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Identification of Cdc37 as a Novel Regulator of the Stress-Responsive Mitogen-Activated Protein Kinase

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A mitogen-activated protein kinase (MAPK) cascade is a signaling module ubiquitous among eukaryotes that transmits extracellular stimuli to the nucleus. A MAPK cascade is composed of three conserved kinases, MAPK, MAPK kinase (MAPKK), and MAPKK kinase (MAPKKK), and signals are transmitted through sequential activation of the three kinases by phosphorylation; stimulus-activated MAPKKK phosphorylates MAPKK, which in turn phosphorylates and activates MAPK. Activated MAPK phosphorylates downstream effector proteins, such as transcription factors, and modulates their function (6, 20, 27, 41). A subfamily of MAPKs dedicated for transmitting environmental stress stimuli, such as high-osmolarity stress, oxidative stress, heat shock, and genotoxic agents, are also known as stress-activated protein kinases (SAPKs). Among those are Hog1 in budding yeast Saccharomyces cerevisiae (4), Spc1 (also known as Sty1) in fission yeast Schizosaccharomyces pombe (29, 43), and mammalian p38 (19, 24). Genetic studies of yeast SAPKs indicate that SAPKs play key roles in cellular stress resistance (18, 34), and mammalian SAPKs are also implicated in inflammation and the response of cancer cells to cytotoxic treatments (23, 53).

The structures and functions of SAPK cascades are highly conserved throughout evolution; like the mammalian p38 pathway, the fission yeast Spc1 cascade, which consists of Wis4 and Win1 MAPKKks, Wis1 MAPKK, and Spc1 MAPK, is activated by a wide range of stress, including osmostress, oxidative stress, and heat shock (34). Interestingly, the highly homologous Hog1 SAPK cascade in budding yeast is responsive mostly to high-osmolarity stress (42), although a recent report also indicates heat shock-induced activation of Hog1 (55). Upon environmental stress, fission yeast Spc1 phosphorylates and activates the Atf1 transcription factor (44, 54), a fission yeast ortholog of mammalian ATF2, which is also a key substrate of the mammalian SAPKs (17, 39). Atf1 is responsible for expression of a number of stress resistance genes, including those in osmoregulation and oxidative stress responses (9, 38, 44, 54). Because of the high similarity between the fission yeast and mammalian SAPK cascades, the genetic study of the Spc1 pathway provides an excellent opportunity to identify and analyze novel elements in stress signaling by SAPK cascades.

CDC37 was first identified in budding yeast as a gene essential for cell cycle progression in G1 (13, 40), and structurally related proteins were subsequently identified in Drosophila melanogaster and mammalian cells (7, 8, 50). Although the function of Cdc37 was not apparent from its amino acid sequence, recent studies revealed that Cdc37 is a part of the chaperone complex required for the stability and/or activity of some protein kinases (reviewed in reference 21). Budding yeast Cdc28, a cyclin-dependent kinase (Cdk), shows significantly shorter half-lives in cdc37 mutants (12). Cdc37 is also required for maintaining the oncogenic tyrosine kinase v-Src in a soluble, biologically active form when expressed in budding yeast (11). Mammalian Cdc37 was found as a 50-kDa protein that forms a complex with diverse protein kinases, such as v-Src, Raf, and Cdk (16, 37, 50), while no MAPK has been identified as a target of Cdc37. Complexes of Cdc37 with different protein kinases often contain a molecular chaperone, Hsp90, and it has been proposed that Cdc37 is a kinase-targeting subunit of Hsp90 (50). On the other hand, in vitro experiments using budding yeast Cdc37 suggest that Cdc37 itself has a Hsp90-like chaperone activity (22) and, therefore, Cdc37 may also function independently of Hsp90.

Here we report a genetic screen in fission yeast to identify novel components of the Spc1 SAPK cascade. We have identified cdc37 as a mutation that inhibits the Spc1 function in response to the ectopic expression of activated Wis1 MAPKK.
The Cdc37 protein physically interacts with Spc1 MAPK, and the cdc37 mutation brings about significant decreases in both the abundance and stress-induced phosphorylation of Spc1. Our study suggests that Cdc37 functions as a molecular chaperone to maintain the stability of Spc1 MAPK as well as to facilitate the phosphorylation of Spc1 by Wis1 MAPKK.

**MATERIALS AND METHODS**

**Yeast strains and general techniques.** S. pombe strains used in this study are listed in Table 1. Growth media and basic techniques for S. pombe have been described previously (2, 30). S. pombe cells were grown in YES extract medium and EMM2 synthetic medium.

