stf1: Non-Wee Mutations Epistatic to *cdc25* in the Fission Yeast *Schizosaccharomyces pombe*

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ABSTRACT

In *Schizosaccharomyces pombe, cdc25* is a cell cycle regulated inducer of mitosis. *wee1* and phenotypically wee alleles of *cdc2* are epistatic to *cdc25*. Mutant alleles of a new locus, *stf1* (suppressor of *t*wenty-*f*ive), identified in a reversion analysis of conditionally lethal *cdr1-76 cdc25-22* and *cdr2-96 cdc25-22* double mutant strains, also suppress both temperature-sensitive and gene disruption alleles of *cdc25*. These mutants, by themselves, are phenotypically indistinguishable from wild type strains; hence they represent the first known mutations that are epistatic to *cdc25* and do not display a wee phenotype. *stf1* genetically interacts with other elements of mitotic control in *S. pombe*. *stf1-1* is additive with *wee1-50, cdc2-1w* and *cdc2-3w* for suppression of *cdc25-22*. Also, like *wee1*$^-$ and *cdc2-w, stf1*$^-$ suppression of *cdc25* is reversed by overexpression of the putative type 1 protein phosphatase *bws1*$^+$/*dis2*$^+$. Interaction with various mutants and plasmid overexpression experiments suggest that *stf1* does not operate either upstream or downstream of *wee1*. Similarly, it does not operate through *cdc25* since it rescues the disruption. *stf1* appears to encode an important new element of mitotic control.

IN *Schizosaccharomyces pombe*, cell cycle progression is dependent on *cdc2* gene function, both in G1, prior to the initiation of DNA synthesis, and at the G2/M boundary, for the initiation of mitosis (NURSE, THURIAUX and NASMYTH 1976; NURSE and BISSETT 1981). The *cdc2* gene encodes a 34-kD serine/threonine protein kinase (HINDLEY and PHEAR 1984; SIMANIS and NURSE 1986) which has been highly conserved among eukaryotes, and forms a part of maturation or M-phase promoting factor (MPF) (reviewed in NURSE 1990). Dominant *cdc2* mutants show premature entry into mitosis, suggesting that the *cdc2* gene product regulates the timing of mitotic initiation (THURIAUX, NURSE and CARTER 1978; FANTES 1981).

Genetic and biochemical analyses have identified several proteins which interact with p34^{cdc2}. The products of the *suc1* (p13) and *cdc13* (cyclin) genes physically interact with p34^{cdc2} prior to or during mitotic initiation (HAYLES, AVES and NURSE 1986; HAYLES *et al.*, 1986; EVANS *et al.*, 1983; BOOHER and BEACH 1987; BRIZUELA, DRAETTA and BEACH 1987; GOEBL and BYERS 1988; SOLOMON *et al.* 1988; BOOHER *et al.* 1989; DRAETTA *et al.* 1989). The *cdc25* and *wee1* gene products appear to regulate the timing of activation of the p34^{cdc2} kinase. *cdc25* encodes a cell cycle regulated inducer of mitosis, loss of which causes cell cycle arrest, whereas overexpression results in early entry into mitosis (NURSE, THURIAUX and NASMYTH 1976; RUSSELL and NURSE 1986; MORENO, NURSE and RUSSELL 1990; DUCOMMUN *et al.* 1990). The requirement for *cdc25* function is correlated with tyrosine dephosphorylation of *cdc2* and activation of the p34^{cdc2} complex (GOULD and NURSE 1989). *wee1* encodes a putative protein kinase (RUSSELL and NURSE 1987a) which acts antagonistically to *cdc25*; loss of *wee1*$^+$ function overcomes the requirement for *cdc25* function (FANTES 1979, 1981). Another gene, *bws1/dis2* reverses *wee1* epistasis of *cdc25* when present on a multicopy plasmid (BOOHER and BEACH 1989). Comparison of nucleotide sequences reveals that *bws1*$^+$/*dis2*$^+$ is highly similar to previously identified mammalian type 1 protein phosphatases (OHKURA *et al.* 1989). Mutations in *win1* and *mcs3* also reverse this epistasis, though the gene products have not yet been identified (OGDEN and FANTES 1986; MOLZ *et al.* 1989).

