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Sgt1 Associates with Hsp90: an Initial Step of Assembly of the Core Kinetochore Complex

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The kinetochore, which consists of DNA sequence elements and structural proteins, is essential for high-fidelity chromosome transmission during cell division. In budding yeast, Sgt1, together with Skp1, is required for assembly of the core kinetochore complex (CBF3) via Ctf13 activation. Formation of the active Ctf13-Skp1 complex also requires Hsp90, a molecular chaperone. We have found that Sgt1 interacts with Hsp90 in yeast. We also have determined that Skp1 and Hsc82 (a yeast Hsp90 protein) bind to the N-terminal region of Sgt1 that contains tetratricopeptide repeat motifs. Results of sequence and phenotypic analyses of *sgt1* mutants strongly suggest that the N-terminal region containing the Hsc82-binding and Skp1-binding domains of Sgt1 is important for the kinetochore function of Sgt1. We found that Hsp90's binding to Sgt1 stimulates the binding of Sgt1 to Skp1 and that Sgt1 and Hsp90 stimulate the binding of Skp1 to Ctf13, the F-box core kinetochore protein. Our results strongly suggest that Sgt1 and Hsp90 function in assembling CBF3 by activating Skp1 and Ctf13.

The centromere is essential for chromosome inheritance and requires DNA sequence elements and structural and regulatory proteins (the structural protein complex is called the kinetochore) for its activity and coordination within the cell cycle. In budding yeast (*Saccharomyces cerevisiae*), the kinetochore consists of more than 30 different proteins, and several of these proteins are conserved in mammals (21). The budding yeast centromere DNA is a 125-bp region that contains three conserved regions, CDEI, CDEII, and CDEIII (8, 13). CDEIII (25 bp) is essential for centromere function (12) and was the site shown to be bound by CBF3, a protein complex that contains the major proteins p110 (encoded by *NDC10*/*CTF14*/*CBF2*), p64 (encoded by *CEP3*/*CBF3b*), p58 (encoded by *CTF13*) (4, 5, 11, 17, 23, 24, 37, 39), and Skp1 (4, 37). All four are essential for viability, and mutations in any one abolish the CDEIII-binding activity of CBF3 (18, 36). No known kinetochore protein, except for CDEI-binding Cbf1, can localize to kinetochores when the CBF3 complex is disrupted. Therefore, the CBF3 complex is the fundamental structure of the kinetochore, and the mechanism of CBF3 assembly is of great interest.

SGT1 was isolated as a dosage suppressor of *skp1-4*, a kinetochore-defective mutant (22). By activating Ctf13, Sgt1 and Skp1 are required for assembly of the CBF3 complex (22). However, the mechanism of activation is not well characterized. Stemmann et al. showed that formation of the active Ctf13-Skp1 complex requires Hsp90, a molecular chaperone (38). Sgt1 has highly conserved tetratricopeptide repeat (TPR) (22) and CS (CHORD protein and Sgt1-specific) motifs—in other proteins these motifs are required for interaction with Hsp90 (2, 6). Here we report that molecular chaperones from the Hsp90 and Hsp70 families form a complex with Sgt1 in yeast. Our results strongly suggest that Hsp90's interaction with Sgt1 is required for kinetochore assembly in yeast. On the basis of our findings, we propose a model in which Sgt1 and its interaction with Hsp90 are required for assembly of the core kinetochore complex, an initial step in kinetochore activation.

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MATERIALS AND METHODS

Yeast strains and medium. Table 1 lists the genotypes of the yeast strains used in this study. The medium used for yeast growth and sporulation was described previously (33). Yeast transformation was done as described by Ito et al. (16). Strains that expressed Sgt1-20myc or Sgt1-13myc were generated in accordance with the procedure of Longtine et al. (26). Sgt1-13myc contains 13 myc tags; Sgt1-20myc contains 20. The numbers of tags were confirmed by direct sequencing. Regions encoding the myc tags were inserted at the 3′ end of the endogenous *SGT1* locus.

Plasmid construction and primers. Table 2 lists the plasmids used in this study. Details of their construction (19) and primer sequences are available upon request.

Generation and integration of temperature-sensitive alleles. Temperature-sensitive mutant forms of *sgt1* were generated by a PCR-based method as described previously (22). New temperature-sensitive alleles (*sgt1-6*, *sgt1-7*, *sgt1-8*, *sgt1-9*, *sgt1-10*, *sgt1-11*, and *sgt1-12*) were isolated, and the recovered mutated plasmids were retested to determine their effect on cell viability at 37°C. All alleles were integrated into the *SGT1* locus in the yeast genome (Table 1). All nucleotides of the mutant alleles in plasmids were sequenced. All portions of the mutant alleles that were integrated into the yeast genome were amplified by PCR, and the PCR fragments were sequenced. We did not find any additional mutations after integration (Table 3).

Synthetic lethality. Strain Y1129 (*mat*α *hsc82*Δ::*KAN hsp82*Δ::*TRP1 pYEp24-HSC82* 2μm/URA) and strain YKK54 (*mat***a** *sgt1-3*::*leu2*) or YKK45 (*mat***a** *sgt1-5*::*leu2*) were mated. After diploid selection and sporulation, Ura^+ spores were isolated. From these selected spores, those representing all possible single or double combinations of $G418^+$, Trp^+, or Leu^+ were selected. To test synthetic lethality, these haploid strains harboring the *pYEp24-HSC82* 2μm/*URA* plasmid were restreaked on synthetic medium that contained 5-fluoroorotic acid (Sc+5-FOA) and on Sc that lacked uracil (Sc−Ura). The plates were incubated at 25°C for 2 days.

Mass spectrometry. Mass spectrometry (under the direction of Clive Slaughter) was performed by the Hartwell Center for Bioinformatics and Biotechnology at St. Jude Children's Research Hospital. Immunopurified proteins (see description of immunoprecipitation below) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the proteins in excised gel pieces were reduced, alkylated with iodoacetamide, and digested with trypsin. For Hsc82 identification, the unfractionated digest was subjected to mass spec-

TABLE 1. Yeast strains used in this study

Strain	Genotype	Reference
YPH499	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1*	34a
YKK54	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgtI-3::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	22
YKK45	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-5::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	22
Y37R	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-1::LEU2*	22
Y40R	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-2::LEU2*	22
Y36R	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-4::LEU2*	22
Y310	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 SGT1-20Myc::H1S3-MX6*	This study
Y1019	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 SGT1-13Myc::HIS3-MX6*	This study
Y1015	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-6::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1016	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-7::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1134	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-8::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1135	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-9::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1136	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-10::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1137	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-11::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
Y1018	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-12::LEU2 CFIII(CEN3.L.YPH983) TRP1 SUP11*	This study
YPH972R	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 ctf13-30*	5
PY694	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 met30-6::KAN*	17a
K699	*mat***a** *ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 GAL$^+$ psi$^+$*	29b
MTY871	*mat***a** *ura3 trp1-1 his3-11 15 can1-100 leu2-3 112 GAL psi$^+$ cdc53-1*	41
US104	*mat***a** *ura3 trp1-1 his3-11 leu2-3 112 cdc4-1*	11a
US504	*mat***a** *a ura3 trp1-1 his3-11 leu2-3 112 cdc34-1*	11a
US581	*mat***a** *ura3 trp1-1 his3-11 leu2-3 sic1Δ::LEU2*	11a
US893	*mat***a** *ura3 trp1-1 his3-11 leu2-3 112 cdc4Δ::URA3 pGAL-CDC4/TRP1/CEN*	11a
US1198	*mat***a** *ura3 trp1-1 his3-11 leu2-3 112 cdc4Δ::URA3 pcdc4-12/TRP1/CEN*	11a
YPH1161	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 skp1Δ1::TRP1 skp1-4::LEU2 CFIII(CEN3.L.YPH983) HIS3 SUP11*	4
YPH1172	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 skp1Δ1::TRP1 skp1-3::LEU2 CFIII(CEN3.L:YPH983) HIS3 SUP11*	4
ΔPLCDa(T1011)	*mat***a** *ade2-1 can1-100 his3-12,16 leu2-3,112 trp1-1 ura3 hsp82::LEU2 hsc82::LEU2 pRS314-hsp82(T1011)*	29c
Y1124	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 pYEp24-HSC82.2μm/URA*	This study
Y1125	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsp82Δ::TRP1 pYEp24-HSC82 2μm/URA*	This study
Y1126	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsc82Δ::KAN pYEp24-HSC82 2μm/URA*	This study
Y1127	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-3::LEU2 pYEp24-HSC82 2μm/URA*	This study
Y1128	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 sgt1-5::LEU2 pYEp24-HSC82 2μm/URA*	This study
Y1129	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsc82Δ::KAN hsp82Δ::TRP1 pYEp24-HSC82 2μm/URA*	This study
Y1130	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsp82Δ::TRP1 sgt1-3::LEU2 pYEp24-HSC82 2μm/URA*	This study
Y1131	*matα ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsp82Δ::TRP1 sgt1-5::LEU2 pYEp24-HSC82 2μm/URA*	This study
Y1132	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsc82Δ::KAN sgt1-3::LEU2 pYEp24-HSC82 2μm/URA*	This study
Y1133	*mat***a** *ura3-52 lys2-801 ade2-101 trp1-Δ63 his3-Δ200 leu2-Δ1 hsc82Δ::KAN sgt1-5::LEU2 pYEp24-HSC82 2μm/URA*	This study

