Sum1, a Highly Conserved WD-Repeat Protein, Suppresses S-M Checkpoint Mutants and Inhibits the Osmotic Stress Cell Cycle Response in Fission Yeast

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ABSTRACT

The S-M checkpoint ensures that entry into mitosis is dependent on completion of DNA replication. In the fission yeast *Schizosaccharomyces pombe*, the S-M checkpoint mutant *cdc2-3w* is thought to be defective in receiving the checkpoint signal. To isolate genes that function in the checkpoint pathway, we screened an *S. pombe* cDNA library for genes that, when overexpressed, could suppress the checkpoint defect of *cdc2-3w*. Using this approach, we have identified a novel gene, *sum1*$^+$ (suppressor of uncontrolled mitosis). *sum1*$^+$ encodes a highly conserved WD-transducin repeat protein with striking sequence similarity to the human transforming growth factor (TGF)-β-receptor interacting protein TRIP-1 and to the translation initiation factor 3 subunit eIF3-p39, encoded by the *TIF34* gene in *Saccharomyces cerevisiae*. *S. pombe sum1*$^+$ is an essential gene, required for normal cell growth and division. In addition to restoring checkpoint control, overexpression of *sum1*$^+$ inhibits the normal cell cycle response to osmotic stress. Furthermore, we demonstrate that inactivation of the stress-activated MAP kinase pathway, required for cell cycle stress response, restores the S-M checkpoint in *cdc2-3w* cells. These results suggest that Sum1 interacts with the stress-activated MAP kinase pathway and raise the possibility that environmental conditions may influence the checkpoint response in fission yeast.

CHECKPOINTS maintain the order of cell cycle events by blocking late events when early events have not been completed (Hartwell and Weinert 1989). The S-M checkpoint ensures that initiation of mitosis (M) is dependent on completion of DNA synthesis (S). In fission yeast, inhibitory tyrosine phosphorylation of Cdc2, the catalytic subunit of cyclin-dependent kinase (CDK), is required for the S-M checkpoint (Enoch *et al.* 1991; Enoch and Nurse 1990). Mutations that completely eliminate Cdc2 tyrosine phosphorylation, such as *cdc2F15*, result in loss of S-M checkpoint control and lethal premature mitosis known as "mitotic catastrophe" (Enoch *et al.* 1991). Other *cdc2* mutations disrupt checkpoint control while altering mitotic regulation in a more subtle fashion. One such mutant, *cdc2-3w*, is viable although it divides at a reduced size, indicating that the timing of mitosis is advanced. Unlike wild-type cells, when DNA replication is blocked with hydroxyurea (HU) or mutations in DNA replication enzymes, *cdc2-3w* cells proceed into mitosis, attempting to segregate a single set of chromosomes (Enoch *et al.* 1991; Enoch and Nurse 1990). Mutations that alter the activity of Cdc25, the major Cdc2 tyrosine phosphatase, and/or the Cdc2 tyrosine kinases Mik1 and Wee1 can also disrupt the S-M checkpoint (Enoch and Nurse 1990; Lundgren *et al.* 1991; Sheldrick and Carr 1993). Despite the importance of Cdc2 tyrosine phosphorylation in fission yeast cell cycle control, tyrosine phosphorylation of CDKs in budding yeast is not essential and is required for cell cycle control only under certain circumstances (Lew and Kornbluth 1996).

In addition to cell cycle regulators, six other genes required for the S-M checkpoint have been identified in fission yeast; these are *rad1*$^+$, *rad3*$^+$, *rad9*$^+$, *rad17*$^+$, *rad26*$^+$ and *hus1*$^+$ (Humphrey and Enoch 1995). Another gene, *cds1*$^+$ may also be involved (Murakami and Okayama 1995). Related genes are found in other eukaryotes including *Saccharomyces cerevisiae* and humans (Lieberman *et al.* 1996; Stewart and Enoch 1996). In particular, the *rad3*$^+$ gene is functionally and structurally similar to the *MEC1* gene from *S. cerevisiae* and the human *ATM* gene. Mutations in *ATM* are responsible for the severe cancer prone syndrome, ataxia-telangiectasia (Savitsky *et al.* 1995a). Thus there appears to be an evolutionarily conserved checkpoint pathway in eukaryotes that plays an important role in preventing cancer in human cells (Savitsky *et al.* 1995a,b). *rad3*$^+$ and *ATM* are also related to the gene encoding the catalytic subunit of the DNA dependent protein kinase (DDK), which is activated specifically by breaks in double-stranded DNA (Gottlieb and Jackson 1993; Hartley *et al.* 1995). By analogy it seems likely that Rad3 is activated by DNA structures formed when S-phase is inhibited, and this activation probably requires products of the other checkpoint genes. However, it is not known how Rad3 is activated and what proteins link Rad3 activation to tyrosine phosphorylation of Cdc2.

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To identify novel components of the checkpoint pathway, we have screened for genes that suppress the checkpoint defect of cdc2-*3w* mutants. We reasoned that because this mutant is only partially defective in mitotic control, overexpression of genes encoding positive regulators or upstream components of the S-M checkpoint pathway could suppress the checkpoint defect of *cdc2-3w*. Using this approach, we have identified a high copy suppressor of the *cdc2-3w* checkpoint defect, which we have named *sum1*$^+$ (suppressor of uncontrolled mitosis). *sum1*$^+$ is predicted to encode a highly conserved WD-transducin repeat protein with high sequence similarity to the human TGF-β-receptor interacting protein TRIP-1 (Chen *et al.* 1995), and to the translation initiation factor 3 subunit eIF3-p39, encoded by the *TIF34* gene in *Saccharomyces cerevisiae* (Naranda *et al.* 1997). *sum1*$^+$ is an essential gene, and we show that it is required for normal cell growth and division.

In addition to restoring the *cdc2-3w* checkpoint, overexpression of *sum1*$^+$ disrupts the normal cellular response to osmotic stress. In wild-type fission yeast cells, exposure to osmotic stress stimulates entry into mitosis. This response is controlled by a kinase cascade involving a MAP kinase kinase (MAPKK) encoded by *wis1*$^+$, and a MAP kinase (MAPK) encoded by *sty1*$^+$/*spc1*$^+$ (Millar *et al.* 1995; Shiozaki and Russell 1995). Activation of this pathway is negatively regulated by phosphotyrosine phosphatases encoded by *pyp1*$^+$ and *pyp2*$^+$, which dephosphorylate Sty1/Spc1(Millar *et al.* 1995; Shiozaki and Russell 1995). Like overexpression of *sum1*$^+$, mutations that block activation of the Sty1/Spc1 MAP kinase abolish the normal cell cycle stress response. Cells harboring such mutations undergo cell cycle arrest in response to osmotic stress. Interestingly, mutations that abolish the cell cycle stress response also suppress the checkpoint defect in *cdc2-3w*. These results suggest that Sum1 interacts with the stress-response pathway and raise the intriguing possibility that, because the stress-response and checkpoint response pathways have opposing effects on mitotic initiation, the S-M checkpoint may function less efficiently under stressful conditions.

MATERIALS AND METHODS

Growth of *S. pombe*: Standard media and growth conditions were used as described in Moreno *et al.* (1991), and cells were transformed by electroporation as described by Prentice (1992). Phase contrast, Nomarski and fluorescence micrographs were obtained using a Zeiss Axiophot microscope, and a Photonic Microscope Image Processor C1966 (Hamamatsu Photonic Sys. Corp., Bridgewater, NJ). All strains are listed in Table 1.

Screening for high copy plasmid suppressors of *cdc2-3w*: TE361 (*cdc2-3w ade6-M210 leu1-32 h*$^-$) was transformed with an *S. pombe* cDNA library in which cDNA expression is regulated by the thiamine repressible *nmt1*$^+$ promoter (B. Edgar and C. Norbury, unpublished results). Leu$^+$ transformants were selected on Edinburgh minimal medium (EMM) plates with adenine and 2 μm thiamine (EMM+A+T), and colonies replica plated to EMM+A plates without thiamine for 24 hr to induce the *nmt1*$^+$ promoter (Maundrell 1990). Colonies were replica plated to EMM+A plates with Phloxine B (Fisher Scientific, Pittsburgh) with and without 5 mm HU and grown for 2–3 days at 29°. Colonies that grew both in the presence and absence of HU were examined microscopically. Colonies consisting of cells that were normal in size in the absence of HU and elongated in the presence of HU were analyzed further. Eighteen transformants out of the 40,000 screened formed colonies in the presence and absence of HU. Plasmids were recovered from each of these transformants and 15 were able to retransform the original TE361 strain. One transformant displayed a consistently convincing phenotype. The corresponding gene, *sum1*$^+$, was subject to further analysis. A blunt ended *Sal*I to *Bam*HI DNA fragment encoding the *sum1*$^+$ cDNA from the *S. pombe* expression vector was subcloned into the *Eco*RV and *Bam*HI (blunt-ended) site of the Bluescript SK$^+$ plasmid (Stratagene, La Jolla, CA) to generate plasmid 6.3SK. Complete DNA sequence analysis of the insert revealed the 3′ end of the *sum1*$^+$ cDNA to be fused to a DNA fragment encoding a 50S ribosomal subunit. This 3′ region was deleted from the original *sum1*$^+$ expression plasmid, which was subsequently retested, and found to suppress the HU sensitivity of TE361.

