Genetic interactions among *ZDS1,2*, *CDC37*, and protein kinase CK2 in *Saccharomyces cerevisiae*

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Abstract **We report here the identification of the homologous gene pair *ZDS1,2* as multicopy suppressors of a temperature-sensitive allele (*cka2-13*^{ts}) of the *CKA2* gene encoding the α′ catalytic subunit of protein kinase CK2. Overexpression of *ZDS1,2* suppressed the temperature sensitivity, geldanamycin (GA) sensitivity, slow growth, and flocculation of multiple *cka2* alleles and enhanced CK2 activity in vivo toward a known physiological substrate, Fpr3. Consistent with the existence of a recently described positive feedback loop between CK2 and Cdc37, overexpression of *ZDS1,2* also suppressed the temperature sensitivity, abnormal morphology, and GA sensitivity of a CK2 phosphorylation-deficient mutant of *CDC37*, *cdc37-S14A*, as well as the GA sensitivity of a *cdc37-1* allele. A likely basis for all of these effects is our observation that *ZDS1,2* overexpression enhances Cdc37 protein levels. Activation of the positive feedback loop between CK2 and Cdc37 likely contributes to the pleiotropic nature of *ZDS1,2*, as both CK2 and Cdc37 regulate diverse cellular functions.**

Key words: Budding yeast; Protein kinase CK2; Multicopy suppression

1. Introduction

Protein kinase CK2 is a ubiquitous serine/threonine kinase of unknown function (for reviews, see [1–4]). The enzyme is composed of catalytic α (35–44 kDa) and regulatory β (24–28 kDa) subunits that combine to form an $\alpha_2\beta_2$ tetramer. In *Saccharomyces cerevisiae*, the enzyme consists of two distinct catalytic subunits, α and α′ (encoded by *CKA1* and *CKA2*, respectively), and two regulatory subunits, β and β′ (encoded by *CKB1* and *CKB2*, respectively). CK2 recognizes Ser/Thr (or in exceptional cases Tyr; [5]) in an acidic environment and phosphorylates a broad spectrum of endogenous substrates involved in transcription, translation, signal transduction, and other functions [6].

The *S. cerevisiae ZDS1* and/or *2* genes have been isolated in a bewildering variety of genetic screens (for review see [7]). Genes identified in these screens include: *CDC42*, encoding a Rho family GTPase required for cell polarity [7]; *CDC28*, which encodes the central cell division cycle kinase of *S. cerevisiae* [8]; *CEG1*, encoding the guanylyltransferase responsible for mRNA capping [9]; *SIN4*, which plays a global role in chromatin structure [8]; as well as several other genes involved in multiple processes [10–13]. The diverse nature of these genetic interactions was the basis for naming this gene pair *ZDS1* and *ZDS2* (zillion different screens 1 and 2; [7]).

In spite of the diversity of their interactions, *ZDS1,2* appear to be intimately involved in some aspect/s of cell polarity. First, a strain bearing a deletion of both *ZDS1* and *ZDS2* has elongated, abnormally shaped buds with hyperpolarized actin [7–9]. Second, epitope-tagged Zds1 localizes to presumptive bud sites, the apex of developing buds, and the mother–bud junction of large-budded cells [7]. Third, Zds1,2 have recently been reported to physically interact with diverse gene products involved in cell polarity [14]. The basis for the bewildering array of genetic interactions involving Zds1,2 remains obscure, however. We have recently reported that a positive feedback loop between CK2 and Cdc37 promotes the activity of multiple cellular kinases involved in diverse functions [15]. We now present evidence to show that *ZDS1,2* overexpression augments the function of both CK2 and Cdc37, thus providing a possible explanation for the pleiotropic nature of *ZDS1,2*, since both CK2 and Cdc37 regulate diverse cellular processes.

2. Materials and methods

2.1. Strains and growth media

S. cerevisiae strains used in this study are listed in Table 1 [8,15,16]. Yeast strains were grown in rich glucose medium (YPD: 1% yeast extract, 2% peptone, and 2% glucose) or in YPD medium supplemented with geldanamycin (GA) at 35 μM at different temperatures as indicated in figure legends. GA (Sigma) was dissolved in dimethylsulfoxide and added directly to warm medium. Escherichia coli strain DH5α (Clontech) was grown in Luria broth containing 50 μg/ml ampicillin.