cdr1 and *cdr2* were hypothesized to play a role in transmitting information regarding nutritional status to the mitotic control in *S. pombe* (YOUNG and FANTES 1984, 1987). *cdr* mutants show an altered mitotic response to starvation and show strong genetic interactions with *cdc25* mutants. The recent observation that *cdr1* is allelic to *nim1* (H. FEILOTTER, P. NURSE and P. G. YOUNG, personal communication), a putative protein kinase which appears to downregulate *wee1* inhibition of *cdc2* action (RUSSELL and NURSE 1987b), suggests that these genes play an important role in the control of mitosis.

To identify new genes or novel alleles of known genes involved in the regulation of mitosis, we have collected and analyzed revertants of the conditionally lethal *cdr*$^-$ *cdc25-22* double mutant strains. Among these revertants are three new mutants which can

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TABLE 1

Table of strains

Q206	*h⁻ cdc2-33*	Q724	*h⁻ stf1-1 cdc25-M51*
Q250	*h⁻ 972*	Q725	*h⁻ stf1-1 cdc25-22 wee1-50*
Q257	*h⁻ cdr2-96*	Q726	*h⁺ stf1-1 cdc25-22 cdc2-1w*
Q258	*h⁻ cdr1-76*	Q727	*h⁻ stf1-1 cdc25-22 cdc2-3w*
Q293	*h⁻ cdc2-3w leu1-32*	Q728	*h⁺ stf1-1 suc1-210*
Q307	*h⁺ cdc2-L7*	Q729	*h⁻ stf1-1 cdc25-22 int pcdc25.5(ADH)*
Q323	*h⁺ 975*	Q732	*h⁻ stf1-1 cdc25::URA4 ura4-D18*
Q352	*h⁻ cdc25-22 cdc2-1w leu1-32*	Q733	*h⁻ stf1-1 cdr1-76 cdc25-22*
Q370	*h⁻ cdc25-22 ade6-216*	Q734	*h⁻ stf1-2 cdr2-96 cdc25-22*
Q371	*h⁺ cdc25-22 ade6-216*	Q735	*h⁻ stf1-3 cdr2-96 cdc25-22*
Q376	*h⁺ cdc25M51*	Q744	*h⁻ stf1-2*
Q429	*h⁺ cdc2-M35R20*	Q745	*h⁻ stf1-3*
Q430	*h⁺ cdd2-M35*	Q746	*h⁺ stf1-2 cdc25-22*
Q434	*h⁺ cdr2-40*	Q747	*h⁺ stf1-3 cdc25-22*
Q437	*h⁺ cdc2-59 leu1-32 ade6-216*	Q748	*h⁻ stf1-1 cdc25-22 ade6-210*
Q480	*h⁻ wee1-50*	Q749	*h⁺ stf1-1 cdc25-22 ade6-216*
Q630	*h⁻ cdc25-22 int pcdc25.5(ADH) leu1-32*	Q750	*h⁺ stf1-2 cdc25-22 ade6-210*
Q662	*h⁻ cdc2-1w*	Q751	*h⁻ stf1-2 cdc25-22 ade6-216*
Q682	*h⁻ cdc25-22 bws1::URA4 ura4-D18 leu1-32*	Q752	*h⁻ stf1-3 cdc25-22 ade6-210*
Q703	*h⁺ cdc13-117*	Q753	*h⁺ stf1-3 cdc25-22 ade6-216*
Q704	*h⁻ cdc25-22 wee1-50*	Q754	*h⁻ stf1-1 ade6-210*
Q705	*h⁻ cdc25-22 cdc2-3w*	Q755	*h⁺ stf1-1 ade6-216*
Q706	*h⁻ suc1-210 leu1-32*	Q759	*h⁻/h⁺ stf1-1/stf1-1 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q708	*h⁻ cdc25::URA4 cdc2-3w ura4-D18*	Q760	*h⁻/h⁺ stf1-1/stf1-2 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q709	*h⁻ stf1-1*	Q761	*h⁻/h⁺ stf1-1/stf1-3 cdc25-22/cdc25-22 ade6-210/ace6-216*
Q710	*h⁺ stf1-1*	Q762	*h⁻/h⁺ stf1-2/stf2-1 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q711	*h⁺ stf1-1 cdr1-76*	Q763	*h⁻/h⁺ stf1-2/stf1-3 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q712	*h⁺ stf1-1 cdr2-40*	Q764	*h⁻/h⁺ stf1-3/stf1-3 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q713	*h⁻ stf1-1 cdr2-96*	Q765	*h⁻/h⁺ stf1-1/stf1+ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q714	*h⁺ stf1-1 cdc2-33*	Q766	*h⁻/h⁺ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q715	*h⁻ stf1-1 cdc2-M35*	Q767	*h⁻/h⁺ stf1-2/stf1+ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q716	*h⁻ stf1-1 cdc2-M35R20*	Q768	*h⁻/h⁺ stf1-3/stf1+ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q717	*h⁻ stf1-1 cdc2-L7*	Q769	*h⁻/h⁻ stf1-1/stf1-1 cdc25-22/cdc25-22 ade6-210/ade6-216*
Q718	*h⁺ stf1-1 cdc2-59*	Q770	*h⁻/h⁻ stf1-1/stf1+ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q719	*h⁻ stf1-1 cdc2-1w*	Q771	*h⁻/h⁻ cdc25-22/cdc25-22 ade6-210/ade6-216*
Q720	*h⁻ stf1-1 cdc2-3w*	Q772	*h⁻/h⁺ stf1-1/stf1-1 ade6-210/ade6-216*
Q721	*h⁻ stf1-1 wee1-50*	Q773	*h⁻/h⁺ stf1-1/stf1+ ade6-210/ade6-216*
Q722	*h⁺ stf1-1 cdc13-117*	Q774	*h⁻/h⁺ ade6-210/ade6-216*
Q723	*h⁻ stf1-1 cdc25-22*		