Nucleotide sequence accession number: The cDNA sequence of *sum1*$^+$ has been deposited with GenBank under accession no. Y09529.

Construction of the *sum1::ura4*$^+$ mutant: A *sum1::ura4*$^+$ disruptant mutant was generated by inserting a 1.7-kb *Eco*RI fragment encoding the *ura4*$^+$ gene into the *Eco*RI site of *sum1*$^+$ in plasmid 6.3SK. An *Nde*I to *Age*I DNA fragment was isolated from this plasmid (6.3SKura4) and used to disrupt one copy of the *sum1*$^+$ gene, by the one-step disruption method, in an *h*$^+$/*h*$^+$ *ura4-D18*/*ura4-D18* stable diploid (TE480 gift of G. Cottarel, Mitotix Inc., Boston). Two stable ura$^+$ transformants were selected, and the *Nde*I to *Age*I DNA fragment was confirmed to have been homologously integrated into the *sum1*$^+$ locus of these transformants by Southern blotting. One of these strains (TE557) was crossed to an *h*$^-$/*h*$^-$ *ura4-D18*/*ura4-D18* stable diploid (TE558) to generate sporulating diploids heterozygous for the *sum1::ura4*$^+$ disruption (TE554). These diploids were sporulated on minimal media plates, and 20 tetrads dissected. A 2$^+$:2$^-$ ratio for viability was observed in all dissected tetrads, and the viable colonies failed to grow in the absence of uracil, indicating that disruption of the *sum1*$^+$ gene is lethal. The *sum1::ura4*$^+$ disruption is a recessive mutation because a wild-type phenotype was observed in the heterozygous diploid strain. Furthermore, overexpression of *sum1::ura4*$^+$ from a pREP3X promoter (Basi *et al.* 1993) cannot suppress the checkpoint defect of TE361 and does not cause any obvious phenotypes.

Sum1 depletion study: Strain TE578 (*sum1::ura4*$^+$ *ura4-D18 leu1-32 ade6-M210 h*$^+$ pREP41X-*sum1*$^+$ *LEU2*) was grown in EMM+A media in the absence of thiamine to midexponential phase. This culture was diluted to an optical density (OD) 595 of 0.02, split into two halves, and thiamine (2 μm final concentration) was added to one half of the culture to repress *sum1*$^+$ transcription from the weak *nmt1*$^+$ REP41X promoter (Basi *et al.* 1993). The OD 595 and cell number (cells/ml) of the cultures were recorded for 24 hr after addition of thiamine, and strains were photographed after 36 hr.

Cloning and expression of *sum1*$^+$ homologues in fission yeast: The TRIP-1 gene was PCR amplified from a human cardiac cDNA library (a generous gift from the J. Seidman Lab, Harvard Medical School, Boston) using the primers: 5′CCGGGAT GAAGCCGATCCT 3′ and 5′GTTGGTCAGGCTGGTCTTAA 3′ to give a 1088nt PCR product and subcloned into the pCRII vector (Invitrogen, San Diego). An *Spe*I (blunt-ended) to *Not*I

TABLE 1

***S. pombe* strains used in this study**

Strain no.	Genotype
TE361	*cdc2-3w ade6-M210 leu1-32 h*$^-$
TE480	DIPLOID *ade6-M216/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^+$*/h*$^+$ (GP109)
TE554	DIPLOID *sum1*$^+$*/sum1::ura4*$^+$ *ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^+$*/h*$^-$
TE557	DIPLOID *sum1*$^+$*/sum1::ura4*$^+$ *ade6-M216/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^+$*/h*$^+$
TE558	DIPLOID *ade6-M210/ade6-M210 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^-$*/h*$^-$
TE577	*sum1::ura4 ura4-D18 leu1-32 ade6-M210 h*$^+$ pREP3X-*sum1*$^+$*(LEU2)*
TE578	*sum1::ura4 ura4-D18 leu1-32 ade6-M210 h*$^+$ pREP41X-*sum1*$^+$*(LEU2)*
TE596	*cdc2-3w ade6-M210 leu1-32 h*$^-$ pREP3X*(LEU2)*
TE742	*cdc2-3w spc1-M13 ura4-D18 leu1-32 h*$^-$
TE805	*cdc2-3w ade6-M210 leu1-32 h*$^-$ pREP3X-*sum1*$^+$*(LEU2)*
TE806	*cdc2-3w ade6-M210 leu1-32 h*$^-$ pREP1-*pyp1*$^+$*(LEU2)*
TE807	*leu1-32 h*$^-$ pREP81X*(LEU2)*
TE808	*leu1-32 h*$^-$ pREP3X-*sum1*$^+$*(LEU2)*
TE809	*leu1-32 h*$^-$ pREP1-*pyp1*$^+$*(LEU2)*
TE810	*spc1HA 6H(ura*$^+$*) leu1-32 ura4-D18 h*$^-$ pREP3X*(LEU2)*
TE811	*spc1HA 6H(ura*$^+$*) leu1-32 ura4-D18 h*$^-$ pREP3X-*sum1*$^+$*(LEU2)*
TE812	*spc1HA 6H(ura*$^+$*) leu1-32 ura4-D18 h*$^-$ pREP1-*pyp1*$^+$*(LEU2)*
TE813	*spc1-M13, ura4-D18, leu1-32 h*$^-$
TE814	*pyp1::ura4*$^+$ *leu1-32 ura4-D18 h*$^+$ pREP3X-*sum1*$^+$*(LEU2)*
TE815	*pyp2::ura4*$^+$ *leu1-32 ura4-D18 h*$^-$ pREP3X-*sum1*$^+$*(LEU2)*

All strains were from the Enoch strain collection except TE480 (GP109), a generous gift from G. Cottarel (Mitotix Inc., Boston). Strains TE742 and TE813 are derived from KS1147. Strains TE810, TE811, and TE812 were derived from KS1376. KS1147 and KS1376 are described in Shiozaki and Russell (1995, 1996) and were generous gifts from P. Russell.

DNA fragment (1154nt) was subcloned into the *Xho*I (blunt-ended) and *Not*I sites of the *S. pombe* expression plasmids REP3X and REP41X, to form plasmids REP3X-TRIP-1 and REP41X-TRIP-1, respectively. The *S. cerevisiae TIF34* gene was PCR amplified from *S. cerevisiae* total DNA using the primers: 5′GCAGAAGAGTTACTGATGAAC3′ and 5′CCCCTCGTG GTTAAAGTGAC 3′, to yield a 1356nt PCR product, which was subcloned into the pCRII vector. An *Xho*I to *Bam*HI DNA fragment (1434nt) was subcloned into the *Xho*I and *Bam*HI sites of the *S. pombe* expression plasmids REP3X and REP41X to form plasmids REP3X-*TIF34* and REP41X-*TIF34*, respectively. To test the ability of these strains to functionally complement the *sum1::ura4*$^+$ disruption, strain TE554 (*sum1*$^+$*/sum1::ura4*$^+$ *ade6-M210/ade6-M216 leu1-32/leu1-32 ura4-D18/ura4-D18 h*$^+$*/h*$^-$) was transformed with these plasmids, and Leu$^+$ transformants were selected and sporulated using standard conditions. Leu$^+$ spores were germinated on EMM+A plates with uracil (EMM+A+U), and colonies replica plated to EMM+A plates. Only colonies transformed with plasmids that can complement the *sum1::ura4*$^+$ disruption should be able to grow in the absence of uracil.

Phosphotyrosine levels of Sty1/Spc1 protein: Strain KS1376 (a generous gift from P. Russell, Scripps Research Institute, La Jolla, California) in which Sty1/Spc1 has been tagged with two copies of the HA epitope and six histidine residues was transformed with either pREP3X, pREP3X-*sum1*$^+$ or pREP1-*pyp1*$^+$ (a generous gift from P. Russell) to generate strains TE810, TE811 and TE812, respectively (see Table 1). These strains were grown to midlog phase in EMM media at 29° following derepression of the *nmt1*$^+$ promoter for 24 hr. KCl was added to a final concentration of 0.9 m for 20 min before harvesting. Pelleted cells were lysed with glass beads (Sigma Chemical Co., St. Louis) into lysis buffer containing 6 m urea, 0.5% NP-40, 0.5% deoxycholate, 50 mm Tris-HCl (pH 7.5), 50 mm NaF, 10% glycerol, 2 mm Na-orthovanadate, 10 mm β-mercaptoethanol, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 100 μm TPCK, 2 mm PMSF, 60 mm β-glycerophosphate, 15 mm paranitrophenol phosphate, and 1 μm okadaic acid. The Sty1/Spc1 protein was isolated by affinity purification using Ni^{2+} charged His-Bind beads (Novagen, Madison, Wisconsin), according to the manufacturer's instructions. The proteins were resolved by 12% sodium dodecyl sulphate (SDS) PAGE and transferred electrophoretically to a nitrocellulose membrane. The membrane was immunoblotted with anti-HA (12CA5) and anti-pTyr (4G10; Upstate Biotechnology, Inc., Lake Placid, NY) antibodies, which were detected by enhanced chemiluminescence (ECL; Amersham, Arlington Heights, IL) according to the manufacturer's instructions.