2.2. Strain manipulations and cell biology

Transformations and other genetic manipulations were carried out using standard yeast procedures [17]. Disruption of the *SWE1* gene was carried out by polymerase chain reaction-mediated gene replacement [18]. For morphological observations, indicated strains were grown to mid-log phase at 23°C and fixed by addition of formalde-

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Abbreviations: GA, geldanamycin

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Table 1
S. cerevisiae strains

Strain	Genotype	Source
YDH6	*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52 cka1-Δ1::HIS3 cka2-Δ1::TRP1* [pRS315 *CKA2*]	[16]
YDH8	*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52 cka1-Δ1::HIS3 cka2-Δ1::TRP1* [pRS315 *cka2-8*]	[16]
YDH11	*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52 cka1-Δ1::HIS3 cka2-Δ1::TRP1* [pRS315 *cka2-11*]	[16]
YDH13	*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52 cka1-Δ1::HIS3 cka2-Δ1::TRP1* [pRS315 *cka2-13*]	[16]
YRM14.0	*MAT***a** *his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52 cdc37-1*	[15]
YSB11	*MAT***a** *leu2 ura3 trp1 cdc37Δ::LEU2* [pRS314 *CDC37*]	[15]
YSB13	*MAT***a** *leu2 ura3 trp1 cdc37Δ::LEU2* [pRS314 *cdc37-S14A*]	[15]
YSB15	*MAT***a** *leu2 ura3 trp1 cdc37Δ::LEU2* [pRS314 *cdc37-S14,17E*]	[15]
YSB49	*MAT***a** *ade2-101 his3-Δ200 leu2-Δ1 lys2-801 trp1-Δ1 ura3-52 cka1-Δ1::HIS3 cka2-Δ1::TRP1 ptp1::kanMX4* [pRS315 *cka2-13*]	[15]
DY3143	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 ssd1-d1 trp1-1 ura3-1 zds1::URA3 zds2::TRP1*	[8]
YSB27	*MAT***a** *leu2 ura3 trp1 cdc37Δ::LEU2 swe1Δ::kanMX4* [pRS314 *cdc37-S14,17E*]	This study
YSB28	*MAT***a** *ade2-1 can1-100 his3-11,15 leu2-3,112 ssd1-d1 trp1-1 ura3-1 zds1::URA3 zds2::TRP1 swe1Δ::kanMX4*	This study

hyde to a final concentration of 3.7%. Cell morphology was visualized using Nomarski optics.

Flocculation was assayed as described previously [19] except that the cultures were grown in selective liquid medium to reduce variation in flocculation values.

2.3. ZDS1 *mutagenesis*

ZDS1(1-358) was prepared by introducing a GAA-to-TAA mutation at position 359 using the mutagenic oligonucleotide 5′-CCATGACATGGCCTTAACGATCGTGAC-3′. The AlwN1 selection oligonucleotide (Stratagene) was used as the selection oligonucleotide. Mutagenesis was confirmed by sequencing. Zds1Δ625-809 was produced by removal of the internal 555 nucleotide *Hin*dIII fragment of *ZDS1*.

2.4. *CK2 assays and Western blotting*

To assay for steady-state Tyr-phosphorylation of Fpr3, the relevant strains were grown at the indicated temperatures to mid-log phase. Five A_{600} units of each culture were pelleted by centrifugation, brought to a final volume of 100 μl, and stored at −80°C for Western analysis. Samples were processed for Western blotting as described elsewhere [15]. Mouse monoclonal anti-Cdc37 (gift from Avrom Caplan, Mt. Sinai Medical Center) was used at 1:1500, mouse anti-phosphotyrosine antibody 4G10 (Upstate Biotechnology) at 1:1000, and rabbit anti-Fpr3 (gift of Jeremy Thorner, University of California, Berkeley, CA, USA) at 1:1000. Immunodetection was performed using the Amplified Alkaline Phosphatase Immun-Star™ kit (Bio-Rad).