trometry, and the mass values of the peptides were matched with the expected values obtained by analysis of the protein sequence. Mass spectrometry of the digested protein in a crystalline matrix of α-cyano-4-hydroxycinnamic acid was performed by matrix-assisted laser desorption/ionization tandem time-of-flight mass spectrometry in a model 4700 Proteomics analyzer from Applied Biosystems (Foster City, Calif.). The MS-Fit program of Protein Prospector was used for the database search. The program and the database that were accessed are located at http://hc-appc1.stjude.org/index-pp.html. NCBInr. was used for protein identification.

For Ssa1 and Ssb1 identification, tryptic peptides were extracted and subjected to combined capillary liquid chromatography-tandem mass spectrometry by a

TABLE 2. Plasmids used in this study

Plasmid	Relevant characteristic(s)	Reference
BKK9	*pRS316 SGT1 CEN/URA*	22
B760	*p413-GPD*	29a
B756	*p413-GPD-HSP82*	29a
pKAT6	*pYEp24-HSC82* 2μm*URA*	29a
BKK222	*pGAL1-His6 HA-CTF13 CEN/URA*	18
B584	*pGEX-4T-2-SKP1*	This study

TABLE 3. *sgt1* mutations

Allele	No. of mutations	Codon	DNA position	Sequence change
sgt1-1	2	119	356	Y (TAC) to C (TGC)
		378	1132	S (TCT) to P (CCT)
sgt1-2	2	243	728	L (CTT) to H (CAT)
		378	1133	S (TCT) to F (TTT)
sgt1-3	3	31	92	L (CTC) to P (CCC)
		99	295	F (TTC) to L (CTC)
		213	638	N (AAT) to I (ATT)
sgt1-4	2	360	1078	K (AAA) to E (GAA)
		385	1153	S (TCA) to T (ACA)
sgt1-5	2	220	659	D (GAT) to V (GTT)
		364	1090	E (GAA) to K (AAA)
sgt1-6	2	26	77	L (CTT) to P (CCT)
		99	295	F (TTC) to L (CTC)
sgt1-7	2	117	350	L (CTT) to P (CCT)
		191	571	Q (CAA) to K (AAA)
sgt1-8	3	360	1078	K (AAA) to E (GAA)
		135	403	L (TTG) to L (CTG)
		288	864	S (TCA) to S (TCG)
sgt1-9	1	360	1078	K (AAA) to E (GAA)
sgt1-10	1	378	1132	S (TCT) to P (CCT)
sgt1-11	3	378	1132	S (TCT) to P (CCT)
		390	1168	M (ATG) to V (GTG)
		196	588	V (GTC) to V (GTT)
sgt1-12	2	74	220	A (GCT) to T (ACT)
		151	451	I (ATT) to V (GTT)

ThermoQuest LCQ-DECA ion trap mass spectrometer with a nanoelectrospray ion source. Fragment ion (MS2) spectra were subjected to a search by the SEQUEST program of Eng and Yates (ThermoQuest). The National Center for Biotechnology Information nonredundant protein database was used.

Antibodies. The anti-Hsc82 antibody used was a gift from Suzan Lindquist (Whitehead Institute for Biomedical Research). The anti-Skp1 and anti-Sgt1 antibodies used were previously described (20, 22). The anti-hemagglutinin (anti-HA; Roche), anti-myc (Roche), and anti-His_6 (QIAGEN) antibodies used were purchased.

Protein expression and immunoprecipitation. Immunoprecipitation with yeast lysates was performed as described previously, except that antibodies were cross-linked to Sepharose by using the Seize Primary Immunoprecipitation kit (Pierce) (20, 22).

For in vitro expression of proteins (Sgt1 deletion proteins [Sgt1-Ds] and HA-Hsp82), PCR products that encoded polypeptides of interest were used as templates in the TnT T7 Quick for PCR DNA in vitro transcription-translation system with rabbit reticulocyte lysates (Promega), and Sgt1 deletion proteins were labeled with [^{35}S]methionine. HA-tagged Skp1 was expressed in insect cells as previously described (20, 22). For Skp1- and Hsc82-binding domain analyses, approximately equal amounts of deletion proteins were used for immunoprecipitation with an anti-HA antibody. Immune complexes were washed five or seven times with 1.5 ml of buffer A (50 mM Tris-HCl [pH 7.5], 0.5% NP-40, 150 mM NaCl, 10 mM β-glycerophosphate, protease inhibitor cocktail [Roche]) before they underwent SDS-PAGE and immunoblotting. Total reticulocyte lysates (T) were represented by material in a volume that was equivalent to 2 to 6% of that of the starting material.

For protein expression in bacteria, BL21(DE3) cells were transformed with pGEX (a glutathione *S*-transferase [GST] gene fusion vector; Amersham Biosciences) or with pGEX-Skp1; expression was induced by addition of isopropyl-β-D-thiogalactopyranoside (1 mM) and incubation for 3 h at 37°C. The GST-tagged proteins were affinity purified in accordance with the manufacturer's instruction and then subjected to immunoprecipitation studies.

Expression and purification of the His_6-Sgt1, His_6-Sgt1(59-395), and HA-His_6-Ctf13 proteins were done in accordance with the manufacturer's (QIAGEN) instructions. Human Hsp90 protein purified from HeLa cells was purchased from Stressgen. Approximately 200 ng of the purified proteins was mixed with 500 μl of buffer B (50 mM Tris-HCl [pH 7.5], 0.2% Triton X-100, 50 mM NaCl, protease inhibitor cocktail [Roche]) and incubated at 30°C for 1 h. The proteins were then mixed with an anti-His_6 or anti-HA antibody and Sepharose A and incubated at 4°C for 2 h. Immune complexes were washed five or seven times with 1.5 ml of lysis buffer before they underwent SDS-PAGE and immunoblotting. Total protein mixtures (T) were represented by material in a volume that was equivalent to 5% of that of the starting material.

^{35}S-labeled proteins were detected by autoradiography and quantified with a Molecular Dynamics Phospho-Imager with Image Quant software. Nonradiolabeled proteins were detected by immunoblotting with ECL reagents (Amersham) and quantified with a Chemidoc EQ system (Bio-Rad) with Quantity One 1-D Analysis Software (Bio-Rad). All signals were measured within the linear ranges of detection.