RNA analysis: Strains TE554 (*sum1::ura4*$^+$ *ura4-D18 leu1-32 ade6-M210 h*$^+$ pREP41X-*sum1*$^+$*LEU2*), TE807 (*leu1-32 h*$^-$ pREP81X), TE808 (*leu1-32 h*$^-$ pREP3X-*sum1*$^+$*LEU2*) and TE809 (*leu1-32 h*$^-$ pREP1-*pyp1*$^+$*LEU2*) were grown in 200-ml cultures to midlog phase in EMM+A. Strain TE554 was grown in the presence of 2 μm thiamine for 18 hr to repress *sum1*$^+$ transcription from the REP41X promoter. KCl was added to a final concentration of 0.9 m for 90 min to half of each culture before harvesting. The pelleted cells were lysed with glass beads in 1 ml of solution containing 0.32 m sucrose, 20 mm Tris-HCL (pH 7.5) 10 mm EDTA and 0.5 mg/ml heparin, and diluted with 10 ml of the above solution containing 1% SDS. Phenol extraction was performed at 60° for 3 min followed by further phenol/chloroform extraction at 22°, and total RNA was ethanol precipitated. For Northern hybridizations, 10 μg of total RNA was separated on a denaturing formaldehyde gel according to the method of Rave *et al.* (1979). Following transfer to nitrocellulose (Genescreen, Life Science Products, Boston), the bound RNA was hybridized to either *gpd1*$^+$ or *act1*$^+$ probes. The *gpd1*$^+$ probe was generated from a 1253 bp *Hin*dIII to *Xba*I DNA fragment from the plasmid PCR-*gpd1*$^+$. The *act1*$^+$ probe was generated from a 1126 bp *Eco*RV to *Hin*dIII DNA

fragment from the plasmid PCR-*act1*$^+$. Primers were labeled as described in Feinberg and Vogelstein (1984).

Genetic analysis of the role of *sum1*$^+$ in stress response: To determine whether the lethality of the *sum1::ura4*$^+$ disruption was suppressed by mutations in the *sty1*$^+$/*spc1*$^+$ gene, strain TE577, in which the *sum1::ura4*$^+$ mutation is complemented by the plasmid-borne, thiamine-repressible REP3X-*sum1*$^+$ gene, was crossed to strain TE813 (derived from KS1147, a generous gift from P. Russell), carrying a mutation in the Spc1/Sty1 MAP kinase, *spc1-M13*. The products of this cross were examined by free spore analysis using standard genetic techniques. No ura$^+$ colonies were isolated from this cross on plates containing thiamine, indicating that the lethality of *sum1::ura4*$^+$ is not suppressed by the *spc1-M13* mutation. To determine whether overexpression of *sum1*$^+$ could suppress the lethality of a *pyp1 pyp2* double mutant, *pyp1* (TE814) and *pyp2* (TE815) strains in which *sum1*$^+$ was overexpressed, were crossed, and the progeny were examined by tetrad analysis. No viable double mutants were obtained from this experiment, indicating that overexpression of *sum1*$^+$ cannot suppress the lethality of a *pyp1 pyp2* double mutant.

RESULTS

Identification of genes that suppress the *cdc2-3w* checkpoint defect: To identify genes that positively regulate the S-M checkpoint in fission yeast, we screened for genes that could restore the S-M checkpoint when overexpressed in *cdc2-3w* cells. Such genes might encode upstream components of the checkpoint pathway or positive regulators of the checkpoint response. As the *cdc2-3w* mutant is defective in the S-M checkpoint, exposure of *cdc2-3w* to HU causes cells to enter mitosis inappropriately, with incompletely replicated chromosomes (Enoch *et al.* 1992; Enoch and Nurse 1990). In these cells the septum bisects the single nucleus resulting in aneuploid or anucleate cells; this resembles the morphology of "cut" mutants (Hirano *et al.* 1986). As a result, *cdc2-3w* cells fail to form colonies in the presence of HU (Enoch and Nurse 1990). In contrast, wild-type cells initially undergo cell cycle arrest in the presence of HU, and then resume the cell cycle with a longer S-phase, forming slow-growing colonies consisting of highly elongated cells. Overexpression of genes encoding positive regulators of checkpoint function may allow growth of *cdc2-3w* on HU as elongated cells. These can be distinguished from genes that counteract the effects of HU, such as the catalytic subunit of ribonucleotide reductase, as cells overexpressing these genes divide at a normal length on HU (T. Enoch, unpublished results). Suppressors can also be distinguished from general negative regulators of the cell cycle, as such genes will block cell division both in the presence and absence of HU.

To identify genes encoding positive regulators of checkpoint function, the strain TE361 (*cdc2-3w, ade6-M210, leu1-32 h*$^-$) was transformed with an *S. pombe* cDNA library under the control of the thiamine-repressible *nmt1*$^+$ promoter (Maundrell 1990) on a vector encoding the *LEU2* gene (B. Edgar and C. Norbury, unpublished results). Expression of cDNA is conditional and can be activated by replica plating cells to media without thiamine. Leu$^+$ transformants were selected that could form colonies on minimal media plates in the presence of 5 mm hydroxyurea following derepression of the *nmt1*$^+$ promoter (see materials and methods). Colonies were examined microscopically, and transformants were selected that elongated in the presence of HU but were not significantly elongated in its absence. Using this approach, 40,000 transformants were screened, and six of these transformants appeared to delay the cell cycle in a HU-dependent manner. The strongest of these suppressors was found to be encoded by a novel gene, *sum1*$^+$, and was subject to further analysis.

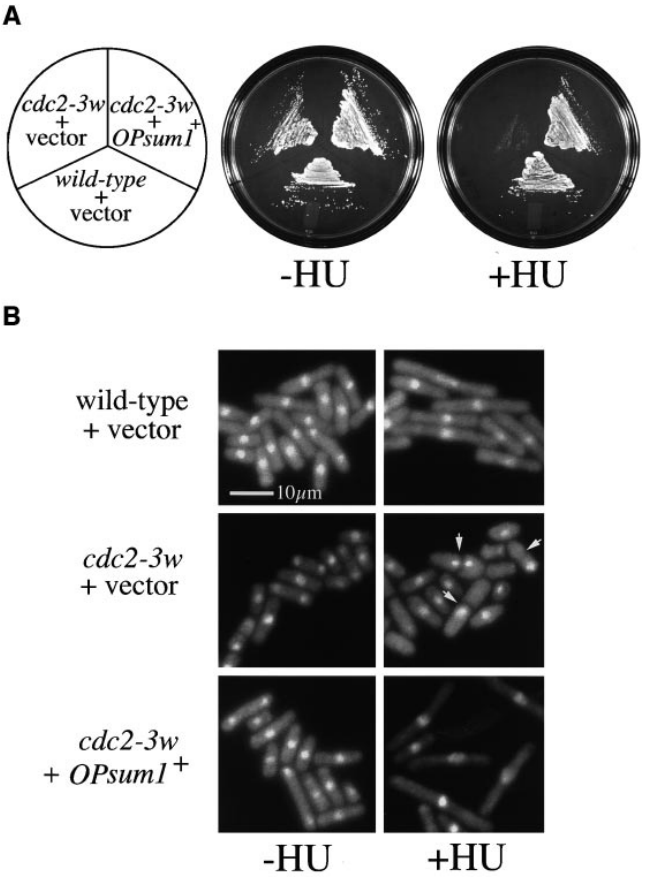

Figure 1.—*OPsum1*$^+$ suppresses the HU sensitivity of *cdc2-3w*. (A) TE361 transformed with pREP3X plasmid (vector) or pREP3X-*sum1*$^+$ (*OPsum1*) and grown at 29°, on EMM+A in the absence (−HU) or presence (+HU) of 5 mm HU. Wild-type cells transformed with vector alone (TE807) are shown (wild-type + vector) as a positive control. (B) Overexpression of *sum1*$^+$ delays the cell cycle of *cdc2-3w* in HU. The above strains were grown in EMM+A liquid media in the absence (−HU) or presence (+HU) of 5 mm HU for 6 hr at 29°. Cells were fixed and DAPI stained to visualize the nuclear material as described in materials and methods. White arrows indicate cells showing a "cut" phenotype. Bar, 10 μm.