**Integration of the spc1+ gene at the h7 locus.** The 2.9-kb EcoRI-Spel genomic DNA fragment containing the spc1+ gene was cloned into the NotI site of pBluescript II (Stratagene), to which the h7+ marker gene was inserted at the Smal site. The resultant plasmid was used to transform a wild-type (CA7) S. pombe strain. Stable His+ transformants were selected, and the integration of the plasmid construct at the h7 locus was verified by Southern hybridization analysis. The integrated spc1+ gene was able to suppress the osmosensitivity and the temperature sensitivity of the Δspc1 strain (data not shown).

**Isolation of mutants resistant to Wis1 overexpression.** Strains CA1725 and CA1726 were transformed with the pREP1-WIS1DD-HA6H plasmid, which expresses Wis1DD (46), a constitutive active form of Wis1 MAPKK under the control of the thiamine-repressible nmt1 promoter (28). The transformants were grown in liquid EMM2 lacking thiamine (EMM2–T) at 30°C for 16 h and plated onto EMM2–T at the concentration of ~2 × 107 cells per plate. After incubation at 30°C for 7 days, growing colonies were picked up and streaked to confirm their growth on EMM2–T. Resultant colonies were subjected to anti-hemagglutinin (anti-HA) immunoblotting to select mutants that had not lost the ability of Wis1 overexpression.

**Cloning of the cdc37+ gene.** A temperature-sensitive strain, CA1388, isolated from the above screen was transformed with an S. pombe genomic library and plated onto EMM2 medium. After incubation at 25°C for 7 days, transformants were replica plated onto YES medium and incubated at 37°C for 1 day. The transformants were further replica plated on fresh YES medium, which was followed by incubation at 37°C for 2 days. Forty-one out of ~80,000 transformants showed colony formation after the second replica plating at 37°C. DNA sequencing and PCR analyses of the recovered plasmids from all of the t° colonies showed that they all contained the cdc37+ gene.
MAPKK results in a lethal phenotype accompanied by cell lysis, partly due to a defect in cellular osmoregulation (43, 44). This lethal phenotype is caused by deregulated hyperactivation of Spc1 MAPK and is suppressed by the \( \text{snc1} \) null (Δ9004) mutation. However, Wis1 overexpression brings about a severe growth defect even in the absence of Atf1, the only known target transcription factor for Spc1, although the cell lysis phenotype is suppressed by the Δaff1 mutation (Fig. 1B) (44). This observation implies that hyperactivated Spc1 MAPK phosphorylates an unknown factor in addition to Atf1, leading to a growth defect. Therefore, we screened for mutations that rescue the lethality of Wis1 overexpression in Δaff1 strains (Fig. 1C), because they would include mutations in (i) the unknown factor downstream of Spc1, or (ii) novel factors required for activation or function of Spc1 MAPK.

Three classes of unwanted mutants could be isolated in the suppressor mutation screen described above. First, mutants defective in transcription from the Wis1 overexpression plasmid would be isolated. We used HA epitope-tagged Wis1 for overexpression and eliminated this class of mutants by measuring the Wis1-HA protein level by anti-HA immunoblotting. Second, mutations in upstream components that positively regulate Wis1, such as Wis4 and Win1 MAPKKKs, would weaken the toxicity of Wis1 overexpression. To eliminate this possibility, we overexpressed the constitutively active form of Wis1, Wis1DD, in which the MAPKKK phosphorylation sites in Wis1 are substituted with aspartic acid residues that mimic phosphorylation (46). Third, as mentioned above, snc1 mutations completely repress the lethality by Wis1 overexpression (43). To avoid isolating snc1 mutations, we used strains in which an additional copy of the snc1 gene was integrated in the genome, as it is unlikely that two copies of snc1 are mutated at the same time when we screen for spontaneous mutations.

Out of ~10^6 cells plated, about 1,000 viable colonies appeared spontaneously under the Wis1 overexpression condition. When 184 isolates were examined by anti-HA immunoblotting, 61 of them were confirmed for the overexpression of Wis1DD-HA. Subsequent genetic analyses showed that a mutant, named sws1-681 (suppressor of Wis1 overexpression), exhibited a temperature-sensitive (ts) growth phenotype (see Fig. 3B) in addition to the resistance to Wis1 overexpression. This report focuses on the detailed study of the sws1 mutation.