rescue temperature-sensitive and gene disruption alleles of *cdc25*. By themselves, these mutations do not affect cell size and are not allelic to *wee1* or *cdc2*. These mutants, *stf1-1,2,3* (*s*uppressor of *t*wenty-*f*ive), thus represent the first phenotypically non-wee suppressors of *cdc25*.

MATERIALS AND METHODS

Strains: All strains were derived from standard wild-type *S. pombe* strains 972 (h^{-S}) or 975 (h^{+N}) introduced by U. LEUPOLD (1950). The isolation of *cdc2-33*, *cdc13-117*, *cdc25-22* (NURSE, THURIAUX and NASMYTH 1976), *cdc2-1w*, *wee1-50*, *cdc2-L7*, *cdc2-M35* (NURSE and THURIAUX 1980), *cdc2-3w* (FANTES 1981), *cdc25-M51* (NASMYTH and NURSE 1981), *cdc2-M35R20* (CREANOR and MITCHISON 1982), and *cdc2-59* (BOOHER and BEACH 1987) mutations has been previously described. The construction and characterization of *cdc25-disruption* and *cdc25-overexpresser* strains have been previously described (RUSSELL and NURSE 1986; DUCOMMUN *et al.* 1990). Standard genetic techniques as described by GUTZ *et al.* (1974) were employed. All diploid strains were constructed using the method of complementing *ade6-210* and *ade6-216* mutations as described by GUTZ et al. (1974). A strain list is included in Table 1.

Media: Strains were grown in complete media (YEA: 0.5% yeast extract, 3% glucose, 100 μg/ml adenine) or minimal media, EMM (MITCHISON 1970 as modified by NURSE 1975), containing appropriate auxotrophic supplements (100 μg/ml). Matings were performed on 3% malt extract.