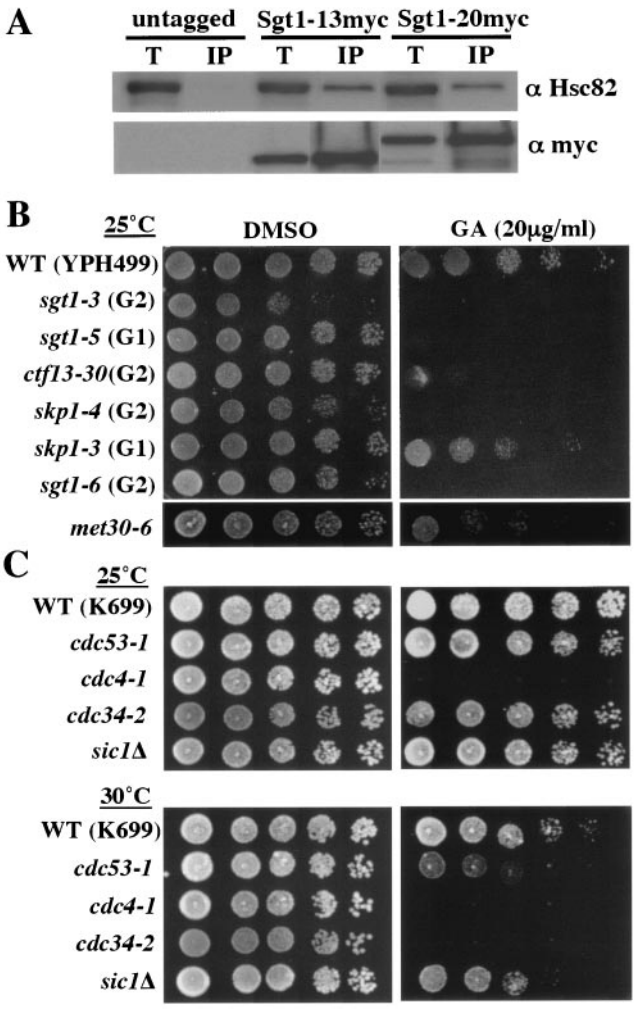
RESULTS

Association of Sgt1 with Hsp90 in budding yeast. Sgt1, together with Skp1, is required for formation of CBF3 (the core kinetochore complex) in budding yeast (22). To further investigate the function of Sgt1, we performed immunoprecipitation-mass spectrometry analysis of strains expressing myc-tagged Sgt1. Sgt1 is essential for viability, and strains expressing the myc-tagged proteins showed no growth defect or chromosome missegregation (data not shown); therefore, we concluded that myc-tagged Sgt1 was functional. Hsc82 (a yeast Hsp90 protein) and other cochaperones were found specifically in precipitates of myc-tagged Sgt1 (unpublished data). The specificity of the immunoprecipitation was confirmed by Western blot analysis with anti-Hsc82 antibody (Fig. 1A). Skp1 was also present in the myc-tagged Sgt1 immunoprecipitates (unpublished data), a result consistent with our previous finding (22). These results indicate that Sgt1 forms a complex with Hsp90 in vivo.

Sensitivity of *sgt1* mutants to a specific inhibitor of Hsp90. Five *sgt1* temperature-sensitive mutants were isolated from the initial screen as described previously (22). One mutant, *sgt1-3*, was arrested in the G_2 phase at the nonpermissive temperature (therefore, *sgt1-3* is called a G_2 allele), and this mutant showed substantial chromosome missegregation (22). The other mutants, *sgt1-1*, *sgt1-2*, *sgt1-4*, and *sgt1-5*, underwent arrest in the G_1 phase at the nonpermissive temperature (therefore, *sgt1-1*, *sgt1-2*, *sgt1-4*, and *sgt1-5* are called G_1 alleles) (22). Genetic and biochemical experiments indicated that *sgt1-3* is defective in kinetochore (CBF3) assembly and that *sgt1-5* is deficient in SCF ubiquitination and adenylyl cyclase activity (6, 22). To further characterize the role of Sgt1 in kinetochore function, we obtained seven new temperature-sensitive mutants: *sgt1-6*, *sgt1-7*, *sgt1-8*, *sgt1-9*, *sgt1-10*, *sgt1-11*, and *sgt1-12*.

To examine whether the function of Sgt1 involves Hsp90, we tested whether *sgt1* temperature-sensitive mutants are sensitive to geldanamycin, an Hsp90 inhibitor. All 12 *sgt1* temperature-sensitive mutants showed substantial sensitivity to geldanamycin (Fig. 1B and unpublished data). These results are consistent with the previous finding that *skp1* temperature-sensitive mutants and *ctf13-30* have marked sensitivity to geldanamycin (38). Together with the interaction between Sgt1 and Hsp90, these results suggest that Hsp90 is required for the function of Sgt1.

Because Sgt1 is involved in the function of the SCF ubiquitin ligase complex that targets cell cycle regulators, i.e., Sic1 (a



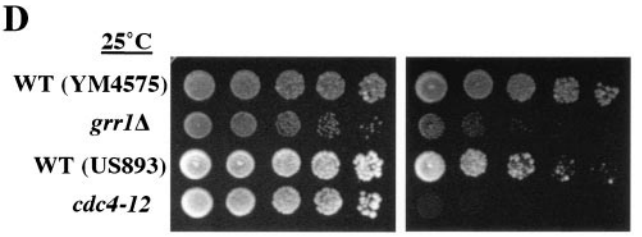


FIG. 1. Sgt1 associates with Hsp90 in yeast. (A) Proteins from strains expressing untagged Sgt1 or myc-tagged Sgt1 (Sgt1-13myc and Sgt1-20myc) were immunoprecipitated with anti-myc antibody and then immunoblotted with anti-Hsc82 antibody. T, total lysate; IP, immunoprecipitate. (B) The *sgt1* mutants and SCF mutants are sensitive to geldanamycin. The indicated strains were grown on yeast extract-peptone-dextrose plates containing 20 μg of geldanamycin per ml (GA) or dimethyl sulfoxide (DMSO) only. The numbers of cells that were spotted onto each plate (left to right) were approximately 5×10^4, 1×10^4, 2×10^3, 4×10^2, and 8×10^1. The plates were incubated at the indicated permissive temperatures for 3 days. (B) Strains in the YPH499 background. (C) Strains in the K699 background. (D) Strains in the YM4575 or US893 background. WT, wild type.

yeast CDK inhibitor) and Clns (yeast G_1 cyclins), we tested the sensitivity of other SCF mutants to geldanamycin. Our observation that *skp1-3* cells (deficient in SCF function) are moderately sensitive to geldanamycin (Fig. 1B) is consistent with the findings of Stemmann et al. (38). However, in contrast to their finding that *cdc34-1*, *cdc4-1*, and *cdc4-2* did not show any growth defects on geldanamycin-containing medium (38), the SCF mutants, especially the F-box protein mutants *cdc4-1*, *cdc4-12*, *met30-6*, and *grr1*Δ, showed substantial sensitivity to geldanamycin at the permissive temperatures (Fig. 1C and D). Our results indicate that Hsp90 is required for cell viability when the SCF activity is compromised.

Genetic interaction between *SGT1* and *HSP90*. Because inhibition of yeast Hsp90 by geldanamycin resulted in a lethal phenotype in *sgt1* mutants, we examined whether *SGT1* and *HSP90* show genetic interaction. The two budding yeast Hsp90 proteins, Hsc82 and Hsp82, are functionally equivalent (3), and Hsc82 is expressed 10 times as highly as Hsp82 at 25°C (3). The *sgt1-3 hsp82*Δ, *sgt1-3 hsc82*Δ, *sgt1-5 hsp82*Δ, and *sgt1-5 hsc82*Δ double mutants and the single mutants harbored *YEP-HSC82* (*URA HSC82*). All of the mutants were streaked onto 5-FOA plates to select cells that could lose the *HSC82* plasmid. When the cells were incubated at 25°C, *sgt1-3 hsc82* and *sgt1-5 hsc82* did not grow, although all of the other types of cells grew (Fig. 2A). These results indicate that *sgt1* mutations exert synthetic lethality with *hsc82*Δ.