3. Results

A genetic screen was conducted to isolate multicopy suppressors of the temperature sensitivity of a *cka2-13* allele at 35°C. In addition to *CDC37* [15], we also isolated *ZDS1* as a multicopy suppressor of *cka2* mutants. As shown in Fig. 1A, overexpression of *ZDS1* suppressed *cka2-13* at 35°C, but not at 37°C. The introduction of a stop codon at position 359 of the Zds1 open reading frame inactivated suppressor function, confirming the identification of *ZDS1* as the relevant gene. An in-frame *Hin*dIII deletion construct was fully active as a suppressor, indicating that residues 625–809 are dispensable for suppressor function. Overexpression of *ZDS1* or *CDC37* also suppressed the flocculation (Fig. 1B) and slow-growth phenotype (data not shown) of multiple *cka2* alleles. Although *ZDS1* overexpression did not suppress the flocculation phenotype of *cka2-11* (Fig. 1B), it did suppress its temperature sensitivity and slow-growth phenotype. Multicopy Zds1 was unable to suppress a null (data not shown), indicating that it does not bypass the requirement for CK2. The Zds1 homolog, *ZDS2*, was also tested and found to be able to suppress *cka2-13* (data not shown).

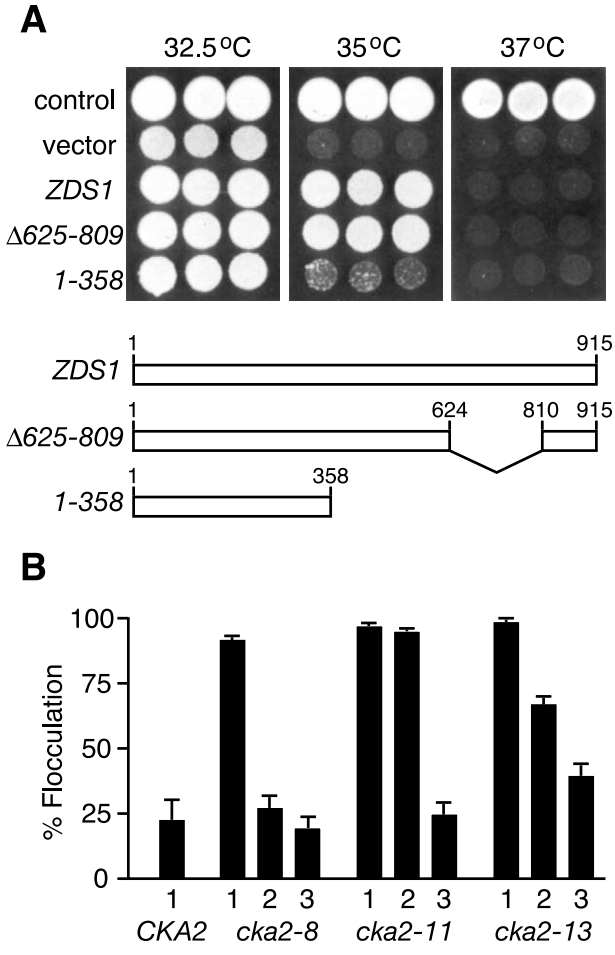

Fig. 1. Multicopy suppression of *cka2*^{ts} by *ZDS1* and *CDC37*. A: Effect of multicopy *ZDS1* alleles on the temperature sensitivity of *cka2-13*. Strains used in order were YDH6 (*cka1*Δ *CKA2*) transformed with pRS426 and YDH13 (*cka1*Δ *cka2-13*^{ts}) transformed with pRS426 or pRS426 expressing *ZDS1*, *ZDS1*-(Δ*625-809*), or *ZDS1*-(*1-358*). B: Effect of multicopy *ZDS1* and *CDC37* on the flocculation phenotype associated with three *cka2*^{ts} alleles. Strains YDH6 (*cka1*Δ *CKA2*), YDH8 (*cka1*Δ *cka2-8*^{ts}), YDH11 (*cka1*Δ *cka2-11*^{ts}), and YDH13 (*cka1*Δ *cka2-13*^{ts}) were transformed with pRS426 (1), pRS426-*ZDS1* (2), or with pRS426-*CDC37* (3).