Atf1-dependent gene expression is defective in the sws1 mutant. As described above, the sws1 mutation may represent an unknown, Atf1-independent branch downstream of Spc1 MAPK or a novel factor required for activation and/or function of Spc1 (Fig. 1C). In order to distinguish these two possibilities, we examined whether the sws1-681 mutation
affects the activity of Atf1 in stress-induced gene expression. Three genes of which transcription is induced by the Spc1-Atf1 pathway upon stress were studied by Northern blotting analyses: \textit{gpd1} (encoding glycerol-3-phosphate dehydrogenase), \textit{pyp2} (encoding tyrosine-specific phosphatase, which dephosphorylates and inactivates Spc1 MAPK), and \textit{ctt1} (encoding catalase, an enzyme which decomposes hydrogen peroxide) \cite{10, 32, 44, 54}. In wild-type cells, \textit{gpd1} mRNA was induced within 10 min of oxidative stress by 0.3 mM H$_2$O$_2$ and reached the maximum level at 40 min (Fig. 2A). On the other hand, in the \textit{sws1-681} mutant, the level of \textit{gpd1} mRNA was reduced to approximately 50% of wild type, while the kinetics of induction was similar to that in wild-type cells. The effect of the \textit{sws1} mutation was more obvious in the \textit{pyp2} expression. In the wild-type strain, \textit{pyp2} expression was detected at 5 min after exposure to the stress, with maximum induction at 20 min. In the \textit{sws1-681} mutant, \textit{pyp2} expression upon stress was significantly compromised, and the maximum level of \textit{pyp2} mRNA was approximately 25% of that in wild-type cells (Fig. 2). We observed that the stress-induced expression of \textit{ctt1} was also defective in \textit{sws1-681} cells (data not shown). These results indicate that expression of the Atf1-dependent genes is compromised in the \textit{sws1-681} mutant.

The conclusion above implies that the \textit{sws1} mutation represents a factor required for activation and/or function of Spc1 MAPK, rather than an Atf1-independent pathway downstream of Spc1. Although the \textit{sws1-681} mutation was originally isolated in the \textit{Delta1} background, this model predicts that, like \textit{spc1} mutations, the \textit{sws1} mutation suppresses the phenotypes of \textit{Wis1} MAPKK overexpression even in the presence of \textit{atf1}. As expected, the \textit{sws1-681 atf1} strain formed viable colonies even when \textit{Wis1} was overexpressed (Fig. 3A). Taken altogether, these results imply that the \textit{sws1} mutant is defective in the activation and/or function of Spc1 MAPK.

\textit{sws1} is allelic to \textit{cdc37}. In addition to the resistance to \textit{Wis1} overexpression, \textit{sws1-681} cells exhibited a ts growth phenotype, and they stopped dividing at temperatures above 36°C (Fig. 3B). Heterozygous diploids constructed by mating wild-type and \textit{sws1-681} strains showed neither of the phenotypes, indicating that \textit{sws1-681} is a recessive mutation (data not shown). In order to identify the \textit{sws1} gene, haploid \textit{sws1-681} cells were transformed with an \textit{S. pombe} genomic library, and the plasmid
clones that complement the sws1 ts phenotype were isolated. Among ~80,000 transformants screened, 41 of them showed growth at 37°C, and all the plasmids recovered from the ts colonies were found to contain the cdc37 gene (GenBank accession number AJ132376). As shown in Fig. 4A, one of the plasmids isolated from the library, pDB248-CDC37, which contains cdc37 and an adjacent open reading frame, SPBC9B6.09c, suppressed the sws1 ts phenotype. We subcloned the open reading frame of cdc37 into the S. pombe expression vector pREP1 (28), and the resultant plasmid, pREP1-CDC37, was also capable of suppressing the ts phenotype of sws1 cells (Fig. 4A), indicating the complementation of sws1-681 by the cdc37 gene. In addition, a 1-bp substitution that changes Leu-285 of the Cdc37 protein to proline was found in the cdc37 gene cloned from the sws1-681 mutant (Fig. 4B). Replacement of this mutated sequence with the wild-type cdc37 sequence by homologous recombination rescued the sws1 ts phenotype (data not shown). Taken together, we concluded that sws1 is identical to cdc37 and, hereafter, we refer to sws1-681 as cdc37-681.