The phenotype of *cdc2-3w* cells overexpressing *sum1*$^+$ is shown in Figure 1. *cdc2-3w* transformed with vector alone fails to grow on plates in the presence of HU. In contrast, *cdc2-3w* transformed with an *nmt1*$^+$ (pREP3X) plasmid expressing *sum1*$^+$ (*OPsum1*$^+$) is able to form colonies in the presence of HU. These cells fail to grow in the

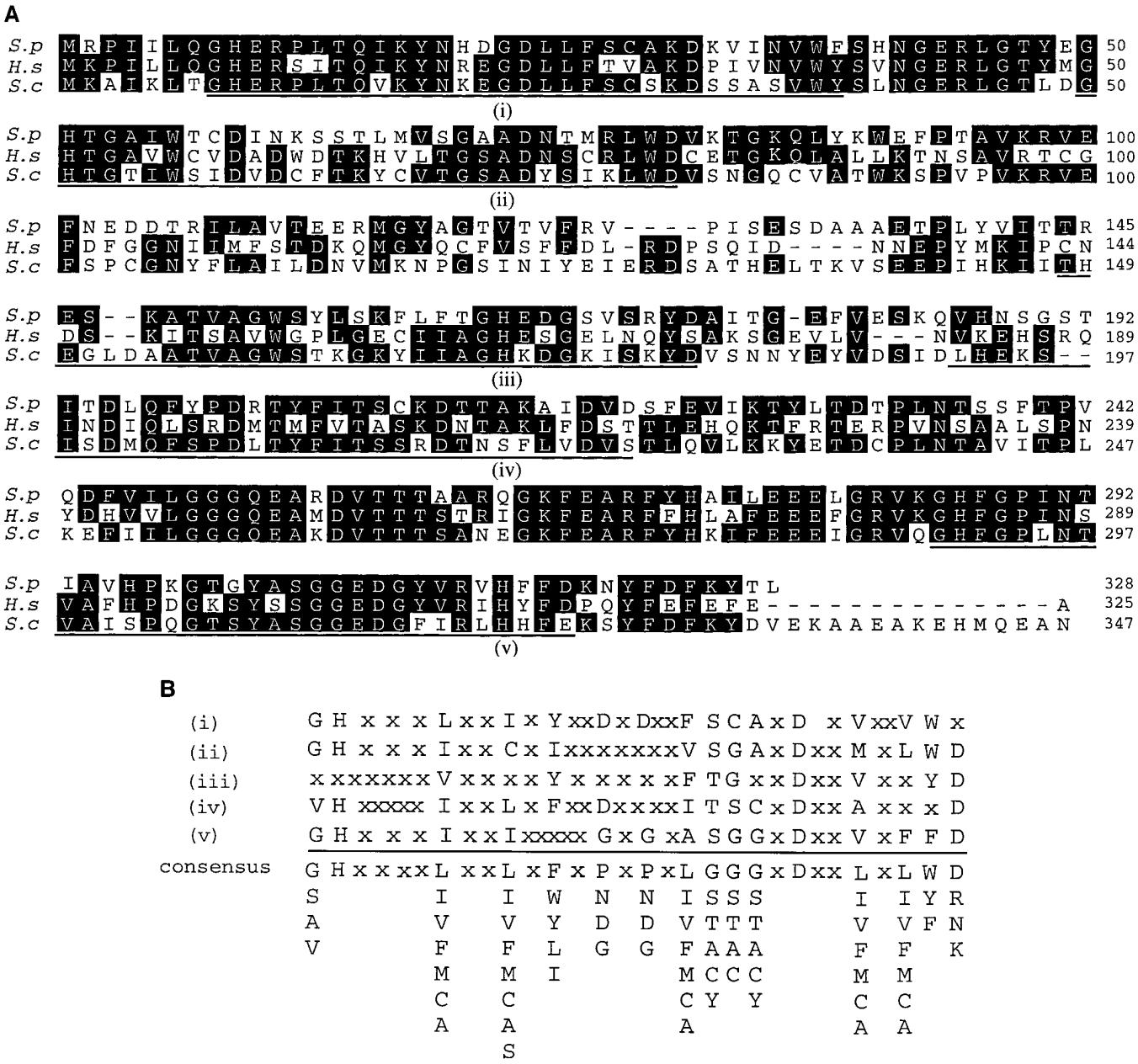

Figure 2.—*sum1*$^+$ encodes a highly conserved WD-transducin repeat protein. (A) Alignment of the amino acid sequence from the *S. pombe* (*S.p*) Sum1 ORF with that of TRIP-1 from *H. sapiens* (*H.s*) and *TIF34* from *S. cerevisiae* (*S.c*). Boxes indicate identities among all three proteins. The WD/transducin repeats are underlined. The sequence alignment was performed using the DNASTAR "Megalign" program. The nucleotide sequence of the *sum1*$^+$ cDNA is found in the GenBank nucleotide sequence data base under accession number Y09529. (B) Alignment of the WD-transducin repeats of Sum1 with the WD-transducin repeat consensus sequence, as defined by Neer *et al.* (1994). The WD transducin domains (i–v) correspond to those underlined in (A).

presence of HU when the *nmt1*$^+$ promoter is transcriptionally repressed (data not shown). Cytological examination reveals that overexpression of *sum1*$^+$ in *cdc2-3w* cells in the absence of HU induces a modest G2 delay compared to *cdc2-3w* transformed with vector alone (compare Figure 1B, middle and lower left panels; FACS data not shown). In the presence of HU, *cdc2-3w* cells transformed with vector alone form a "cut" phenotype, as described previously (Figure 1B, middle right panel). However, *cdc2-3w* cells in which *sum1*$^+$ is overexpressed became elongated in HU (Figure 1B, lower right panel), resembling wild-type cells in HU (Figure 1B, upper right panel). Thus, overexpression of *sum1*$^+$ restores checkpoint function in *cdc2-3w* mutants. Overexpression of *sum1*$^+$ also restores checkpoint function in *wee1.50 mik1* at the permissive temperature and restores strains overexpressing *cdc25*$^+$ (data not shown). However, checkpoint function is not restored in checkpoint *rad* mutants, *hus1* or *rad3* (data not shown).

***sum1*$^+$ encodes a highly conserved WD-repeat protein:** The *sum1*$^+$ cDNA has been sequenced and is predicted to encode a protein of 328 amino acids (Figure 2A). The

cDNA sequence of *sum1*$^+$ has been deposited with GenBank under accession number Y09529. (This sequence has also been deposited by S. Yoshioka, K. Kato and H. Okayama as part of the complete sequencing of *Schizosaccharomyces* cDNA project under GenBank accession no. D89187.) A search through the protein databases reveals Sum1 to be related to proteins from a wide range of eukaryotes. In particular, genes predicted to encode proteins with a striking degree of amino acid identity to *sum1*$^+$ were identified in the genomes of humans and *S. cerevisiae* (Figure 2A). The human homologue, TRIP-1, shares 49% amino acid identity and 65% similarity to Sum1 and was identified as a protein that is phosphorylated by the transforming growth factor (TGF)-β type II receptor (Chen *et al.* 1995). The *S. cerevisiae* homologue, encoded by *TIF34*, is predicted to be 53% identical and 72% similar to Sum1. *TIF34* has recently been shown to encode the 39 kD subunit of the eukaryotic translation initiation factor 3 (Naranda *et al.* 1997). All three genes encode members of the WD-repeat family of proteins (Neer *et al.* 1994) and each possess five putative core WD-repeat domains (underlined in Figure 2A). These WD-transducin domains have been found in a number of regulatory proteins and are thought to be involved in protein-protein interactions. A comparison of the five Sum1 WD-repeat domains with the WD-repeat consensus sequence is shown in Figure 2B. The region of sequence homology is extended throughout these proteins and is not restricted to the WD-repeat regions.

***Sum1*$^+$ is an essential gene:** To examine the *sum1* null phenotype in fission yeast, a construct was generated in which *sum1*$^+$ was disrupted with a copy of the *ura4*$^+$ gene (see Figure 3A; materials and methods). A DNA fragment encoding the disrupted *sum1*$^+$ gene was transformed into a stable *h*$^+$/*h*$^+$*ura4-D18*/*ura4-D18* diploid (TE480). Ura+ transformants were selected, and homologous integration was confirmed by Southern blotting. Transformants were crossed to an *h*$^-$/*h*$^-$ *ura4-D18*/*ura4-D18* diploid (TE558) to generate sporulating diploids heterozygous for the *sum1::ura4*$^+$ disruption. The heterozygous diploids had a wild-type phenotype, indicating that the *sum1::ura4*$^+$ disruption was a loss of function mutation. Tetrad analysis of two independent ura$^+$ diploid transformants revealed a 2$^+$:2$^-$ segregation for viability in each of 20 asci dissected on yeast extract plates. The viable colonies were all found to be ura$^-$, indicating that disruption of the *sum1*$^+$ gene is lethal. Upon microscopic examination, the nonviable segregants were found to be indistinguishable from ungerminated spores, suggesting that *sum1*$^+$ is required for either germination, cell growth, or both.