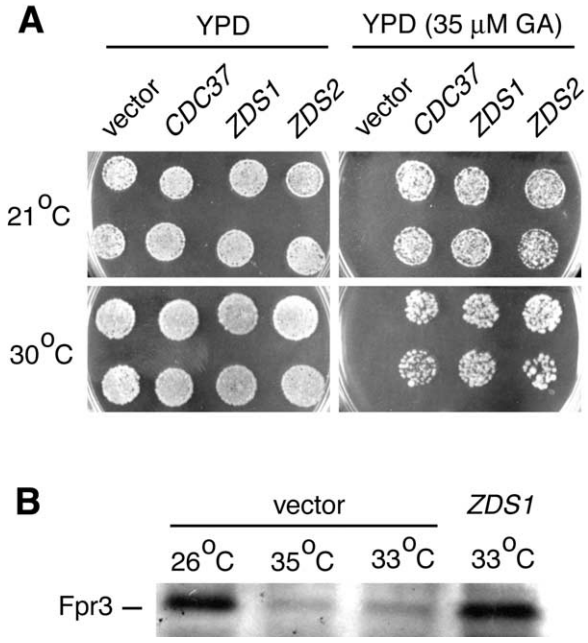

Fig. 2. *ZDS1,2* overexpression suppresses GA sensitivity of *cka2-13* and enhances CK2 activity toward Fpr3 in vivo. A: YDH13 (*cka1*Δ *cka2-13*^{ts}) transformed with pRS426 or pRS426 expressing *CDC37*, *ZDS1*, or *ZDS2* was spotted at 5000 cells/spot on YPD or YPD supplemented with GA (35 μM) and incubated at 21°C or 30°C for 3 days. B: YDH13 transformed with pRS426 or pRS426-*ZDS1* was grown at permissive temperature (26°C) or semipermissive temperature (33°C). Half of the culture grown at 26°C was shifted to non-permissive temperature (35°C) for 3 h. Identical amounts of cell lysates were probed with anti-P-Tyr antibody (4G10 at 1:1000 dilution) to assay for levels of phosphorylated Fpr3 as a measure of CK2 activity in vivo.

Overexpression of *ZDS1,2* might suppress *cka2-13* strains either by stimulating residual CK2 activity or by decreasing the cellular requirement for CK2. We have previously shown that *cka2-13* mutants are sensitive to the Hsp90-specific inhibitor GA and that overexpression of *CDC37* suppresses this sensitivity by promoting CK2 activity [15]. As shown in Fig. 2A, overexpression of either *ZDS1* or *ZDS2* suppressed the GA sensitivity of a *cka2-13* mutant, suggesting a role for *ZDS* in maintaining or enhancing CK2 activity. The level of suppression observed is comparable to that seen with *CDC37* (Fig. 2A). As a more direct measure of the effect of *ZDS1* overexpression on CK2 activity, we monitored tyrosine phosphorylation of Fpr3 as an indicator of CK2 activity in vivo. CK2 specifically catalyzes tyrosine phosphorylation of Fpr3, a nuclear immunophilin, on Tyr^{184}, and this phosphorylation is reversed by the protein tyrosine phosphatase encoded by *PTP1* [5]. In a *ptp1*Δ background, phosphorylated Fpr3 represents a major band in a Western blot of whole cell extract probed with anti-P-Tyr antibody. As shown in Fig. 2B, a *cka2-13 ptp1*Δ strain arrests at 35°C with very little Tyr-phosphorylated Fpr3 and has only marginally higher activity toward Fpr3 at 33°C, a semipermissive temperature for this mutant. *ZDS1* overexpression (at 33°C) increases the relative amount of Tyr-phosphorylated Fpr3 to a level approximately equal to that seen in the mutant at permissive temperature (all lanes in Fig. 2B contain comparable levels of Fpr3 protein; data not shown). Thus, overexpression of Zds1 enhances CK2 activity.