Reversion analyses: Double mutant strains *cdr1-76 cdc25-22*, *cdr1-34 cdc25-22*, *cdr1-76 cdc2-M35R20* and *cdr2-96 cdc25-22* were constructed. Cells were grown on minimal media at a permissive temperature and then shifted to and held at a restrictive temperature. Colonies that appeared four to eleven days after the temperature shift, representing spontaneous revertants, were collected for analysis.

Analysis of revertants: All revertants were crossed to wild-type strains. Strains that segregated phenotypically wee progeny (by free spore analysis) were discarded. The remaining strains were analyzed by tetrad analysis of crosses to wild-type and *cdc25-22* strains.

Cell length measurements: Cells were grown in liquid YEA at 25° and 35°. Lengths of cells displaying a complete

TABLE 2

Summary of reversion analyses

Strain	Reverted strain			
	cdr1-76 cdc25-22	*cdr1-34 cdc25-22*	*cdr1-76 cdc2-M35R20*	*cdr2-96 cdc25-22*
Rescued by mutations in				
wee1 or *cdc2*	194	174	82	359
cdc25	0	0	0	2
cdr1,2	0	2	0	0
Non-wee third site[a]	2	4	0	13
Total analyzed	196	180	82	374

[a] Some of these strains mate very poorly and the possibility that some revertants placed in this class contain intragenic revertants in *cdr* or *cdc25* has not been rigourously excluded.

medial septum under phase contrast microscopy were measured. A total of 12 to 90 cells were measured for each strain at each temperature. Appropriate strains were compared statistically using Student's *t*-tests.

Plasmids and transformations: All plasmids are derived from *S. pombe* transformation vectors DB248X, pWH5, and YEp13. pcdc25.1 carries an 8-kb *Hin*dIII genomic insert encompassing *cdc25*$^+$ in pWH5 (MOLZ *et al.* 1989). pcdc2.14 contains a 3.4 kb *Pst*I genomic fragment encompassing the *cdc2*$^+$ gene in DB248X (BOOHER and BEACH 1988). pwee1.1 carries an 8.4-kb genomic fragment containing the *wee1*$^+$ gene inserted into the *Bam*HI site of YEp13 (RUSSELL and NURSE 1987a). pbws1-1 contains a 5.2-kb genomic insert in DB248X encompassing the *bws1*$^+$ gene (BOOHER and BEACH 1989).

All transformations were performed as described in BEACH and NURSE (1981).

RESULTS

Reversion analyses: The conditional lethality of *cdr1-76 cdc25-22*, *cdr1-34 cdc25-22*, *cdr1-76 cdc2M35R20* and *cdr2-96 cdc25-22* double mutant strains has been previously described (YOUNG and FANTES 1984, 1987; H. FEILOTTER, P. NURSE and P. G. YOUNG, personal communication). Each of these strains were grown at a permissive temperature (20–25°) on minimal media, then shifted to, and held at a restrictive temperature (29° except 36° for *cdr1-76 cdc2-M35R20*). Spontaneous intragenic and extragenic revertants were collected for analysis.

Most of these reverted strains segregated phenotypically wee progeny when backcrossed to wild-type strains (Table 2). This was not unexpected; recessive mutations in *wee1* and dominant mutations in *cdc2* confer a wee or small cell size phenotype in *S. pombe*, and are known to phenotypically rescue *cdr1*$^-$, *cdr2*$^-$ and *cdc25*$^-$ mutants (FANTES 1979, 1981; YOUNG and FANTES 1987).

A total of 19 strains did not segregate wee progeny yet still segregated phenotypically *cdr1*$^-$ and *cdc25*$^-$ progeny when outcrossed to wild type strains, indicating that these strains were rescued by non-wee mutations extragenic to *cdr1* and *cdc25*. Three of these strains were further analyzed by tetrad analysis of crosses to wild-type and *cdc25-22* strains.