To determine whether *sum1*$^+$ is necessary for normal cell growth and division, a strain was constructed (TE578) in which the lethality of the *sum1::ura4*$^+$ disruption was rescued by plasmid expression of the *sum1*$^+$ gene under the control of a weakened *nmt1*$^+$ (REP41X) promoter (Basi *et al.* 1993; see materials and methods). This strain cannot form colonies in the presence of thiamine. To examine the effects of Sum1 depletion on cell growth and division, an exponentially growing culture was diluted, and thiamine was added to one half of the culture to transcriptionally repress the complementing pREP41X-*sum1*$^+$ gene. It took approximately 18 hr to deplete *sum1*$^+$ function under these conditions. Cell mass (OD 595) and cell number (cells/ml) of these cultures were recorded for 24 hr following addition of thiamine (Figure 3B). An exponential increase in both cell mass and cell number is observed over a 24-hr period in the absence of thiamine (promoter on). In contrast, the exponential increase in cell mass and cell number is no longer observed following transcriptional repression of pREP41X-*sum1*$^+$ in thiamine. Instead, cell growth is greatly retarded in this culture (OD-promoter off), and no further cell division is observed after 22 hr following addition of thiamine (cells/ml promoter off). In addition, transcriptional repression of the pREP41X-*sum1*$^+$ gene also resulted in irreversible loss of viability (data not shown). These results indicate that *sum1*$^+$ is required for normal cell growth and cell division in fission yeast. The cell cycle distribution of the cells in which *sum1*$^+$ was transcriptionally repressed is indistinguishable from that of wild-type cells indicating that Sum1 depletion does not arrest the cell cycle (FACS data not shown). Furthermore, no "cut" phenotype was observed in this strain following transcriptional repression of *sum1*$^+$ in the presence of HU (data not shown).

Microscopic analysis of cells in which *sum1*$^+$ is transcriptionally repressed (Figure 3C, promoter off) reveals them to be morphologically distinct from cells expressing *sum1*$^+$ (Figure 3C, promoter on). Sum1 depleted cells are swollen, pear-shaped, and slightly larger than wild-type cells (Nomarski used). Moreover, DAPI staining of these cells, which weakly stains the cytoplasm in addition to the nuclear material, reveals dark nonstaining regions within these cells appearing with time. These regions may be vacuolar or may represent regions in which the cytoplasm has become dissociated from the cell wall.

Because the lethality of the *sum1::ura4*$^+$ strain can be rescued by plasmid expression of the *sum1*$^+$ gene, we examined whether the human TRIP-1 and yeast *TIF34* genes could also functionally complement the *sum1::ura4*$^+$ strain. These genes were subcloned into *S. pombe* expression vectors and transformed into a diploid strain heterozygous for the *sum1::ura4*$^+$ disruption mutation (see materials and methods). Sporulation of these diploid transformants revealed that, in contrast to cells expressing the *sum1*$^+$ gene, human TRIP-1 and yeast *TIF34* genes were unable to functionally complement the *sum1::ura4*$^+$ strain. In addition, the fission yeast *sum1*$^+$ gene and the human TRIP-1 gene were unable to functionally complement the lethality of a *TIF34::HIS3 S. cerevisiae* strain (data not shown), consistent with findings re-

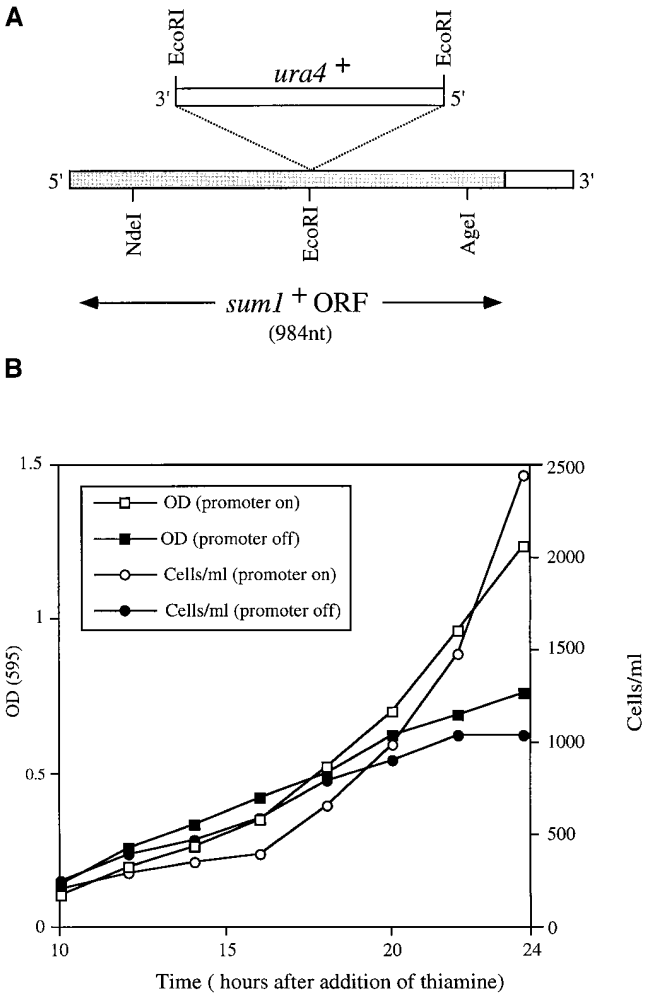

Figure 3.—Construction and analysis of the *sum1* disruption mutant. (A) Diagram of the DNA construct used to make the *sum1::ura4*$^+$ disruption mutant. The *sum1*$^+$ ORF is indicated in grey. The *ura4*$^+$ gene was inserted into the *Eco*RI site of *sum1*$^+$ in the orientation shown as described in materials and methods. (B) Effects of Sum1 depletion on cell growth and division. The optical density (OD 595; □, ■) and cell number (cells/ml) (○, ●) of an exponential culture of TE578 (*sum1::ura4*$^+$, *ura4-D18*, *leu1-32 ade6-M210 h*$^+$ pREP41X-*sum1*$^+$ *LEU2*) in EMM+A was recorded in the absence (promoter on; □, ○) and presence (promoter off; ■, ●) of 2 μm thiamine added at 0 hr. (C) Phenotype of Sum1 depleted cells. Strain TE578 was grown in EMM+A in the absence (promoter on) or presence (promoter off) of 2 μm thiamine as described above. Cells were fixed, DAPI-stained and photographed using Nomarski and fluoresence (DAPI) microscopy, as indicated. Bar, 10 μm.

ported by others (Naranda *et al.* 1997). Overexpression of the TRIP-1 and *TIF34* genes was also unable to suppress the checkpoint defective phenotype of *cdc2-3w* (data not shown). Thus, despite a high degree of sequence similarity, functional complementation is not observed between species.

Overexpression of *sum1*$^+$ inhibits the normal cell cycle response to osmotic stress: During the course of these experiments we noticed that the phenotypes caused by *sum1* mutations are similar to phenotypes caused by mutations altering expression of the *S. pombe* phosphotyrosine phosphatases (PTPases) Pyp1 and Pyp2. The Pyp1 and Pyp2 PTPases, encoded by the *pyp1*$^+$ and *pyp2*$^+$ genes, function to negatively regulate the highly conserved stress-activated MAP kinase encoded by the *sty1*$^+$/*spc1*$^+$ gene in fission yeast (Millar *et al.* 1995; Shiozaki and Russell 1995). This kinase is activated in response to environmental stress including heat shock, oxidative stress, and osmotic stress (Degols *et al.* 1996; Millar *et al.* 1995; Shiozaki and Russell 1995). Activation of the stress-activated MAP kinase pathway stimulates entry into mitosis (Hannig *et al.* 1994; Millar *et al.* 1992). The Pyp1 and Pyp2 PTPases negatively regulate this pathway through tyrosine dephosphorylation of the Sty1/Spc1 stress-activated MAP kinase (Millar *et al.* 1995; Shiozaki and Russell 1995). Overexpression of either *pyp1*$^+$ or *pyp2*$^+$ inactivates the MAP kinase pathway, causing a cell cycle delay in G2/M under normal growth conditions, and cell cycle arrest in response to stress (Millar *et al.* 1995; Shiozaki and Russell 1995). As overexpression of *sum1*$^+$ also causes a G2/M delay, it resembles the *pyp*$^+$ genes in this respect. Furthermore, simultaneous inactivation of Pyp1 and Pyp2 PTPases, like disruption of *sum1*$^+$, gives rise to inviable spores that do not germinate (Millar *et al.* 1992), and inhibition of *pyp1*$^+$ expression in a *pyp2* strain results in a similar phenotype to Sum1-depleted cells; the cells exhibit greatly retarded growth and are large and swollen (Millar *et al.* 1995). Thus, loss of Sum1 or Pyp1 and Pyp2 leads to severe growth defects, whereas overexpression of these genes results in an extended G2 phase.