We have recently reported the existence of a positive feedback loop between protein kinase CK2 and the protein kinase-specific chaperone, Cdc37 [15]. Hence, we wished to test if *ZDS1,2* might stimulate CK2 activity in a *cka2-13* strain by enhancing Cdc37 function. Consistent with such a possibility, we found that overexpression of *ZDS1* or *ZDS2* was able to suppress the temperature sensitivity (Fig. 3A) and also the abnormal morphology (Fig. 3B) of *cdc37-S14A*, an allele of *CDC37* in which the evolutionarily conserved CK2 phosphorylation site at serine-14 has been mutated to an alanine. Unlike the wild-type protein, Cdc37-S14A is deficient in promoting the activity of multiple protein kinases, including CK2 [15]. Although impaired in Cdc37 function, *cdc37-S14A* overexpression suppressed the temperature sensitivity of this strain more efficiently than did *ZDS1,2* overexpression (Fig. 3A). The significance of this result is discussed below. As shown in Fig. 3B, *ZDS1,2* overexpression also suppressed the abnormal morphology of *cdc37-S14,17E*, an allele in

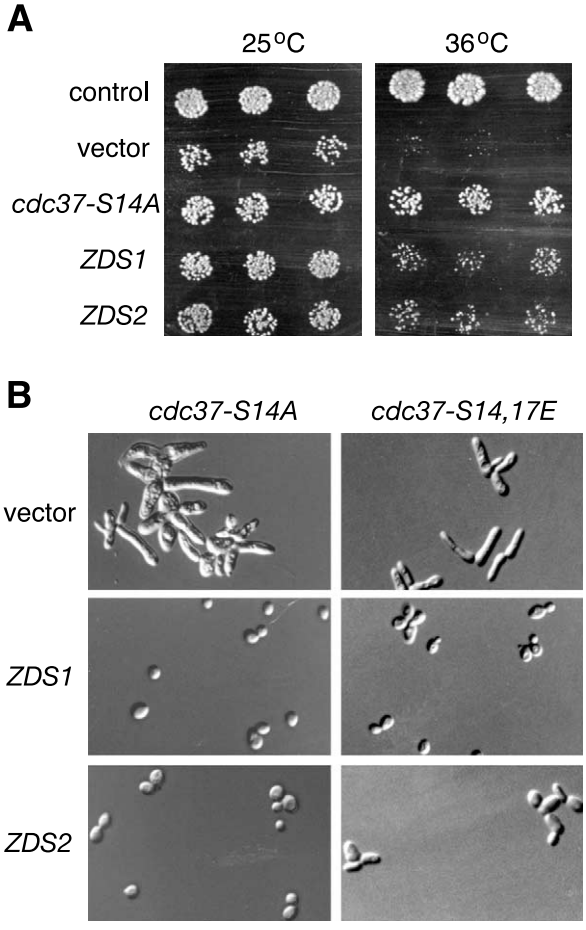

Fig. 3. Multicopy suppression of *cdc37* mutants by *ZDS1,2*. A: *ZDS1,2* overexpression suppresses the temperature sensitivity of *cdc37-S14A*. YSB11 (*CDC37*) transformed with pRS426 or YSB13 (*cdc37-S14A*) transformed with pRS426 or with pRS426 expressing *cdc37-S14A*, *ZDS1*, or *ZDS2* was spotted at 5000 cells/spot and grown at the indicated temperatures for 3 days before being photographed. B: *ZDS1,2* overexpression suppresses abnormal bud morphology of *cdc37-S14A* or *cdc37-S14,17E*. YSB13 (*cdc37-S14A*) and YSB15 (*cdc37-S14,17E*) bearing relevant overexpression plasmids as indicated were grown at 27°C to mid-log phase, and photographs of cells were taken on a Zeiss IM 35 epifluorescence microscope fitted with Nomarski optics.

which both serine-14 and an additional CK2 consensus site at serine-17 are replaced by glutamate, which partially restores Cdc37 function [15].