When we investigated the reciprocal phenotypes, we found that overexpression of Hsp82 can suppress the temperature sensitivity of *sgt1-3* (a G_2 allele, G_2/M arrest at the nonpermissive temperature, kinetochore deficient) but not that of *sgt1-5* (a G_1 allele, G_1 arrest at the nonpermissive temperature) (Fig. 2B and results not shown). Note that overexpression of yeast Hsp90 does not protect cells from heat stress (15). Importantly, overexpression of Hsp82 can suppress the chromosome mis-segregation phenotype of *sgt1-3* (Fig. 2C). These results strongly suggest that Hsp90 activity is relevant to the functions of Sgt1, including its kinetochore function.

Binding of Skp1 and Hsc82 to the N-terminal domain of Sgt1. Previous analyses of amino acid sequences revealed that Sgt1 has three conserved domains (Fig. 3C and 4A). The first is located at the N-terminal end (amino acids 13 to 141) and contains TPR motifs that are homologous (27% similarity) to those of HOP (Sti1 in budding yeast), an Hsp90-Hsp70-organizing protein (22). The second domain is the CS motif (amino acids 184 to 279), which is homologous (18% similarity) to a domain in the cochaperone p23 (6, 9). The TPR domain of HOP and the CS motif of p23 are required for each protein's interaction with Hsp90. Therefore, we hypothesized that one or both of these domains in Sgt1 are responsible for the association between Hsp90 and Sgt1.

We performed immunoprecipitation experiments with truncated Sgt1 proteins to identify the Skp1-binding and Hsc82-binding domains. Various deletion proteins (Fig. 3A and B) were expressed and labeled with [^{35}S]methionine by the in vitro translation system, and the reticulocyte lysates containing the deletion proteins were mixed with a protein extract containing HA-tagged Skp1 that was expressed separately. HA-tagged Skp1 was immunoprecipitated by using Sepharose to which anti-HA antibody was conjugated (Fig. 3A). Analysis of the binding activity of the deletion proteins revealed that there

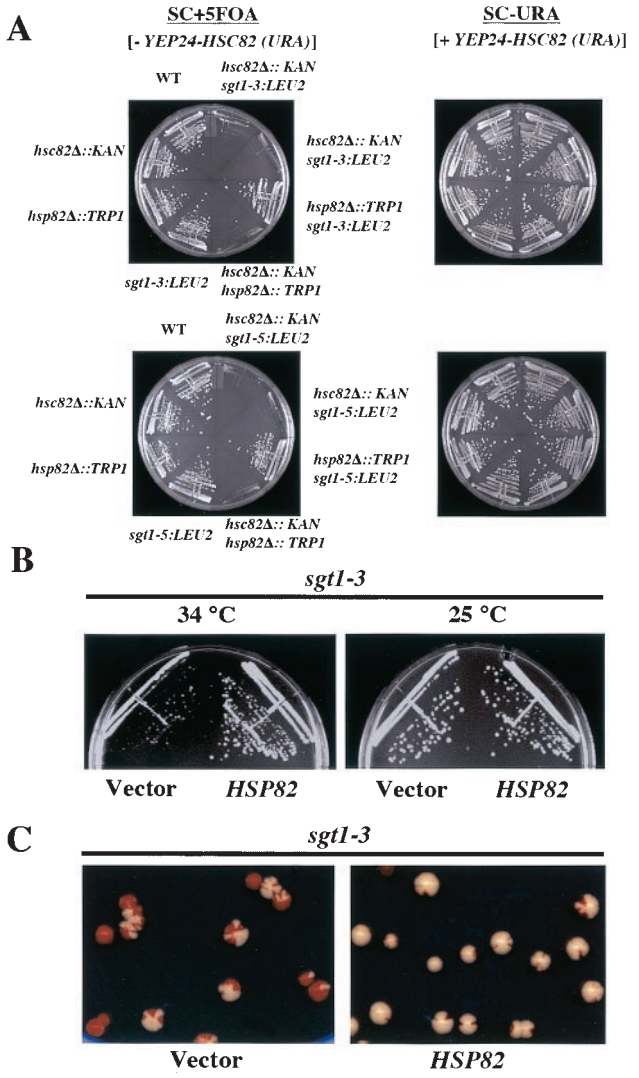


FIG. 2. (A) The *sgt1* and *hsc82* mutations exert synthetic lethality. The indicated single and double mutants carrying *YEP24-HSC82* were streaked onto plates containing Sc+5-FOA and Sc−Ura, respectively. The plates were incubated at 25°C for 2 days. (B) Hsp82 overexpression suppressed the temperature sensitivity of *sgt1-3*. The *sgt1-3* cells containing p413-GPD-Hsp82 (GPD promoter/CEN/HIS3) and the vector alone were streaked onto Sc-His plates and incubated at the indicated temperatures for 3 days. (C) Hsp82 overexpression suppressed the chromosome missegregation phenotype of *sgt1-3* cells. Shown are the results of a colony color-sectoring assay to analyze *sgt1-3* cells that contained the vector alone and that contained p413-GPD-Hsp82. WT, wild type.

is an inhibitory region between amino acids 270 and 312 (Fig. 3A). The Skp1-binding activity of the deletion protein (amino acids 1 to 270) is approximately twice that of the shorter deletion proteins (amino acids 1 to 241 or amino acids 1 to 211), a result that suggests that part of the CS domain contributes to the binding activity (Fig. 3A). Because the absence of the first 58 amino acids did not affect the binding activity (Fig. 3A and B), we conclude that the Skp1-binding domain of Sgt1 is located between amino acids 59 and 211 (Fig. 3C). The Skp1-binding domain contains two of the three repeats of the putative TPR domain and a short part (amino acids 184 to 211) of the CS domain (Fig. 3C).

By using the same immunoprecipitation method and HA-tagged Hsc82 instead of HA-tagged Skp1, we found that the N-terminal region (amino acids 1 to 211) of Sgt1 is able to bind to Hsc82 (Fig. 3B). However, the binding activity of the deletion protein (amino acids 1 to 211) is less than that of the longer deletion protein (amino acids 1 to 312) (Fig. 3B), a result that suggests that the CS domain contributes to the binding activity. Furthermore, a deletion protein lacking amino acids 1 through 58 but containing the Skp1-binding domain (amino acids 59 to 211) or the rest of the protein (amino acids 59 to 395) bound weakly to Hsc82 (Fig. 3B). Thus, we conclude that the full-length TPR domain and a short part (amino acids 184 to 211) of the CS domain are required for Sgt1's binding to Hsc82 (Fig. 3C). Together, these results indicate that the Hsc82-binding domain and the Skp1-binding domain are located within the N-terminal region of Sgt1.

Requirement of the N-terminal domain of Sgt1 for kinetochore function. To assess the biological significance of the domains of Sgt1, we analyzed the phenotypes and sequences of *sgt1* temperature-sensitive mutants. We previously showed that *sgt1-3* cells, which undergo arrest in the G_2/M phases at the nonpermissive temperature, lack the ability to assemble CBF3 and thus exhibit a chromosome-missegregation phenotype; in addition, *sgt1-5* cells, which undergo arrest in the G_1 phase at the nonpermissive temperature, are deficient in the function of the SCF complex and in activation of adenylyl cyclase (6, 22). Therefore, we categorized all 12 mutants on the basis of their chromosome stability.

The colony color-sectoring assay, which measures the stability of a marker chromosome fragment, revealed that only *sgt1-3* (22), *sgt1-6*, *sgt1-7*, and *sgt1-12* showed increased chromosome missegregation (Fig. 4A and B; unpublished data). These mutants consistently exhibited sensitivity to benomyl, a microtubule-destabilizing agent; this phenotype is typical of a kinetochore mutant. Except for *sgt1-1*, the other temperature-sensitive mutants were not sensitive to benomyl and underwent cell cycle arrest in the G_1 phase at a nonpermissive temperature (Fig. 4A and B; unpublished data).