Fission yeast cdc37 encodes a 466-amino-acid, 53-kDa protein with a significant sequence similarity to orthologs in humans, mice, chickens, flies, worms, and budding yeasts. To investigate the cellular function of fission yeast Cdc37, we performed a gene disruption experiment. The entire open reading frame of one of the cdc37 genes in wild-type diploid cells was replaced with the ura4 marker gene by homologous recombination (see Materials and Methods). Sporulation of the resultant heterozygous diploid followed by tetrad analysis revealed that each tetrad produced two viable segregants of the cdc37 mutant and two inviable segregants, indicating that the cdc37 gene is essential for cellular viability. Most of the mutant cdc37 haploid segregants divided several times after germination to form microcolonies of very short cells with one or two elongated cells (Fig. 4C). Thus, Cdc37 has functions essential for vegetative cell growth of S. pombe, which contrasts with the fact that the Spc1 MAPK cascade is dispensable for cell viability in the absence of environmental stress (29, 43).

Both the amount of and the stress-induced phosphorylation of Spc1 are reduced in the cdc37 mutant. Cdc37 is important for the stability and/or activity of several kinases, such as v-Src, Raf, and Cdk (see introduction). Therefore, it is possible that Cdc37 may also regulate Spc1 MAPK or Wis1 MAPK in fission yeast, although no MAPK or MAPKK has been reported to require Cdc37 for the kinase function. To examine this possibility, we compared wild-type and cdc37 mutant cells for the amounts of Spc1 and Wis1 as well as Spc1 activation in response to stress. The cell lysate was prepared from wis1:myc and cdc37-681 wis1:myc strains, in which chromosomal wis1 is tagged with the sequence encoding the myc epitope (14), and the protein levels of Spc1 and Wis1 were evaluated by anti-Spc1 and anti-myc immunoblotting, respectively (Fig. 5A). In the cdc37 mutant, the amount of Spc1 protein was reduced to 30 to 50% of that in wild-type cells, whereas the level of Wis1 MAPKK showed little difference between the two strains. Northern blotting experiments showed that the spe1 mRNA...
was not affected by the cdc37-681 mutation (Fig. 5B), and Cdc37 may affect the stability of the Spc1 protein, as previously reported for other kinases (12, 50). On the other hand, while Cdc37 is known to function together with Hsp90 in the chaperoning of Raf1, Cdk, and other kinases, we found that Spc1 MAPK was not affected by a defect in Hsp90. S. pombe has only one Hsp90 gene, of which mutation, swo1-26, brings about the destabilization of the protein kinase Wee1 in cell cycle regulation (3); Swo1 Hsp90 binds to the Wee1 kinase and probably functions as a molecular chaperone. As shown in Fig. 5C, the protein level of Spc1 was affected by the cdc37 mutation but not by swo1-26, implying that Cdc37 regulates Spc1 MAPK independently of Hsp90.

In order to examine whether cdc37 affects the stress-induced activation of Spc1 MAPK, phosphorylation of Spc1 was monitored in wild-type and cdc37-681 mutant strains exposed to high osmolarity and oxidative stress. In these strains, the chromosomal spc1\(^+\) gene was tagged with the sequence encoding the HA epitope followed by six consecutive histidine residues (HA6H), so that Spc1 was easily purified by Ni-nitrilotriacetic acid beads and analyzed by immunoblotting with anti-HA antibodies as well as antibodies that cross-react with the phosphorylated, active form of Spc1 (45). As shown in Fig. 6A, stress-induced phosphorylation of Spc1 dramatically decreased in the cdc37 mutant. Quantification of Spc1 phosphorylation followed by normalization with the amount of Spc1 protein indicated that the level of Spc1 phosphorylation upon oxidative stress in cdc37-681 was only 20% of that in wild-type cells (Fig. 6A, lower panel). A significant reduction in Spc1 phosphorylation was also detected in cdc37-681 cells exposed to high-osmolarity stress (data not shown). Consistently, immunofluorescence microscopy with anti-Spc1 antibodies showed that osmostress-induced nuclear accumulation of Spc1, which is dependent on Spc1 phosphorylation (14), was also significantly compromised in cdc37-681 cells (data not shown).