TABLE 3

Linkage between alleles of *stf1*

Cross	Phenotype of progeny	
	cdc25-22	*stf1*$^-$ *cdc25-22*
stf1-1 cdc25-22 × *cdc25-22*	260	243
stf1-1 cdc25-22 × *stf1-2 cdc25-22*	0	1024
stf1-1 cdc25-22 × *stf1-3 cdc25-22*	0	1081

stf1$^-$ *cdc25-22* strains were intercrossed and spore progeny were obtained after digestion of parental cells with snail gut enzyme. Spores were germinated and small colonies were formed at 25°, then shifted to 35° for 8 hr and scored.

Isolation of *stf1* alleles: A *cdr1-76 cdc25-22* revertant (Q733) was crossed to a wild-type strain and a tetrad was identified which segregated a phenotypically *cdc25-22*, *cdr1-76*, parental triple mutant, and wild type strain. The phenotypically wild type strain was crossed to a *cdc25-22* tester strain; progeny were phenotypically wild type, *cdc25-22* and of a third heterogeneous phenotype (ranging from approximately wild type to *cdc*$^-$ at 35°). Cells of this third heterogeneous phenotype were crossed to wild type and *cdc25-22* tester strains; the ratios of phenotypes of progeny were consistent with this heterogeneous strain being *cdc25-22* plus an extragenic *cdc25-22* suppressing mutation. This suppressing mutation was named *stf1-1* (*s*uppressor of *t*wenty-*f*ive). Two additional *cdc25-22* suppressor mutations were isolated in a similar manner from revertants of *cdr2-96 cdc25-22* (Q734, Q735, respectively). In a *cdc25*$^+$ genetic background, each of these mutant strains was phenotypically indistinguishable from wild type.

To determine the number of linkage groups, each mutant, in a *cdc25-22* background, was crossed with the other two. These crosses did not yield observable nonrescued *cdc25-22* progeny (Table 3) indicating that these three mutations were tightly linked. The second and third suppressor mutations were hence named *stf1-2* and *stf1-3*, respectively.

***stf1*$^-$ diploids:** Strains were constructed which were homozygous for *cdc25-22* and homozygous *stf1*$^-$/*stf1*$^-$, heterozygous *stf1*$^-$/+ or homozygous +/+. Since homozygous *cdc25-22/cdc25-22* diploid strains showed a cdc$^-$ phenotype at 32° (in contrast to *cdc25-22* haploid strains which appear cdc$^-$ at 34–35°), the diploids were tested at 32°. At this temperature, the homozygous +/+ *cdc25-22/cdc25-22* appeared as single cdc$^-$ or colonies of two or three cdc$^-$ cells. The heterozygous diploids *stf1*$^-$/+ *cdc25-22/cdc25-22* grew as very long cells in comparison to the homozygous *stf1*$^-$/*stf1*$^-$ *cdc25-22/cdc25-22* diploids. Both the homozygous *stf1*$^-$/*stf1*$^-$ *cdc25-22/cdc25-22* and heterozygous *stf1*$^-$/+ *cdc25-22/cdc25-22* strains arrested as colonies of 20 to 200 cells; *stf1-1/stf-1 cdc25*$^+$/*cdc25*$^+$ diploid strains continued to grow and form

TABLE 4

Interactions between *stf1* and *cdc25*

Strain	Mean cell lengths (in μm) 25°	35°
972	13.4 ± 0.2	12.6 ± 0.2
stf1-1	13.6 ± 0.3	12.4 ± 0.2
cdc25-22	17.5 ± 0.3	cdc⁻
cdc25-22 stf1-1	16.6 ± 0.5	18.1 ± 0.5
cdc25-M51	17.6 ± 0.4	cdc⁻
cdc25-M51 stf1-1	19.3 ± 0.4	19.1 ± 1.2
cdc25-disruption[a]	cdc⁻	cdc⁻
cdc25-disruption stf1-1	20.9 ± 0.9	19.0 ± 0.7
cdc25-op	10.7 ± 0.3	9.3 ± 0.5
cdc25-op stf1-1	11.1 ± 0.4	9.8 ± 0.6

Double mutant strains containing *stf1-1* and various alleles of *cdc25* were constructed. Cells were grown in complete media (YEA) at 25° and 35° and cell length measurements were made on cells in which a distinct medial septum was apparent.