We next sought to test the role of Hsp90 in the genetic interaction between *ZDS1,2* and *CDC37*. For this, we again exploited the fact that *cdc37* mutants are hypersensitive to the Hsp90-specific inhibitor GA [15]. As shown in Fig. 4A, both *cdc37-1*, which encodes a C-terminal truncation of approximately the last third of the protein [20], and *cdc37-S14A* are hypersensitive to GA (35 μM). Overexpression of either *ZDS1* or *ZDS2* was able to partially suppress the GA sensitivity of both the *cdc37* mutants tested (Fig. 4A). We independently tested whether Zds1,2 could enhance Hsp90 function, and found that *ZDS1,2* overexpression did not suppress either the temperature sensitivity of *hsp90G170D* or *hsp90T22I* mutants or the GA sensitivity of *hsf1-1* mutants (which are GA sensitive because of reduced Hsp90 function), nor did overexpression of *HSP82* or *HSC82* (which encode the yeast Hsp90 isoforms) suppress the temperature sensitivity of *cdc37-1* or *cdc37-S14A* (data not shown). Thus, our results indicate that Hsp90 is not critical for the genetic relationship between Zds1,2 and Cdc37. These data also indicate that this interaction is not specific to CK2 phosphorylation site mutants of Cdc37.

Western blot analysis revealed that overexpression of

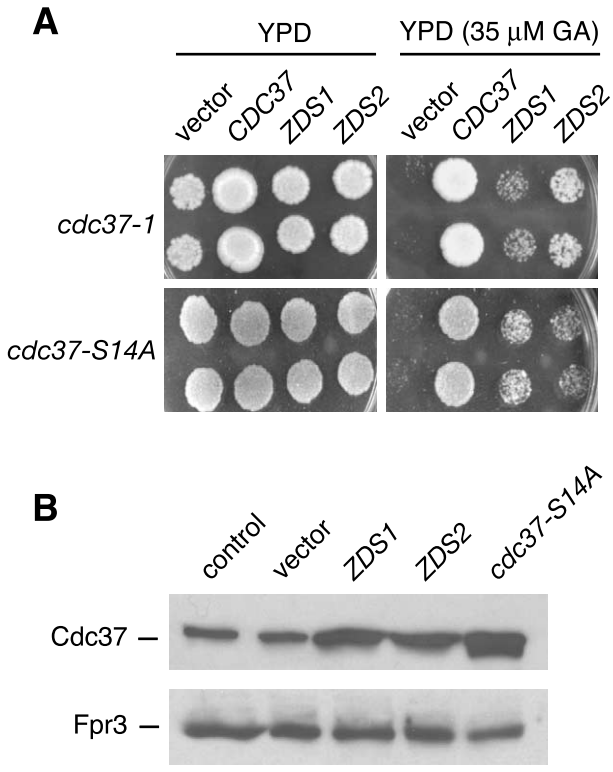

Fig. 4. *ZDS1,2* overexpression increases Cdc37 protein levels. A: YRM14.0 (*cdc37-1*) and YSB13 (*cdc37-S14A*) were transformed with pRS426 or pRS426 expressing *CDC37*, *ZDS1*, or *ZDS2* and spotted on YPD or YPD supplemented with GA (35 μM). Plates were incubated at 27°C (*cdc37-1*) or 30°C (*cdc37-S14A*) for 3 days. B: YSB11 (*CDC37*) transformed with pRS426 or YSB13 (*cdc37-S14A*) transformed with pRS426 or pRS426 expressing *ZDS1*, *ZDS2*, or *cdc37-S14A* was grown to mid-log phase at 27°C, and parallel immunoblots of cell lysates were probed with mouse monoclonal anti-Cdc37 and anti-Fpr3 as a loading control.