Analysis of the *sgt1* sequences in the temperature-sensitive mutants revealed that mutations in the strains with the colony color-sectoring and G_2 arrest phenotypes (G_2 alleles) are in the N-terminal region of Sgt1, which contains the Skp1-binding and Hsc82-binding domains (Fig. 4A and B). In contrast, mutations in the strains with the nonsectoring and G_1 arrest phenotypes (G_1 alleles) are in the C-terminal region of Sgt1, which contains the SGS motif (2) that is highly conserved in Sgt1 homologs of eukaryotes (Fig. 4A and B). These results are consistent with our previous observation that Skp1 binds to sgt1-5 (G_1 allele) protein but not to sgt1-3 (G_2 allele) protein in vitro (22) and with our two-hybrid finding that Skp1 binds to G_1 allele proteins but not to G_2 allele proteins (Fig. 4B and unpublished data).

These results strongly suggest that the N-terminal region of Sgt1 containing the Skp1- and Hsc82-binding domains is important for kinetochore function and that the C-terminal region containing the SGS domain appears to be important for the G_1 function of Sgt1.

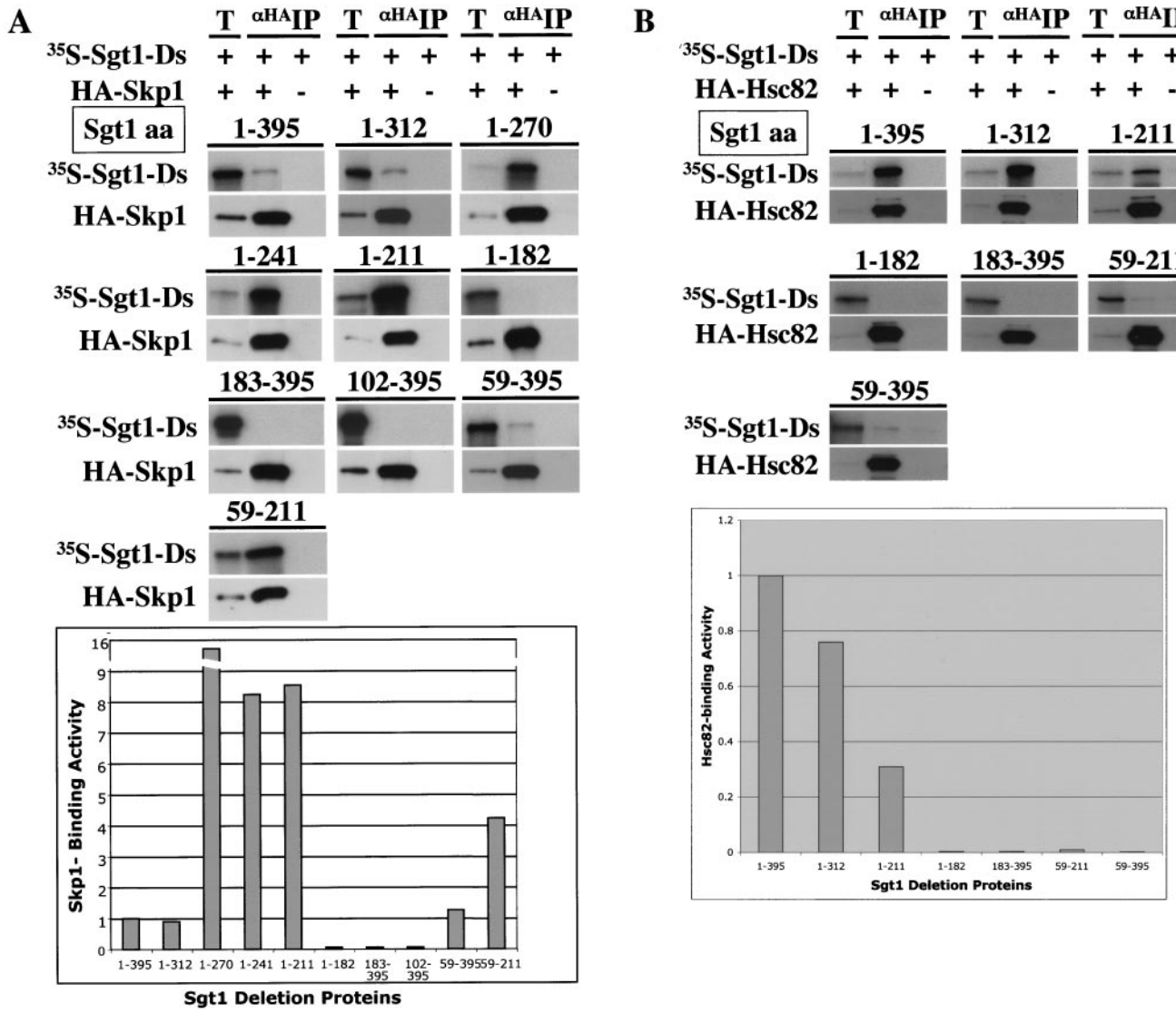


FIG. 3. Skp1-binding and Hsc82-binding domains of Sgt1. (A, top) The indicated proteins were expressed and labeled with [^{35}S]methionine in the in vitro translation system with rabbit reticulocyte lysates, and the radiolabeled proteins were mixed and incubated for 1 h at 30°C with an extract containing HA-tagged Skp1 that was not radiolabeled (20, 22). HA-tagged Skp1 was immunoprecipitated by using anti-HA–Sepharose, the immunoprecipitates were eluted and subjected to SDS-PAGE, and the radioactive bands were identified by autoradiography. HA-tagged Skp1 (HA-Skp1) was detected by immunoblotting with anti-HA antibody. T, total lysates (6% of the starting material); IP, immunoprecipitate; Ds, deletion proteins. The numbers above the lanes indicate the positions of the N- and C-terminal amino acids (aa) in each Sgt1 deletion protein. (A, bottom) Relative Skp1-binding activities of Sgt1 mutant proteins. Binding activity was defined as the ratio of the amount of coprecipitated protein to the amount of input protein. The binding activity of Sgt1 (wild type) was defined as 1, and the binding activities of the mutants were normalized to that value. (B, top) Essentially the same experiments as in panel A but with HA-tagged Hsc82 in place of HA-tagged Skp1. HA-tagged Hsc82 was expressed in the in vitro translation system but not radiolabeled. HA-tagged Hsc82 was detected by immunoblotting with anti-HA antibody. The numbers in parentheses indicate the positions of the N- and C-terminal amino acids in each Sgt1 deletion protein. (B, bottom) Relative Hsc82-binding activities of Sgt1 mutant proteins. Binding activity was calculated as described for panel A. (C) Illustration of the Sgt1 deletion proteins used in the Skp1- and Hsc82-binding assays and the results of these assays. The results of the Skp1-binding domain (Skp1-BD) and the Hsc82-binding domain (Hsc82-BD) analyses are summarized. Plus signs indicate significant binding activity. Minus signs indicate no significant binding activity. Plus-minus signs indicate weak binding activity.

Hsp90 stimulates Sgt1's binding to Skp1. The analyses of the Skp1- and Hsc82-binding domains of Sgt1 raised the possibility that the chaperone protein Hsp90 may stimulate Sgt1's binding to Skp1. To test this hypothesis, we determined whether the addition of purified human Hsp90 protein stimulates Sgt1's binding to Skp1. GST-Skp1 and His_6-Sgt1 were expressed in *Escherichia coli* and partially purified. These proteins were mixed together and incubated at 30°C for 1 h, and the interaction was tested by immunoprecipitation. We observed a weak but specific interaction between GST-Skp1 and His_6-Sgt1 (Fig. 5A, left side; unpublished data). The addition of human Hsp90 stimulated Sgt1's binding to Skp1 (Fig. 5A, left side). The stimulation was approximately 4.8-fold (unpublished data). To confirm the specificity of this stimulation, we used a dele-