[a] The phenotype of this strain was scored after outcrossing a *cdc2-3w cdc25-null* double mutant strain.

TABLE 5

Interactions between *stf1* and *cdc2*

Strain	Mean cell length (in μm) 25°	35°
cdc2-33	14.8 ± 0.2	cdc⁻
cdc2-33 stf1-1	14.3 ± 0.3	cdc⁻
cdc2-L7	14.4 ± 0.3	cdc⁻
cdc2-L7 stf1-1	14.2 ± 0.2	cdc⁻
cdc2-M35	20.1 ± 0.3	cdc⁻
cdc2-M35 stf1-1	21.1 ± 0.8	cdc⁻
cdc2-M35R20	17.5 ± 0.3[a]	18.0 ± 0.3[b]
cdc2-M35R20 stf1-1	16.8 ± 0.3[a]	16.7 ± 0.2[b]
cdc2-59	cdc⁻	10.5 ± 0.4
cdc2-59 stf1-1	cdc⁻	10.1 ± 0.4
cdc2-1w	9.9 ± 0.3	9.3 ± 0.4
cdc2-1w stf1-1	10.5 ± 0.4	10.0 ± 0.4
cdc2-3w	10.3 ± 0.2	8.7 ± 0.2[c]
cdc2-3w stf1-1	10.6 ± 0.2	9.3 ± 0.2[c]
cdc2-1w cdc25-22	12.2 ± 0.3	23.3 ± 1.1[d]
cdc2-1w cdc25-22 stf1-1	12.7 ± 0.6	12.1 ± 0.4[d]
cdc2-3w cdc25-22	14.1 ± 0.2	14.8 ± 0.2[e]
cdc2-3w cdc25-22 stf1-1	14.0 ± 0.2	9.7 ± 0.3[e]

Double and triple mutant strains containing *stf1-1* and various alleles of *cdc2* were constructed and grown in complete media; lengths of cells at septation were measured.

[a,c] Significantly different ($0.025 < P < 0.05$).

[b,d,e] Significantly different ($P < 0.005$).

viable colonies at this temperature indicating that the growth defects of these strains were only exhibited in a *cdc25*⁻ genetic background (similar phenotypes were observed in h^-/h^- diploids).

When tested at 30°, all diploid strains formed viable colonies; although each strain was quite heterogeneous. Heterozygous *stf1*⁻/+ *cdc25-22/cdc25-22* diploids were intermediate in size between *stf1*⁻/*stf1*⁻ *cdc25-22/cdc25-22* and +/+ *cdc25-22/cdc25-22* diploids. The diploids *stf1-1/stf1-2 cdc25-22/cdc25-22*, *stf1-1/stf1-3 cdc25-22/cdc25-22* and *stf1-2/stf1-3 cdc25-22/cdc25-22* were also constructed and tested. Each of these diploid strains was phenotypically similar to the homozygous *stf1*⁻/*stf1*⁻ *cdc25-22/cdc25-22* strain.

Allelism tests with other mitotic control genes: We then tested whether *stf1* was allelic to genes known to be closely associated with the mitotic control. *stf1-1* recombined freely with alleles of *cdc2*, *wee1*, *cdc25*, *cdr1*, *cdr2*, *cdc13*, *suc1*, and a *URA4*⁺ marked deletion strain of *bws1* (see below). These results indicate that *stf1* may represent a novel gene. Allelism to other *cdc*, *win1* and *mcs* genes was not tested due to the absence of suitably marked strains.

Since *stf1-1*, *stf1-2* and *stf1-3* were first identified as suppressors of conditionally lethal interactions between mitotic mutants, the interactions of *stf1-1* and various alleles of known elements of the mitotic control in *S. pombe* were analyzed.