Wis1 has a MAPK-docking sequence, and the interaction between Wis1 and Spc1 contributes to the efficient phosphorylation of Spc1 by Wis1 (31). Since the cdc37 mutant showed
Cdc37 physically interacts with Spc1 MAPK in vivo. The Cdc37 protein binds to protein kinases, such as v-Src and Cdk, as a molecular chaperone important for the stability and/or activity of those kinases. Results described above suggest that Cdc37 is also important for the stable expression and function of Spc1 MAPK in S. pombe, implying a role for Cdc37 as a molecular chaperone for this SAPK. In order to examine whether Cdc37 interacts physically with Spc1, we tested the copurification of Cdc37 with Spc1 from the cell lysate. Spc1 was isolated by anti-myc immunoprecipitation from a spc1myc cdc37HA6H strain, in which chromosomal spc1+ and cdc37+ are tagged with the sequences encoding the myc epitope and HA6H, respectively. As shown in Fig. 7, anti-HA immunoblotting detected Cdc37HA6H coprecipitating with Spc1myc, while Cdc37HA6H was not detectable in the immunoprecipitates from a control strain expressing untagged Spc1 (Fig. 7, spc1+). Similar experiments using the lysate prepared from cells exposed to high osmolarity and oxidative stress were also performed; anti-myc antibodies precipitated phosphorylated Spc1myc and a slightly reduced amount of Cdc37HA6H, while the protein level of Cdc37 in the cell lysate showed little change before and after stresses (Fig. 7, bottom panel). Thus, Cdc37 physically interacts with Spc1 in vivo, a result consistent with the notion that Cdc37 functions as a molecular chaperone for Spc1 MAPK.

Cellular localization of the Cdc37 protein. In response to stress, both Spc1 MAPK and Wis1 MAPKK show dynamic changes in their cellular localization (14, 15, 31). Under normal growth conditions, Spc1 is found throughout the cell, while Wis1 is found exclusively in the cytoplasm due to its nuclear export signal sequence. Once cells are exposed to osmotic stress, both proteins are translocated into the nucleus within a few minutes. Because of the detected interaction between Cdc37 and Spc1, the cellular localization of Cdc37 was studied both in the presence and absence of stress. We constructed an S. pombe strain in which the chromosomal cdc37+ gene was tagged with the sequence encoding green fluorescent protein (GFP). The resultant cdc37-GFP strain showed no apparent growth defect at different temperatures tested (data not shown), indicating that the Cdc37-GFP fusion protein is functional. Fluorescence microscopy of Cdc37-GFP in living cells showed that the Cdc37 protein was located throughout the cell, with prominent localization in the chromatin region of the nucleus (Fig. 8A, upper panel, and B); within the chromatin region, one or two bright dots of Cdc37-GFP signal were consistently observed (Fig. 8A, upper panel, and B). The cytoplasmic staining of Cdc37-GFP was somewhat uneven, probably due to subcellular compartments in the cytoplasm. In contrast to Spc1 and Wis1, we did not observe a dramatic change in the localization of Cdc37 when cells were treated by high-osmolarity stress (Fig. 8A, lower panel), although the Cdc37-GFP signal in the chromatin region became less marked.

DISCUSSION

Previous studies strongly suggest that Cdc37 is an evolutionarily conserved molecular chaperone specific for protein kinases (21), and its functions are essential for cell growth in both budding yeast (40) and fission yeast (this study). Cdc37 is expressed at high levels in some cancer cells (51), and the
ectopic overexpression of Cdc37 in mice promotes cellular transformation (49). However, only a limited number of protein kinases have been demonstrated as clients for the Cdc37 chaperone. In this study, we identified Cdc37 as a positive regulator of the fission yeast Spc1, a member of the evolutionarily conserved stress-activated MAPK subfamily.

In the present study, we have obtained genetic and biochemical data suggesting that Cdc37 plays an important role in the SAPK pathway in *S. pombe*. First, *cdc37* was identified as a mutation that suppresses aberrant Spc1 signaling induced by overexpression of Wis1 MAPKK. Second, Cdc37 forms a complex with Spc1 in vivo, and in the *cdc37* mutant the protein level of Spc1, but not the *spc1* mRNA, is reduced. Third, the interaction of Spc1 with Wis1 MAPKK is compromised in the *cdc37* mutant, and stress-induced phosphorylation of Spc1 by Wis1 is significantly reduced. Consistently, the expression of *gpd1, ctt1*, and *pyp2* genes, which is induced upon stress by activated Spc1 through the Atf1 transcription factor, is also compromised in the *cdc37* mutant. These results support the notion that Spc1 MAPK is a novel target for the Cdc37 chaperone. Interaction with Cdc37 may stabilize the Spc1 protein and maintain Spc1 in a properly folded state competent for the interaction with Wis1 MAPKK.