These similarities prompted us to analyze the effects of overexpression of *sum1*$^+$ on the response of cells to osmotic stress. Although in wild-type cells osmotic stress stimulates entry into mitosis, cells overexpressing *pyp1*$^+$, or lacking functional Sty1/Spc1 MAP kinase become highly elongated in the presence of 1 m KCl or 1.5 m sorbitol (Shiozaki and Russell 1995). As shown in

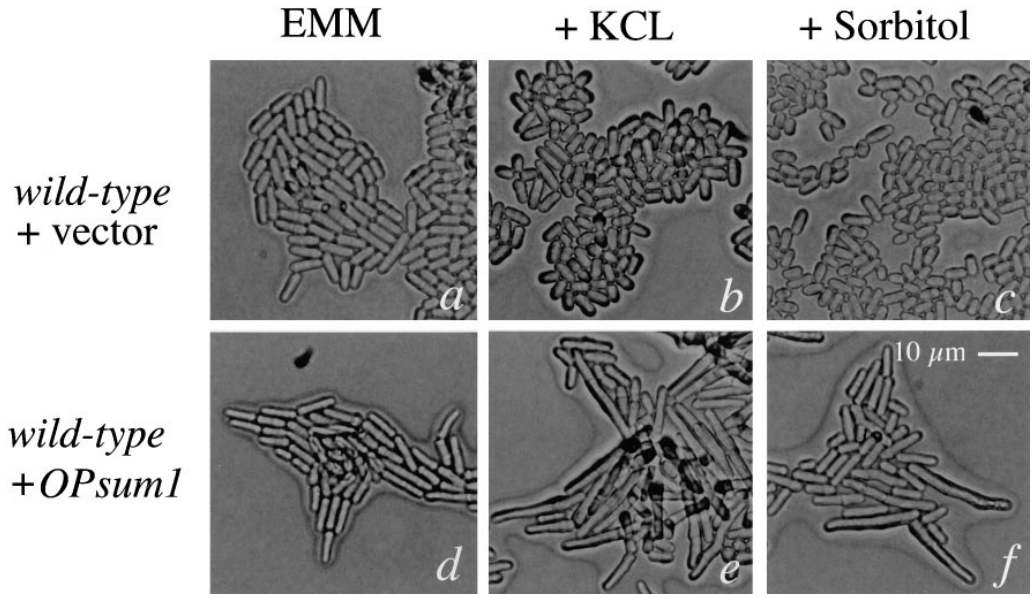

Figure 4.—Overexpression of *sum1*$^+$ disrupts the cell cycle response to osmotic stress. Strains TE807 (wild-type +vector) and TE808 (wild-type +*OPsum1*$^+$) were grown on EMM+T plates, restreaked onto EMM plates for 24 hr to derepress the *nmt1*$^+$ promoter, and replica plated to EMM plates containing either 1 m KCl or 1.5 m sorbitol. These plates were incubated for 24 hr at 29° and cells photographed. Bar, 10 μm.

Figure 4, wild-type cells overexpressing *sum1*$^+$ in the presence of 1 m KCl or 1.5 m sorbitol display a remarkably similar phenotype. Wild-type cells transformed with vector alone (Figure 4, +vector) divide at a smaller size in response to osmotic stress (Figure 4, b and c). In contrast, cells overexpressing *sum1*$^+$ (Figure 4, *OPsum1*) become highly elongated (3–4× the length of controls) in the presence of 1 m KCl or 1.5 m sorbitol (Figure 4, compare e and f with b and c, respectively). These results demonstrate that overexpression of *sum1*$^+$, like inactivation of the MAP kinase pathway, interferes with the normal cell cycle response to osmotic stress.

Overexpression of *sum1*$^+$ does not inhibit stress-activation of the Sty1/Spc1 MAP kinase: The similarities between Sum1 and Pyp1 suggest that these proteins may perform a similar function. Pyp1 and Pyp2 negatively regulate the stress-response pathway by dephosphorylation of a phosphotyrosine residue required for Sty1/Spc1 MAP kinase activity (Millar *et al.* 1995; Shiozaki and Russell 1995). Stimulation of the stress-activated MAP kinase pathway results in tyrosine phosphorylation of the Sty1/Spc1 MAP kinase (Degols *et al.* 1996; Millar *et al.* 1995; Shiozaki and Russell 1995). When *pyp1*$^+$ is overexpressed in stressed cells, greatly reduced levels of tyrosine phosphorylated Sty1/Spc1 are observed (Millar *et al.* 1995; Shiozaki and Russell 1995). To determine whether overexpression of *sum1*$^+$ regulates the stress-activated MAP kinase pathway by a similar mechanism, the Sty1/Spc1 phosphotyrosine levels were examined in cells overexpressing *sum1*$^+$ before and after exposure to osmotic stress. A strain expressing an HA epitope-tagged Sty1/Spc1 MAP kinase was transformed with a control plasmid (Figure 5A, vector) or with plasmids from which the *sum1*$^+$ (Figure 5A, *OPsum1*$^+$) or *pyp1*$^+$ (Figure 5A, *OPpyp1*$^+$) genes were highly expressed. Cells were subjected to osmotic stress where indicated (Figure 5A, +KCl), and phosphotyrosine levels of Sty1/Spc1 from these cells determined by Western blot analysis (Figure 5A). As described previously (Degols *et al.* 1996; Millar *et al.* 1995; Shiozaki and Russell 1995), increased HA-tagged Sty1/Spc1 phosphotyrosine levels are observed when wild-type cells, transformed with vector alone, are exposed to osmotic stress (compare Figure 5A, lanes 1 and 4). In contrast, a considerable decrease in HA-tagged Sty1/Spc1 phosphotyrosine levels is observed in cells overexpressing *pyp1*$^+$ under these conditions (Figure 5A, compare lanes 1 and 3). In cells overexpressing *sum1*$^+$, in KCl, the phosphotyrosine level of the HA-tagged Sty1/Spc1 resembled that of cells transformed with vector alone (compare Figure 5A, lanes 1 and 2). Thus, overexpression of *sum1*$^+$ does not inhibit tyrosine phosphorylation of the Sty1/Spc1 MAP kinase, unlike overexpression of *pyp1*$^+$.

To investigate further the possible role of *sum1*$^+$ in regulating the MAP kinase pathway, we examined the transcriptional response of cells to stress in cells overexpressing or lacking *sum1*$^+$. In wild-type cells, activation of the Sty1/Spc1 MAP kinase leads to transcriptional induction of a number of genes, including the *gpd1*$^+$ gene, encoding glycerol-3-phosphate dehydrogenase (Degols *et al.* 1996; Shiozaki and Russell 1996; Wilkinson *et al.* 1996). Northern blot analysis was thus performed to examine *gpd1*$^+$ mRNA levels in cells overexpressing *sum1*$^+$ or *pyp1*$^+$, and in cells lacking *sum1*$^+$ function (see materials and methods). Figure 5B demonstrates that *gpd1*$^+$ mRNA levels are greatly stimulated by addition of KCl in wild-type cells (Figure 5B, compare lanes 2 and 6). In contrast, transcriptional activation of *gpd1*$^+$ is inhibited in cells overexpressing *pyp1*$^+$ (Figure 5B, compare lanes 4 and 8). However, in cells overexpressing *sum1*$^+$ (Figure 5B, lanes 3 and 7), and cells lacking *sum1*$^+$ (Figure 5B, lanes 1 and 5),