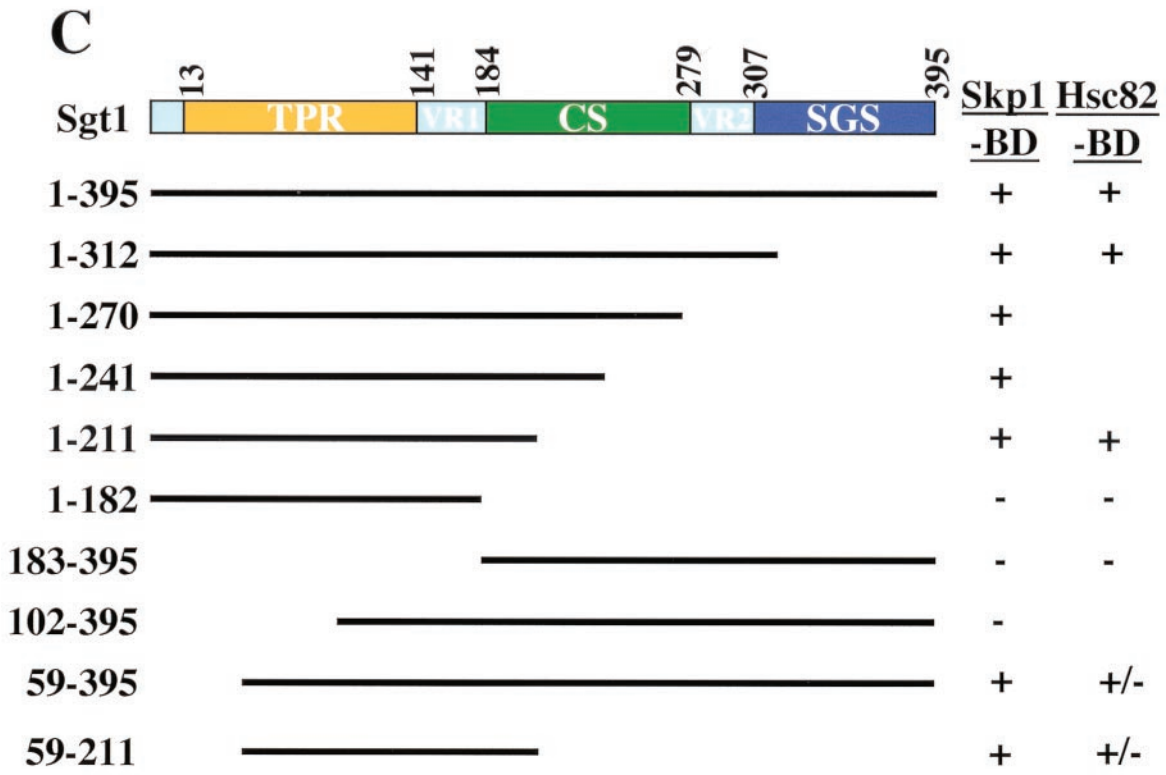


FIG. 3—*Continued.*

tion protein that lacks amino acids 1 through 58 and has lost the ability to bind to Hsp90 substantially; the deletion protein was expressed in *E. coli* and partially purified. As expected on the basis of results of the binding domain analyses (Fig. 3A), we observed a weak but specific interaction between GST-Skp1 and His_6-Sgt1(59-395). However, addition of human Hsp90 did not stimulate the binding of Sgt1(59-395) to Skp1 (Fig. 5A, right side, and unpublished data).

To confirm the importance of Sgt1-Hsp90 association for Sgt1's binding to Skp1, two of these three proteins were first incubated together and the other was added later. As expected, when His_6-Sgt1 and Hsp90 were incubated together before Skp1 was added, the result was stimulated binding of Sgt1 to Skp1 (Fig. 5B). In contrast, no such stimulated binding was observed when either GST-Skp1 and His_6-Sgt1 or GST-Skp1 and Hsp90 were incubated together initially. Taken together, these results support the hypothesis that Hsp90's binding to Sgt1 is required for the activation of Sgt1 in binding to Skp1.

Hsp90 and Sgt1 stimulate Skp1's binding to Ctf13. Next we tested whether this Hsp90-induced activation of Sgt1's binding to Skp1 affects Skp1's binding to Ctf13, which is the F-box protein of the core kinetochore. We observed a weak but specific interaction between GST-Skp1 and His_6-Ctf13 (Fig. 5C; unpublished data). The addition of His_6-Sgt1 and human Hsp90 stimulated the binding of Skp1 to Ctf13 (Fig. 5C), a finding that strongly suggests that Hsp90 plays a role in promoting the association of the core kinetochore proteins. The stimulation was approximately 3.2-fold (unpublished data). On the basis of these in vitro data, we hypothesized that Hsc82 activates Sgt1 to bind to Skp1, and then Skp1 is activated to bind to Ctf13 (see Discussion; Fig. 5F).

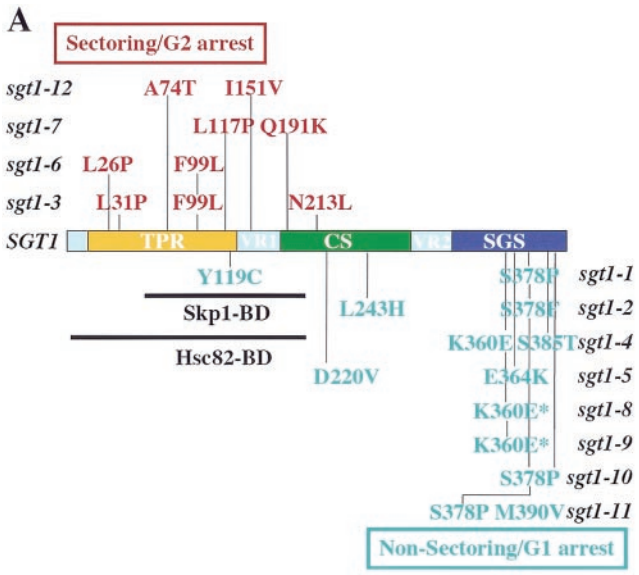
The CBF3 complex fails to form in *sgt1-3* cells, and the sgt1-3 protein cannot bind to Skp1 (22). Therefore, we tested whether the sgt1-3 protein can bind to Hsc82. We performed immunoprecipitation experiments by using Sgt1 (wild-type) and sgt1-3 proteins that were expressed and labeled with [^{35}S]methionine by the in vitro translation system, and we found that the sgt1-3 protein cannot bind to Hsc82 in vitro (Fig. 5D). If our hypothesis (the Ctf13-Skp1 interaction is activated by Sgt1 and Hsp90) is true, the Skp1-Ctf13 interaction should be altered in *sgt1-3* cells. In addition, because the CBF3 complex fails to form in *hsp82T101I* mutant cells (38), the Skp1-Ctf13 interaction should be reduced in *hsp82T101I* mutant cells. To confirm the biological relevance of our in vitro observations, we tested the Ctf13-Skp1 interaction in *sgt1-3* cells and in *hsp82T101I* cells in vivo. Because the level of endogenous expression of Ctf13 is extremely low in cells, HA-tagged Ctf13 was overexpressed through the use of a galactose-inducible promoter and immunoprecipitated with anti-HA antibody. The amounts of Skp1-Ctf13 complex detected in *sgt1-3* and *hsp82T101I* cells, respectively, were significantly less than that detected in wild-type cells (Fig. 5D, bottom, and E). These results strongly support the biological significance of our in vitro data that indicate the activation of the Ctf13-Skp1 interaction by Sgt1 and Hsp90. Taken together, these results strongly suggest that Sgt1 and its interactor Hsp90 are required for proper interactions among Sgt1, Skp1, and Ctf13; these interactions are the initial steps of assembly of the core kinetochore complex (see Discussion; Fig. 5F).

DISCUSSION

In this study we showed that Sgt1 associates with the molecular chaperone Hsp90 in yeast. Analyses of Sgt1 domains revealed that the N-terminal domain including TRP motifs is able to bind to Skp1 or Hsc82 (a yeast Hsp90 protein), and sequence analysis of *sgt1* temperature-sensitive mutants suggested that the N-terminal domain of Sgt1 is important for kinetochore function. On the basis of this work, we have developed a model of CBF assembly mediated by Sgt1 and Hsp90.