Some studies strongly suggest that the function of Cdc37 is to target the Hsp90 chaperone machinery to protein kinases by interacting with both Hsp90 and kinases (16, 50). In addition, mutational inactivation of Cdc37 and Hsp90 similarly affect the stability and/or function of v-Src and Ste11 MAPKKK in budding yeast (1, 11, 26, 56) and the *sevenless* receptor tyrosine kinase pathway in *Drosophila* (7), indicating the cooperative action of Cdc37 and Hsp90. The genome sequence of *S. pombe* contains only one Hsp90 gene, *swol* (3); Swo1 binds to a protein kinase, Wee1, and the Wee1 protein is destabilized in the *swol* mutant, suggesting the chaperone function of the
Swo1 Hsp90 for the Wee1 kinase. In contrast, no apparent defect in the stability and activation of Spc1 MAPK was observed in the swo1-26 mutant and, therefore, the Hsp90 function does not appear to be important for Spc1. Interestingly, an Hsp90-like chaperone activity of the Cdc37 protein has been detected in vitro, and Cdc37 is able to perform the chaperone function independently of Hsp90 at least when overexpressed in budding yeast (22, 25). It is possible that Cdc37 and Hsp90 have some distinct functions in vivo through the regulation of different protein kinases.

Although the amount of active Spc1 MAPK is dramatically reduced in the cdc37-681 mutant, the mutant cells do not show apparent growth defects under environmental stresses; cdc37-681 cells are not sensitive to high osmolarity of 1 M KCl, and the viability of the mutant cells exposed to oxidative stress by H2O2 is comparable to that of wild-type cells (data not shown). Consistently, in the cdc37-681 mutant, Spc1-dependent phosphorylation of Atf1 is detectable (data not shown), and the stress response genes regulated by the Spc1-Atf1 pathway are induced upon stress, although the induction levels of those genes are lower than those in wild-type cells (Fig. 7). Thus, the remaining activity of Spc1 in the cdc37-681 mutant may be sufficient for the cellular survival of stress at least under the conditions tested. The stress sensitivity is also not obvious in the Wis4 MAPKK null mutant, which is significantly compromised for Spc1 activation (45), and the full activation of the Spc1 pathway does not seem to be necessary for survival under the experimental stress conditions.

Whereas the Spc1 MAPK pathway is not essential unless cells are exposed to environmental stress, cdc37Δ is absolutely required for cell viability and the Δcdc37 mutant is lethal. Therefore, Cdc37 must have functions other than regulating the Spc1 pathway. In budding yeast (12), flies (7), and mammals (36, 50), Cdc37 is important for the activity and/or stability of Cdk, and it is possible that the lethal phenotype of Δcdc37 in S. pombe is caused by the loss of functional Cdc2, an essential Cdk in the fission yeast cell cycle. However, in contrast to cdc2 mutants that show a highly elongated cell morphology caused by cell cycle arrest (35), most Δcdc37 cells stop dividing with short cell length, and cdc37-681 cells also do not show a cdc phenotype at the restrictive temperature (data not shown). Thus, the lethal phenotype of the cdc37 mutants cannot be explained solely by inactivation of Cdc2, and the Cdc37 targets essential for cell growth in fission yeast remain to be identified.

In summary, we have identified Cdc37 as a novel regulator of Spc1 MAPK. Although the chaperone function of Cdc37 has been described for MAPKKKs, Raf in higher eukaryotes (16, 48), and Ste11 in budding yeast (1), this is the first report that a MAPK requires Cdc37. Because of the high conservation of SAPKs between fission yeast and mammalian cells, it will be of interest to examine whether human p38 MAPKs are also clients of Cdc37. In addition to cdc37Δ, we have isolated another locus, named sws2 in the genetic screen described in this report, and the characterization of sws2 may also identify a novel, evolutionarily conserved regulator of SAPKs.

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FIG. 8. Cellular localization of Cdc37 in S. pombe. (A) Strain CA1623, of which the chromosomal cdc37Δ gene was tagged with the sequence encoding GFP, was grown to the early log phase and observed by fluorescence microscopy in the presence (lower panel) or absence (upper panel) of high-osmolarity stress induced by 0.6 M KCl. (B) Chromosomal DNA in strain CA1623 was stained with Hoechst 33342, and cells were incubated in YES liquid medium. After 2 h of incubation, cells were observed with fluorescence microscopy for Cdc37GFP and DNA. Bar, 5 μm.