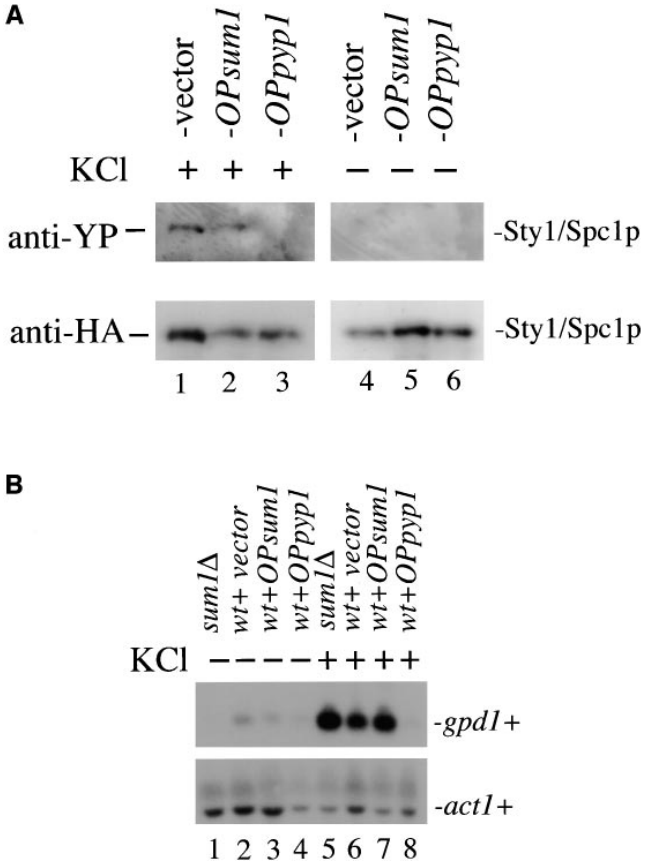

Figure 5.—Overexpression of *sum1*$^+$ does not inhibit Sty1/Spc1 activation or *gpd1*$^+$ transcription in response to osmotic stress. (A) Phosphotyrosine levels of Sty1/Spc1 protein are not altered in strains overexpressing *sum1*$^+$. Strain KS1376 (Shiozaki and Russell 1995), in which Sty1/Spc1p has been tagged with two copies of the HA epitope and six histidine residues, was transformed with either REP3X (vector), pREP3X-*sum1*$^+$ (*OPsum1*) or pREP1-*pyp1*$^+$ (*OPpyp1*). These strains (TE810, TE811, and TE812, respectively) were grown to midlog phase in EMM medium at 29° following derepression of the *nmt1*$^+$ promoter for 24 hr. KCl was added to the medium to a final concentration of 0.9 m, for 20 min before harvesting, as indicated (+). The Sty1/Spc1 protein was isolated by affinity purification and probed by Western blot for the presence of phoshotyrosine (anti-YP) or HA epitope tag (anti-HA). (B) Northern blot analysis of *gpd1*$^+$ transcription levels in strains overexpressing *sum1*$^+$ or *pyp1*$^+$. Strain TE807: *leu1-32 h*$^-$ pREP81X (shown as wt +vector), TE808: *leu1-32 h*$^-$ pREP3X-*sum1*$^+$ *LEU2* (shown as wt +OPsum1), and TE809: *leu1-32 h*$^-$ pREP1-*pyp1*$^+$ *LEU2* (shown as wt +OPpyp1) were grown to mid-log phase in EMM+A. Strain TE578: *sum1::ura4*$^+$, *ura4-D18*, *leu1-32 ade6-M210 h*$^+$ pREP41X-*sum1*$^+$ *LEU2* (shown as sum1), was grown in EMM+A in the presence of 2 μm thiamine for 18 hr to repress *sum1*$^+$ transcription from the REP41X promoter. KCl was added to a final concentration of 0.9 m for 90 min where indicated (+). Total RNA generated from the above strains was separated on a Northern gel, blotted to nitrocellulose and transcripts visualized with *gpd1*$^+$ and *act1*$^+$ probes as indicated.

transcriptional activation of the *gpd1*$^+$ gene is still observed upon addition of KCl. Moreover, loss of Sum1 does not lead to constitutive activation of the MAP kinase pathway as the *gpd1*$^+$ gene is not expressed in the absence of *sum1*$^+$ (Figure 5B, lane 1). These results suggest that the stress-responsive MAP kinase is activated normally when *sum1*$^+$ is overexpressed.

We also performed genetic experiments to determine whether Sum1 negatively regulates the stress-responsive MAP kinase pathway. Mutations in the *sty1*$^+$/*spc1*$^+$ gene, encoding the stress-activated MAP kinase can suppress the lethality of a *pyp1 pyp2* double mutation (Millar *et al.* 1995; Shiozaki and Russell 1995). These results suggest lethality of a *pyp1 pyp2* double mutation results from constitutive activation of the stress-responsive MAP kinase. However, the lethality of the *sum1::ura4*$^+$ disruption cannot be suppressed by mutations in the *sty1*$^+$/*spc1*$^+$ gene (data not shown; see materials and methods). Thus, the lethality of the *sum1::ura4*$^+$ disruption cannot be due solely to constitutive activation of the stress-responsive MAP kinase. In addition, overexpression of *sum1*$^+$ does not rescue the lethality of *pyp1 pyp2* double mutants (data not shown; see materials and methods), demonstrating that *sum1*$^+$ cannot block all the lethal consequences of constitutive activation of the stress-activated MAP kinase pathway. Thus although overexpression of *sum1*$^+$ blocks the cell cycle stress response, genetic and biochemical studies suggest that it does not do this by interfering with activation of the Sty1/Spc1 MAP kinase. Overexpression of *sum1*$^+$ could inhibit the cell cycle stress response downstream of MAP kinase activation. Alternatively, *sum1*$^+$ could be negatively regulating a MAP kinase independent pathway that also activates the cell cycle stress response. To determine whether overexpression of *sum1*$^+$ inhibits the cell cycle stress response independently of Spc1 MAP kinase, we examined whether we would see an additive effect on cell cycle arrest in 1 m KCl following overexpression of *sum1*$^+$ in a *spc1-M13* strain. *spc1-M13* (TE813) was transformed with vector alone or with REP3X-*sum1*$^+$, and cell length was determined following exposure to 1 m KCl. *spc1-M13* cells in which *sum1*$^+$ was overexpressed were found to be marginally longer in 1 m KCl than spc1-M13 transformed with vector alone (data not shown). Such a result might indicate that overexpression of *sum1*$^+$ inhibits the cell cycle stress response independently of Spc1 MAP kinase (Figure 7, pathway b). However, interpretation of this experiment is problematic because the effect is so small. It is not clear whether we would expect to see additivity clearly in this experiment because both single mutations almost completely block cell cycle progression in 1 m KCl.

Inactivating the stress-response pathway suppresses the checkpoint defect of *cdc2-3w*: We have shown that *sum1*$^+$ overexpression disrupts the normal cell cycle response to osmotic stress. A corollary of this observation is that inhibiting the cell cycle stress response could

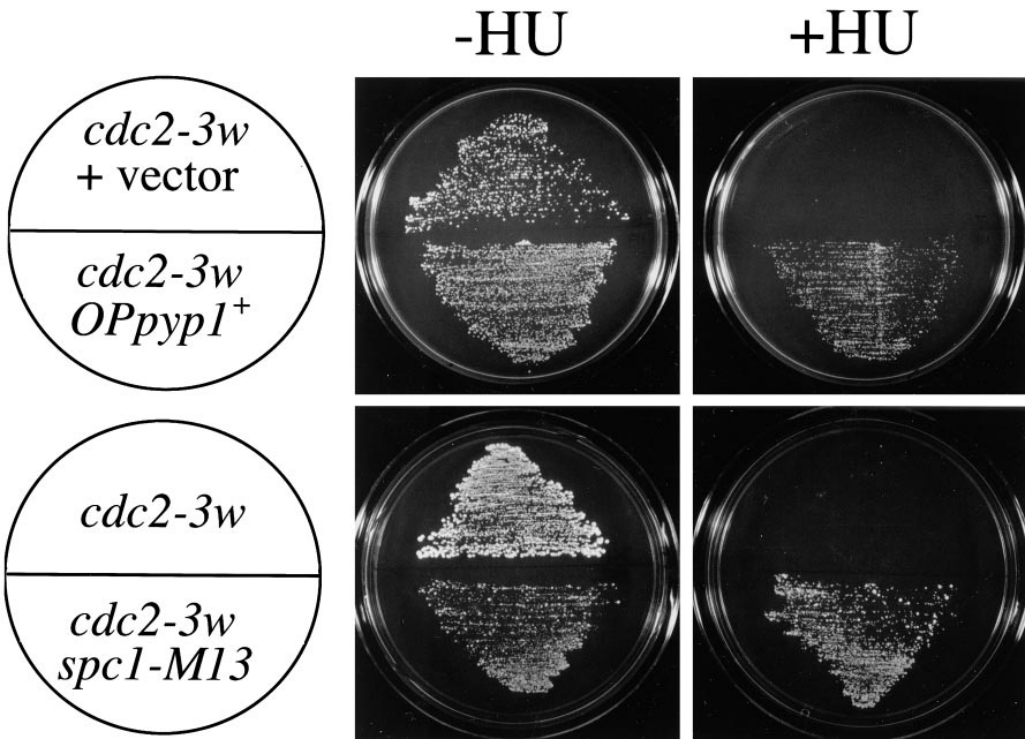

Figure 6.—HU sensitivity of *cdc2-3w* is suppressed by mutants that inactivate the stress-activated MAP kinase pathway. TE596 (*cdc2-3w* + vector) and TE806 (*cdc2-3w* + pREP1-*pyp1*$^+$) were grown at 29° for 3 days on EMM+A plates (in the absence of thiamine) and restreaked to EMM+A plates in the absence (−HU) or presence (+HU) of 5 mm HU. Strains TE361 (*cdc2-3w*) and TE742 (*cdc2-3w spc1-M13*) were grown on EMM+U+A+L plates at 29° for 4 days in the absence (−HU) or presence (+HU) of 5 mm HU.

restore checkpoint control in *cdc2-3w* because *sum1*$^+$ was initially isolated in a screen for genes with this phenotype. To investigate this possibility, we examined the effects of inactivating the stress-response pathway on checkpoint control in *cdc2-3w* mutants. As shown in Figure 6 (upper panels), overexpression of *pyp1*$^+$ enables *cdc2-3w* cells to form colonies in the presence of HU, indicating that overexpression of *pyp1*$^+$ restores the checkpoint response in *cdc2-3w* cells. A loss of function mutation in the Sty1/Spc1 MAP kinase, *spc1-M13*, also restores the checkpoint response, as this double mutant strain (*cdc2-3w spc1-M13*) can form colonies in the presence of HU (Figure 6, lower panels).