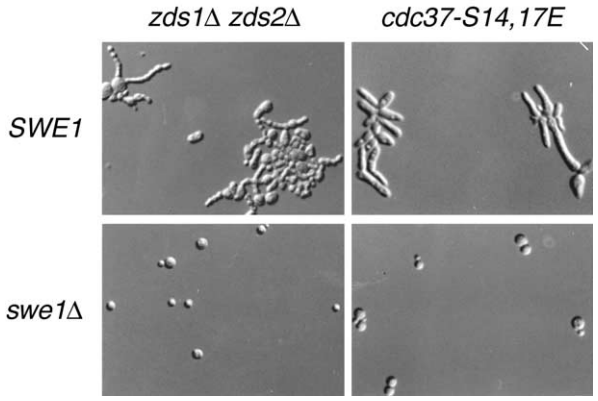

Fig. 5. Abnormal bud morphology of *cdc37-S14,17E* and *zds1*Δ *zds2*Δ is *SWE1*-dependent. YSB15 (*cdc37-S14,17E*), YSB27 (*cdc37-S14,17E swe1*Δ), DY3143 (*zds1*Δ *zds2*Δ), and YSB28 (*zds1*Δ *zds2*Δ *swe1*Δ) were grown at 27°C to mid-log phase in minimal medium and photographed with Nomarski optics.

ZDS1,2 in *cdc37-S14A* resulted in an increased steady-state level of Cdc37-S14A protein, though this increase was less than that attained upon overexpression of *cdc37-S14A* itself in the same strain background (Fig. 4B). These data coincide well with the enhanced suppression achieved upon overexpression of *cdc37-S14A* versus the suppression achieved by overexpression of *ZDS1,2* (Fig. 3A). Thus, we conclude that overexpression of *ZDS1,2* in *cdc37-S14A* achieves suppression by increasing the steady-state level (and hence function) of the partially functional, mutant Cdc37 protein. *ZDS1,2* overexpression was found to enhance Cdc37 levels in a *cka2-13* strain as well (data not shown).

SWE1 encodes a protein kinase that plays a pivotal role in *S. cerevisiae* morphogenesis by negatively regulating Cdc28-Clb activity [21,22]. Interestingly, the hyperpolarized phenotype of *zds1*Δ *zds2*Δ strains is efficiently reversed by deletion of the *SWE1* gene or by mutations of the Cdc28 kinase that render the protein *SWE1* independent [10–12,23]. Consistent with these reports, we find that *SWE1* deletion completely suppresses the abnormal morphology of a *zds1*Δ *zds2*Δ mutant (Fig. 5). Moreover, *ZDS1,2* overexpression inhibits the hyperpolarized morphology seen upon *SWE1* overexpression (data not shown). Thus, *ZDS1,2* behaves genetically as a negative regulator of *SWE1* function. Because of the genetic interactions among *ZDS1,2*, *CDC37*, and CK2, we asked whether the abnormal morphology of *cdc37* and/or CK2 mutants might also be *SWE1* dependent. As shown in Fig. 5, deletion of *SWE1* completely suppressed the abnormal morphology of *cdc37-S14,S17E*, consistent with the activation of Cdc28 by Cdc37 [20] and its inhibition by Swe1. Given the interactions described above, Zds could potentially effect Cdc28 kinase activity via both proteins simultaneously. In contrast, *SWE1* deletion did not suppress any abnormality associated with *cka2-13* mutants (data not shown). The latter result indicates that the interaction between *ZDS1* and CK2 does not result from inhibition of Swe1 by Zds1.

4. Discussion

We report here that *ZDS1,2* overexpression results in stimulation of both nodes of a recently reported, positive feedback

loop between protein kinase CK2 and the protein kinase-specific chaperone, Cdc37 [15]. Given the numerous substrates of CK2 and the diverse clients of Cdc37, activation of the feedback loop between these two proteins may contribute to the pleiotropic interactions displayed by *ZDS* in *S. cerevisiae*. We hasten to add, however, that although *ZDS1,2* enhance CK2 activity and Cdc37 function in *S. cerevisiae* when overexpressed, *zds1*Δ *zds2*Δ mutants exhibit no deficit in either CK2 activity toward Fpr3 or Cdc37 protein levels (S. Bandhakavi and C.V.C. Glover, unpublished). It thus appears unlikely that *ZDS* represents a physiological regulator of the positive feedback loop between these two proteins.