Model of CBF3 assembly. By activating Ctf13, Sgt1, together with Skp1, is required for the formation of the CBF3 complex (22). Hsp90 is also required for activation of the Ctf13-Skp1 complex and thus the formation of the CBF3 complex (38). Here we showed that Sgt1 associates with Hsc82 (a yeast Hsp90 protein) in vivo and that the Hsc82-binding domain of Sgt1 contains its Skp1-binding domain. The addition of Hsp90 stimulated Sgt1-Skp1 binding. Moreover, Sgt1 and Hsp90 stimulated Skp1-Ctf13 binding. Therefore, we propose that in vivo Sgt1 binds to Hsc82 first to form the proper structure for binding to Skp1 and that by binding to Skp1, Sgt1 activates the Skp1-Ctf13 complex for subsequent formation of the CBF3 complex (Fig. 5F) (34). Skp1 can form a complex with Sgt1 and an F-box protein (e.g., Ctf13 is an F-box protein; Fig. 5F, middle) (22). Because Sgt1 is not in the CBF3 complex (22), Sgt1 is expected to dissociate from the complex after assembly (Fig. 5F, bottom).

Possible role of Sgt1 as a cochaperone. Sgt1 participates in multiple activities: kinetochore complex assembly, SCF activity, adenylyl cyclase activity in yeasts, and *R* gene-triggered disease resistance in plants (1, 2, 6, 22). Our present results suggest that Hsp90 stimulates the binding of Sgt1 to Skp1 and the binding of Skp1 to Ctf13 via Sgt1, which is essential for the assembly of the CBF3 complex. Sgt1 did not appear to be unstable in geldanamycin-treated cells (results not shown), an observation that suggests that Sgt1 is not solely an Hsp90 substrate (often called a "client" protein) that is stabilized by the chaperone (30). Hsp90 contains three distinct domains: an amino-terminal ATPase domain, a client-binding domain in the middle, and the C-terminal end containing a dimerization domain and an MEEVD motif that binds to TPR domains of many cochaperones (29). The significant homology of Sgt1's TPR domain to that of the Hsp70-Hsp90-organizing protein HOP (Sti1 in yeast) and the stable physical interactions between Sgt1 and Hsp90 in vivo suggest that Sgt1 acts as a cochaperone (22, 38). However, the expression pattern of Sgt1 in yeast under various stress conditions is very different from those of the known cochaperones, Aha1, Hch1, Sti1, and Crp6 (10, 31). Therefore, Sgt1 does not seem to be required for stress responses. We could not detect physical interactions in yeast cells between Sgt1 and the cochaperones Cdc37, Sba1 (p23 in humans), Aha1, and Sti1 (HOP in humans) (data not shown). Cdc37, p23, and HOP inhibit the ATPase activity of Hsp90, but Aha1 stimulates this activity of this chaperone (31, 32). Therefore, it is of interest to examine whether Sgt1 affects the ATPase activity of Hsp90. In addition, we did not observe any difference in the results of our binding assays when ATP was present or absent (data not shown). Lee et al. also reported that human Sgt1's binding to Hsp90 does not depend on ATP (25).

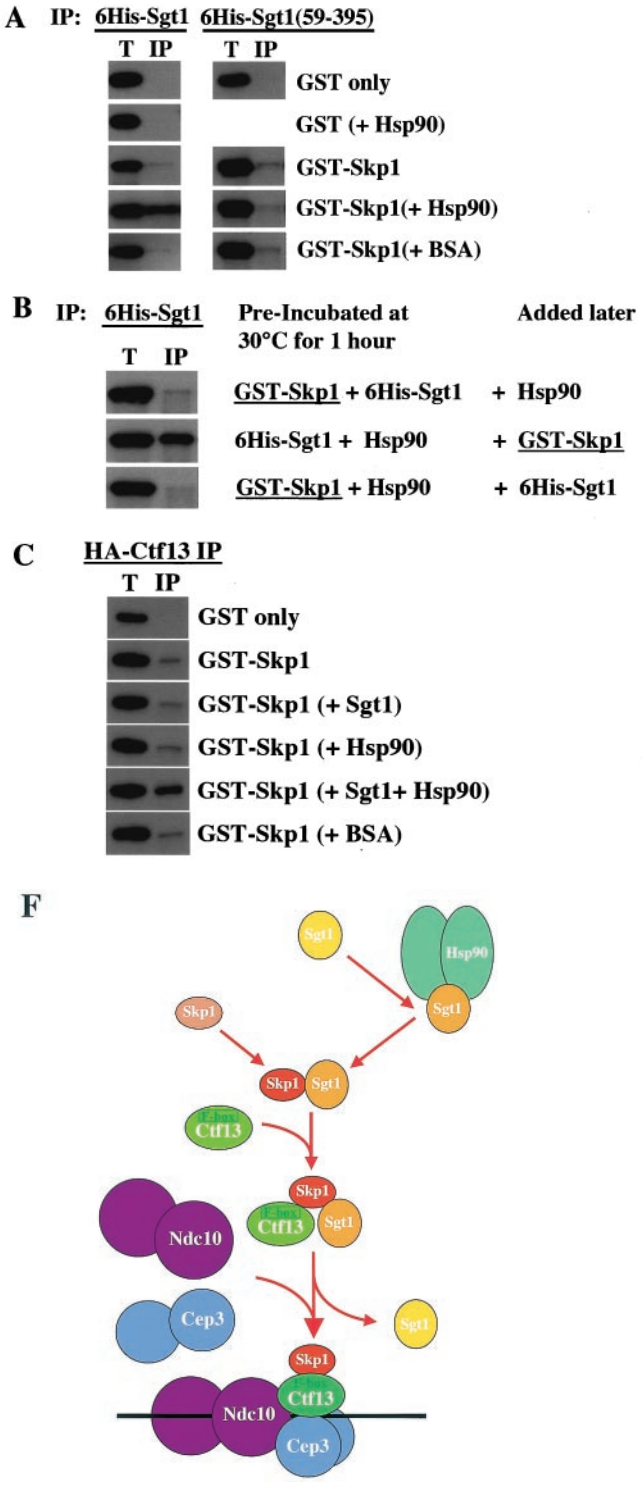


B

Allele	Sectoring	Arrest	Benomyl	GA	TH-Skp1
sgt1-3	+	G2	S	S	-
sgt1-6	+	G2	S	S	-
sgt1-7	+	G2	S	S	-
sgt1-12	+	G2	S	S	-
sgt1-1	-	G1	S	S	ND
sgt1-2	-	G1	-	S	ND
sgt1-4	-	G1	-	S	+
sgt1-5	-	G1	-	S	+
sgt1-8	-	G1	-	S	+
sgt1-9	-	G1	-	S	ND
sgt1-10	-	G1	-	S	+
sgt1-11	-	G1	-	S	ND

FIG. 4. Mutations in temperature-sensitive *sgt1* mutants. (A) The mutations in *sgt1* G_2 alleles are in red; the mutations in *sgt1* G_1 alleles are in blue. "Arrest" indicates the cell cycle stage at which the mutant cells accumulate when incubated at the nonpermissive temperature (unpublished data). Skp1-BD, Skp1-binding domain of Sgt1; Hsc82-BD, Hsc82-binding domain of Sgt1. The mutants indicated by the asterisk in panel A differ: *sgt1-8* has two additional silent mutations at nucleotide positions 403 and 864. (Table 3 contains details of the *sgt1* mutations.) (B) Summary of the phenotypes of *sgt1* mutants. Plus signs in the sectoring column indicate that the mutant cell showed a chromosome missegregation defect that was shown by a colony color-sectoring assay. Minus signs in the sectoring column indicate that the cells did not show substantial chromosome missegregation. The letter S indicates sensitivity to benomyl or geldanamycin (GA); a minus sign indicates resistance. Because the *sgt1-10* mutant, which has only the S378P mutation, is not sensitive to benomyl, we believe that the Y119C mutation is probably responsible for the benomyl sensitivity of the *sgt1-1* mutant, which has two mutations (Y119C and S378P). Also shown are results of the two-hybrid assay (TH-Skp1) to assess the ability of *sgt1* mutant proteins to bind to Skp1 (unpublished data). A plus sign in the TH-Skp1 column indicates that the mutant protein bound to Skp1 in the two-hybrid system. A minus sign indicates that the mutant protein did not do so. ND, not done.

