Cells were grown at 32°C. (b) **ade6-M210 leu1–32h**; Rep3X. (c) **orb2–34 ade6-M210 leu1–32h**; pRep3X. (d) **orb2–34 ade6-M210 leu1–32h** transgenic fragment. (e) **orb2–34 ade6-M210 leu1–32h** pRep3X/**pak1**/**shk1**. (f) **orb2–34 ade6-M210 leu1–32h** pRep3X/**orb6**. Cells were grown exponentially at 25°C in the presence of thiamine for at least eight generations; thiamine was washed away and cells were grown in the absence of thiamine at 25°C for 16 hr, then shifted at 36°C for 6 hr. (Bar = 5 μm.)

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**Fig. 8.** **Orb6** kinase is proposed to be part of a regulatory cascade contributing to the mitotic control and responding to the Ras1 and Cdc42-dependent morphological control pathway via the Pak1/Shk1 kinase. **Orb6** kinase regulates independently cell morphogenesis and the cell cycle, mediating its effect on p34cdc2 kinase directly through the Wee1 protein kinase or through some other mechanism dependent on p34cdc2 tyrosine phosphorylation.
phy of the apical ends. Thus, the warts gene product is important for controlling cellular morphogenesis as well as proliferation. Myotonic dystrophy is also sometimes associated with calcifying epitheliomas, neurofibromas, and parathyroid adenomas, suggesting that the DMPK also may have a role in the control of cell proliferation (25).

What is the role of these kinases in the regulation of the cell cycle and what is its functional significance? One possibility is that the deregulation of cell proliferation is an indirect consequence of cell shape alteration. Our results, though, suggest that a decrease of Orb6 levels leads to mitotic advance before an alteration of cell shape and that Orb6 overexpression can delay onset of mitosis without altering cell morphology. This finding might point to a novel pathway in the control of mitotic onset and in the coordination of cell growth and cell proliferation. Future work will clarify the extent of functional similarity between warts and orb6. If this pathway is conserved, the identification of orb6 in fission yeast, which allows the dissection of the cell cycle machinery by the use of cell cycle (edc) mutants, may provide the opportunity to easily study the mechanism of cell cycle regulation by these kinases.

**Orb6 Protein Kinase Interacts Genetically with pak1/shk1.**

To identify other partners in Orb6 function, we cloned orb2, because orb2 mutants showed a strong synthetic lethality interaction with orb6 mutants and their phenotype displayed similarities to the effects of orb6 loss of function. Cloning of orb2 showed that orb2 is identical to the previously identified pak1/shk1 gene (8, 9). The pak1/shk1 gene encodes a protein kinase that interacts with Cdc42, and is similar to the CDC42 and RAC1-interacting PAK1 protein kinase identified in mammalian cells and STE20 found in budding yeast cells (7). Ras1, Cdc42, and Pak1/Shk1 have a regulatory role in fission yeast cell morphogenesis and are thought to function in a hierarchical order whereby ras1 GTPase interacts functionally with Cdc42 GTase (6) and Pak1/Shk1 kinase acts as an effector of Cdc42 (8, 9).

We identified temperature-sensitive mutants of pak1/shk1, which confirm the morphological role of pak1/shk1 previously indicated by overexpression studies (9). Moreover, our study suggests that Pak1/Shk1 also might have a function in cell cycle control because pak1/shk1/ orb2 cells are short in cell length, and overexpression of pak1/shk1 induces cell elongation in wild type (data not shown). These results are consistent with the observation that Shk1, which interacts with Pak1/Shk1, also causes cell elongation when overexpressed (26). The fact that orb6 overexpression partially suppresses the orb6 phenotype suggests that the Orb6 kinase might function downstream of the Pak1/Shk1 protein kinase, in a pathway that controls and coordinates maintenance of cell polarity with progression through the cell cycle (Fig. 8). Alternatively, pak1/shk1 and orb6 might represent parallel pathway with similar and partially overlapping functions. The former model, though, is supported also by the observation that in pak1/shk1/ orb2 cells, which are defective in the activation of the second tip, the Orb6-HA protein is found only at the one growing tip, whereas another marker of cell polarity, Tea1, is found localized at both ends (27). This finding indicates that pak1/shk1 is required to bring about bipolar growth and to localize Orb6 protein correctly. Future work will address the interaction between Pak1/Shk1 and Orb6 and will clarify whether the Orb6 kinase activity or cortical localization is dependent on Pak1/Shk1.

**CONCLUSION**

We conclude that Orb6 has two roles during the cell cycle: to maintain polarized cell growth and to delay onset of mitosis.

We propose that the Orb6 kinase acts downstream of a morphogenetic control pathway involving Cdc42 and Pak1/Shk1, which maintains the cell in a polarized state during interphase while delaying the onset of mitosis (Fig. 8). When the cell has reached the appropriate size and is ready to undergo mitosis, a reduction in Orb6 kinase activity could remove an inhibitory signal over the p34cdc2 mitotic kinase and thus contribute to bringing about the onset of mitosis. The similarity of orb6 with Drosophila warts, which is thought to function as a tumor suppressor (15, 16) and other genes in mammalian cells that affect cell shape (22, 28), suggests that its function in coordinating cell morphogenesis with the cell cycle may be conserved in higher euakaryotes. Future work will compare the functional properties of this kinase family in different organisms and elucidate their role in the mechanisms integrating cell morphogenesis and proliferation.

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