DISCUSSION

Overexpression of *sum1*$^+$ restores the S-M checkpoint in fission yeast: To identify positive regulators of the S-M checkpoint, we have screened for high-copy plasmid suppressors of the checkpoint mutant *cdc2-3w*. *cdc2-3w* mutants overexpressing *sum1*$^+$ show a modest increase in the length of G2 and are able to arrest normally in the presence of HU. Thus, the presence of excess Sum1 rescues the checkpoint response in these mutants. The restoration of the checkpoint response is not likely to be a trivial consequence of extending G2, as other mutations that extend G2 in *cdc2-3w* do not restore checkpoint control (Enoch *et al.* 1992). Although overexpression of *sum1*$^+$ restores checkpoint control, we are not able to determine whether disrupting Sum1 activity reduces the checkpoint response because loss of Sum1 is lethal. Thus, we do not know whether Sum1 is directly involved in checkpoint control under normal circumstances.

Overexpression of *sum1*$^+$ inhibits the cell cycle response to stress: In fission yeast, entry into mitosis is advanced when cells are subjected to stress, such as changes in nutrient conditions or osmotic pressure (Shiozaki and Russell 1995). Such a response would advance cells into G1 where they may undergo conjugation and sporulation under these adverse conditions. Stress-induced mitotic initiation requires activation of a MAP kinase cascade culminating in the activation of the MAP kinase Spc1/Sty1 (Millar *et al.* 1995; Shiozaki and Russell 1995). Activation of this pathway is negatively regulated by the phosphotyrosine phosphatases, Pyp1 and Pyp2, which dephosphorylate the tyrosine residue of Spc1/Sty1 that is required for its activity (Millar *et al.* 1995; Shiozaki and Russell 1995). We have discovered striking similarities between cells overexpressing Sum1 and mutants that cannot activate the MAP kinase pathway either because the inhibitory tyrosine phosphatases are overexpressed or because *sty1*$^+$/*spc1*$^+$ is mutated. In each of these strains, exposure to osmotic stress results in mitotic arrest (Figure 4). In addition, all of these mutations restore checkpoint control in *cdc2-3w* mutants (Figure 6). Thus, the consequences of overexpressing *sum1*$^+$ are similar to the consequences of inhibiting the stress-activated MAP kinase pathway. Furthermore, the phenotype of mutants lacking Sum1 resembles mutants in which the MAP kinase pathway is constitutively active (Figure 3C). Such strains have similar defects in spore germination, proliferation, and morphology. However, we do not know whether these similarities are circum-

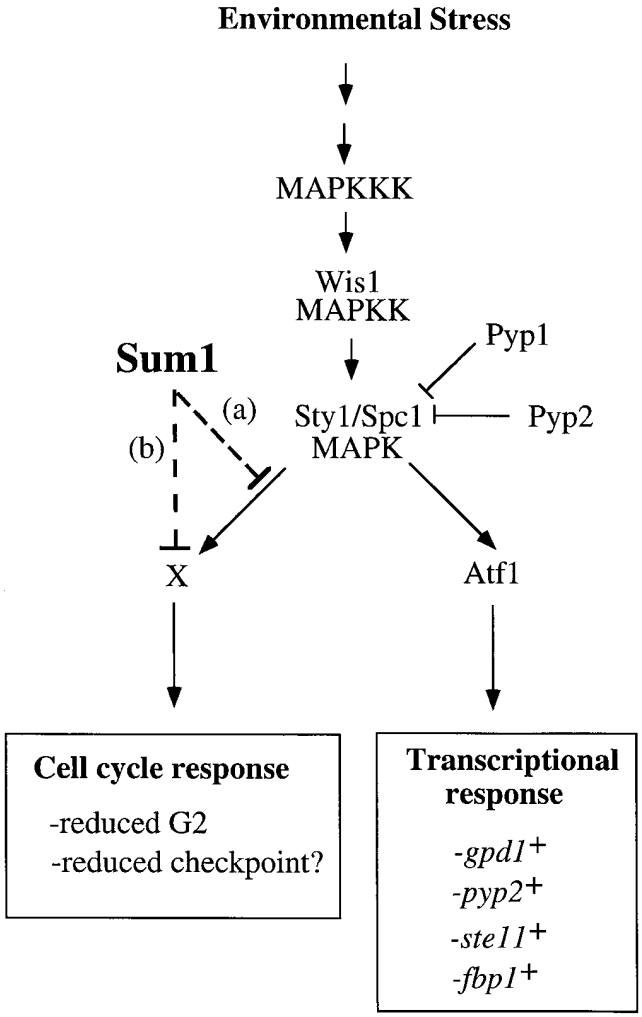

Figure 7.—Model for how *OPsum1*$^+$ disrupts cell cycle stress response in fission yeast. Activation of the Sty1/Spc1 MAP kinase by environmental stress results in a transcriptional response through activation of Atf1, and advanced timing of mitosis by an unkwnown mechanism. Overproduction of Sum1 may disrupt cell cycle stress-response by inhibiting the downstream cell cycle branch of the MAP kinase pathway directly (pathway a) or indirectly (pathway b). See text for details.

stantial or whether they represent similar molecular defects as the cell cycle targets of the constitutively activated MAP kinase have not been identified.

Overexpression of *sum1*$^+$ inhibits the cell cycle stress response but not the transcriptional stress response: We have investigated the mechanism by which overexpression of *sum1*$^+$ interferes with cell cycle response to stress. As shown in Figure 7, the stress response pathway bifurcates after activation of the Sty1/Spc1 MAP kinase. One branch, requiring the transcription factor Atf1, leads to transcriptional activation of genes required for the stress response (Shiozaki and Russell 1996; Wilkinson *et al.* 1996). A second branch leads to stimulation of cell division. In *atf1* mutants, the transcriptional response to stress is abolished, but the cell cycle stress response is not affected (Shiozaki and Russell 1996; Wilkinson *et al.* 1996). Our studies show that *sum1*$^+$ overexpression causes the converse phenotype; the transcriptional response to stress is apparently intact (Figure 5B), but the cell cycle response is blocked (Figure 4). These results suggest that overproduction of Sum1 interferes with the stress response at a point downstream of MAP kinase activation, on the branch of the pathway that leads to activation of mitosis.

How does *Sum1* inhibit the cell cycle stress response? We are considering two explanations for the inhibition of the stress response by Sum1 overexpression. First, *sum1*$^+$ overexpression could directly interfere with phosphorylation of a MAP kinase target. For example, analysis of the homologous *S. cerevisiae* protein *TIF34* has established that it is associated with an essential multiprotein complex, eIF3, required for translational initiation (Naranda *et al.* 1997). This complex could be a MAP kinase target, and overexpression of a subunit could interfere with the ability of the MAP kinase to interact with this target (Figure 7, pathway a). If phosphorylation by the MAP kinase normally inhibits the function of eIF-3, this could explain why constitutive activation of the MAP kinase is lethal and why there are similarities between the phenotypes of such cells and *sum1*$^-$ cells. In this regard, it is interesting that the human gene related to *sum1*$^+$, TRIP-1 associates with and is phosphorylated by the TGF-β type II receptor (Chen *et al.* 1995). Perhaps in humans and yeasts, the Sum1-related proteins function to transduce information from the environment to the translation machinery.

Another possibility is that overexpression of *sum1*$^+$ and inactivation of the MAP kinase pathway inhibit parallel pathways that interact with a common target (Figure 7, pathway b). For example, it is possible that MAP kinase inactivation indirectly causes cell cycle arrest by inhibiting translation. Overexpression of Sum1 may also inhibit translation and thus cause a similar cell cycle response. Distinguishing between these two models will require further analysis of Sum1 function in stress response. It is also possible that the effects of Sum1 on the stress response pathway are indirect. For example, overexpression of many proteins might interfere with translation, and this may result in interference with the cell cycle stress response. However, we think this is unlikely, as we have screened over 100,000 transformants and found only one other gene with the same phenotype as *sum1*$^+$ (K. Forbes and T. Enoch, unpublished results).

The stress response and checkpoint control: Abolishing the cell cycle stress response, either by mutating *sty1*$^+$/*spc1*$^+$ or overexpressing *pyp1*$^+$, restores checkpoint control in *cdc2-3w*. Because abolishing the cell cycle stress response increases the effectiveness of the S-M checkpoint, activation of the stress-response pathway may have the opposite effect; the S-M checkpoint may be less effective under conditions of stress compared to normal growth conditions. We do not think that

suppression of checkpoint defects by mutation of the stress response pathway is a trivial consequence of delaying mitosis as we have demonstrated previously that increasing the length of G2 does not always correlate with suppression of checkpoint defects (Enoch and Nurse 1990). It is therefore possible that the stress-response pathway stimulates mitosis by inhibiting components of the cell cycle machinery that normally restrain mitosis in response to checkpoint signals. Compromising checkpoint control may confer a selective advantage because it allows cells to complete the cell cycle rapidly, enter G1 and prepare for conjugation and sporulation. Normally reducing cell cycle fidelity is deleterious as it increases the frequency of mutations (Hartwell and Weinert 1989). However, perhaps under stressful conditions, a mechanism that stimulates mitosis and generates new mutations is advantageous, as some mutations may promote survival in the harsh environment. Our studies therefore raise the intriguing possibility that the stringency of a checkpoint response is a variable that is modulated by environmental conditions rather than a fixed property of the cell cycle.

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