Sensitivity of SCF mutants to geldanamycin. We found that the N-terminal domain of Sgt1 is required for its interaction with Hsp90 and that the mutations of the *sgt1* G_2 alleles are located in the region that encodes this domain. These findings



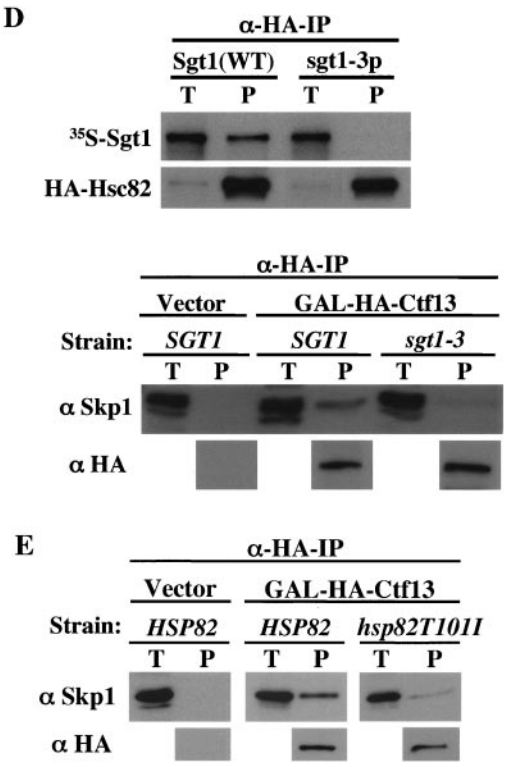


FIG. 5. Sgt1, together with Hsp90, stimulates Skp1-Ctf13 binding. (A) Hsp90 requires the N-terminal region of Sgt1 to promote its interaction with Skp1. Approximately 200 ng of each indicated protein was used for immunoprecipitation (IP). After the samples were incubated at 30°C for 1 h, His_6-tagged Sgt1 or His_6-tagged Sgt1(59-395) was immunoprecipitated with anti-His antibody conjugated to Sepharose. GST-Skp1 and GST proteins were detected by immunoblotting. The bands in the top two panels are GST protein only, and the bands in the bottom three panels are the GST-Skp1 fusion protein. The names of proteins in parentheses indicate that these proteins were present in the reaction mixture but are not shown. BSA, bovine serum albumin. (B) Experiment similar to that described for panel A but with preincubation of two of the three indicated proteins at 30°C for 1 h and addition of the other later (immediately before immunoprecipitation). (C) Sgt1 and Hsp90 stimulate the binding of Ctf13 to Skp1. This experiment was conducted as described for panel A, except that HA-tagged Ctf13 was precipitated with anti-HA antibody conjugated to Sepharose. The bands in the top panel are GST protein only, and the bands in the bottom five panels are the GST-Skp1 protein. (D, top) sgt1-3 protein (sgt1-3p) does not bind to Hsc82 in vitro. Wild-type (WT) Sgt1 protein and sgt1-3 mutant protein were expressed and labeled with [^{35}S]methionine in the in vitro translation system, and the radiolabeled proteins were mixed and incubated for 1 h at 30°C with an extract containing HA-tagged Hsc82 (HA-Hsc82) that was not radiolabeled. HA-Hsc82 was immunoprecipitated by using anti-HA–Sepharose, the immunoprecipitates were eluted and subjected to SDS-PAGE, and the radioactive bands were identified by autoradiography. HA-Hsc82 was detected by immunoblotting with anti-HA antibody. T, total lysates (6% of the starting material); P, immunoprecipitate. (D, bottom) The Skp1-Ctf13 association was reduced in *sgt1-3* cells. HA-tagged Ctf13 expression was induced by addition of galactose; 1 h later, the temperature was shifted to 37°C and the cells were incubated for 90 min. HA-tagged Ctf13 was immunoprecipitated by using anti-HA antibody conjugated to Sepharose. Skp1 was detected by Western blot analysis with anti-Skp1 antibody. (E) The Skp1-Ctf13 association was reduced in *hsp82T101I* cells. HA-tagged Ctf13 expression was induced by addition of galactose; 1 h later, the temperature was shifted to 37°C and the cells were incubated for 90 min. HA-tagged Ctf13 was immunoprecipitated by using anti-HA antibody conjugated to Sepharose. Skp1 was detected by Western blot analysis with anti-Skp1 antibody. (F) Model of CBF3 assembly. Once Sgt1 forms a complex containing Hsp90, Sgt1 is activated to bind to Skp1. By binding to Sgt1, Skp1 is activated to bind to Ctf13 (34).

imply that only the G_2/kineothcore function (but not the G_1 function) of Sgt1 is involved in the activity of Hsp90. However, all of the *sgt1* G_1 alleles are also sensitive to geldanamycin. Because mutations in these strains occurred in the SGS domain, which is located at the C-terminal end, the function of the SGS domain may be related to the function of Hsp90 by an unknown mechanism. Because the *sgt1-5* mutant, one of the

sgt1 G_1 alleles, is deficient in SCF activity (22), we examined whether the SCF mutants are sensitive to geldanamycin. All of the SCF mutants that we tested (*skp1-3*, *cdc53-1*, *cdc34-2*, *cdc4-1*, *cdc4-12*, *met30-6*, and *grr1*Δ) were sensitive to geldanamycin, and the F-box protein mutants (*cdc4-1*, *cdc4-12*, *met30-6*, and *grr1*Δ) were especially sensitive to geldanamycin. Skp1 binds to F-box proteins via the F-box motif (7, 35). Because Sgt1 is required for activation of Ctf13, an F-box protein, Sgt1 may also be required for activation or stabilization of other F-box proteins. Recently, Melville et al. reported that Hsp70 and TriC/CCT chaperones are required for assembly of the von Hippel-Lindau tumor suppressor complex, which is structurally analogous to the SCF complex (28). Further investigation is required to address whether the chaperone activity of Hsp90 or another, related, protein is required for the function of the SCF complex.

Role of human SGT1 in kinetochore function. Human SGT1 (SUGT1) can rescue the *sgt1*-null mutant (22), a result that suggests that the function of Sgt1 is conserved between humans and yeast. SGT1 associates with Hsp90 in human cells (S. Ohta and K. Kitagawa, unpublished data). Very recently, Lee et al. reported that the CS domain, but not the TPR domain, of human SGT1 binds to Hsp90; this result is not consistent with our observation about yeast Sgt1 (25). We do not know whether this discrepancy reflects differences between the two organisms. Further analysis is required to clarify the discrepancy.

Interestingly, Steensgaard et al. found that CENP-I, CENP-F, and Hec1, but not CENP-C, are absent from kinetochores in human Sgt1-depleted cells. This absence suggests that human Sgt1 is a critical factor for assembly of mammalian kinetochores (36a). These data strongly suggest that Sgt1 is functionally conserved between humans and yeast. Additional analysis is needed to investigate the molecular mechanism.

Other functions of the Sgt1-chaperone complex. In addition to its role in the function of the kinetochore and of the SCF complex, Sgt1 is involved in activation of adenylyl cyclase in budding and fission yeasts and in *R* gene-triggered resistance in plants (1, 2, 6, 22). Recently, three research groups independently found that Hsp90 is required for *R* gene-triggered resistance in plants (14, 27, 40). Notably, Sgt1 coimmunoprecipitates with Hsp90 proteins in *Arabidopsis* and barley (40). Sgt1 is highly conserved in eukaryotes, and the Sgt1-chaperone interaction appears to be conserved in yeast, plants, and humans. In this paper, we provide evidence that suggests an initial activation step in the assembly of the CBF3 complex-Sgt1- and Hsp90-mediated activation of the Skp1-Ctf13 association. Similar molecular mechanisms may be involved in the formation of other complexes in which Sgt1 participates. Moreover, these mechanisms may be conserved in other organisms.

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