Because of the existence of the positive feedback between CK2 and Cdc37, it is not currently possible to state unequivocally which of the two proteins (or both) is the target of *ZDS*. However, coupled with our earlier identification of CK2 as a Cdc37 client [15], the observation that *ZDS* overexpression increases Cdc37 protein level (Fig. 4B) suggests a model in which *ZDS* overexpression elevates the steady-state abundance of Cdc37, which in turn stimulates CK2 activity. While additional and possibly direct effects of *ZDS* on CK2 activity cannot be rigorously excluded, the excellent correlation observed between Cdc37-S14A abundance (Fig. 4B) and the extent of *ZDS* suppression of *cdc37-S14A* (Fig. 3A) suggests that such mechanisms, if they exist, may be minor.

How might *ZDS* act to regulate Cdc37 abundance? The absence of allele specificity in the genetic interactions between *CDC37* and *ZDS1,2* provides no evidence for a physical interaction between the two proteins. Consistent with this, we were unable to detect a two-hybrid interaction between Zds1 and Cdc37 (or between either of these proteins and any of the four subunits of yeast CK2) (S. Bandhakavi and C.V.C. Glover, unpublished). Given that *ZDS1,2* suppress mutants of eukaryotic translational initiation factor eIf4A in yeast [9], one possibility is that *ZDS* enhances the translation of Cdc37 mRNA. Alternatively, *ZDS* might inhibit Cdc37 degradation. The ability of *ZDS* to function as a negative regulator of *SWE1* (Fig. 5) [10–12,23] may be of relevance in the latter regard, as degradation of Swe1 is pivotal to its function in G2/M cell cycle progression and the morphogenesis checkpoint [21]. Interestingly, deletion of either *HSL1* or *HSL7*, which are required for Swe1 degradation [24–26], yields a phenotype very similar to that of a *zds1 zds2* strain, and this phenotype is also efficiently suppressed by *ZDS* overexpression [11]. Although negative regulation of *SWE1* transcription by *ZDS* has been proposed to account for these effects [11], a role for *ZDS* in promoting Swe1 degradation would also be consistent with the observations. If the genetic interactions of *ZDS* with *CDC37* and *SWE1* are indeed mediated via an effect on protein turnover, clearly *ZDS* must act differently in the two cases, inhibiting degradation of Cdc37 but promoting degradation of Swe1.

No biochemical function for Zds has yet been defined, and the protein sequence contains no informative motifs. However, a recent screen for protein complexes in *S. cerevisiae* using affinity purification and mass spectrometry [27] provides some intriguing clues. In contrast to two-hybrid results indicating multiple interactions between Zds1 or 2 and cell polarity proteins [14], this screen identified both Zds1 and 2 as components of a multiprotein complex (complex 151) containing virtually all of the known subunits of protein phosphatase 2A (PP2A), including Cdc55, Pph21, Pph22, Rts1, and Tpd3, as well as multiple subunits of the proteasome. The association of Zds with PP2A is intriguing, as a role for Zds in regulation and/or targeting of this protein phosphatase could contribute to the pleiotropic genetic interactions of *ZDS*. Remarkably, *cdc55* or *tpd3* strains display an elongated morphology that is strikingly similar to that of a *zds1 zds2* mutant, and this phenotype is likewise suppressed by a *SWE1*-resistant allele of *CDC28* [10]. The presence of proteasome subunits in the complex is also intriguing in view of the importance of protein phosphorylation/dephosphorylation in targeting proteins for ubiquitin-mediated degradation. Future studies will be necessary to confirm a role for Zds1 and 2 in proteasome-mediated protein degradation and to determine whether PP2A activity is involved in this process.

Computer-based searches of completed eukaryotic genomes reveal that a *ZDS* ortholog is present in diverse fungal species, but absent in plants or metazoans (C.V.C. Glover, unpublished), suggesting a fungal-specific role. Given the importance of *ZDS* to cell polarity and morphogenesis in *S. cerevisiae* and the crucial role of morphology in fungal pathogenesis, *ZDS* may represent an attractive target for development of antifungal agents.

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