Interaction between *stf1* and *cdc25*: Double mutants of *stf1-1* and various alleles of *cdc25* were constructed and analyzed. *stf1-1* rescued the temperature-sensitive defects of *cdc25-M51* and the constitutive defect of a *cdc25* disruption strain, although occasional cdc⁻ cells were still observed in this double mutant strain (Table 4). When tested at 37°, all *stf1-1 cdc25*[ts] and *stf1-1 cdc25-disruption* strains failed to form normal colonies; very heterogeneous colonies of 1–20 cells were observed. Many cells were defective in cell morphology; cells were swollen, bent, and/or asymmetrical. This apparent defect in growth and cell morphology was only exhibited in the absence of *cdc25*⁺; *stf1-1* strains were indistinguishable from wild type at this temperature.

To test if *stf1* mutant strains were still sensitive to increased levels of the wild type *cdc25*⁺ gene, both *stf1-1 leu1-32* and *leu1-32* strains were transformed with pcdc25.1. Colonies of both transformed strains displayed heterogeneous wee phenotypes. To assess the effects of massive overexpression of *cdc25*⁺ on *stf1*⁻, *stf1-1* was crossed to a *cdc25*⁺ overexpresser strain (Q630), in which the wild-type *cdc25*⁺ gene is under the control of the strong ADH promoter. The resultant double mutant *stf1-1 cdc25-overexpresser* was phenotypically indistinguishable from the *cdc25-overexpressor* strain, both displaying heterogeneous wee phenotypes.

Interaction between *stf1* and *cdc2*: Since *cdc25* is believed to exert its action through *cdc2*, the interaction between *stf1-1* and various alleles of *cdc2* was assessed (Table 5). *stf1-1* showed some interaction with *cdc2-3w*, *cdc2-M35* (cells arrested with a shorter terminal phenotype in the double mutant) and its non-cdc⁻ revertant, *cdc2-M35R20*. It is interesting to note that these particular alleles show strong genetic interactions with mutant alleles of *cdc25* (FANTES 1981; H. FEILOTTE, P. NURSE and P. G. YOUNG, personal communication).

cdc2-1w and *cdc2-3w* suppress mutations in *cdc25* by

TABLE 6

Interactions between *stf1* and *wee1*

Strain	Mean cell length (in μm) 25°	35°
wee1-50	12.3 ± 0.4	9.4 ± 0.3
wee1-50 stf1-1	11.6 ± 0.4	8.7 ± 0.3
wee1-50 cdc25-22	15.5 ± 0.3	12.5 ± 0.4[a]
wee1-50 cdc25-22 stf1-1	15.4 ± 0.4	9.0 ± 0.3[a]

[a] Significantly different ($P < 0.005$).

themselves, so the effect of *stf1-1* on this interaction was tested. In both cases, the triple mutants containing *stf1-1* divided at a significantly smaller cell size than their double mutant counterparts at 35°, indicating that both *cdc2-w* alleles and *stf1-1* have an additive effect in rescuing *cdc25-22*.

Since overexpression of $cdc2^+$ affects genetic interactions of *cdc25* (MOLZ *et al.* 1989; H. FEILOTTE, P. NURSE and P. G. YOUNG, personal communication), we also tested whether it could reverse $stf1^-$ suppression of *cdc25* by transforming a *stf1-1 cdc25-22 leu1-32* strain with pcdc2.14. No such effect was observed.

Interaction between *stf1* and *wee1*: *stf1-1* showed no apparent interaction with *wee1-50* by itself (Table 6). Since loss of function mutations in *wee1* are known to suppress mutations in *cdc25* (FANTES 1979) the effect of *stf1* on this interaction was assessed. The triple mutant *stf1-1 wee1-50 cdc25-22* divided at significantly smaller cell size than a *wee1-50 cdc25-22* strain, indicating an additive effect of *stf1-1* and *wee1-50* in suppressing the temperature-sensitive defect of *cdc25-22*.

Overexpression of $wee1^+$ is known to delay mitosis in wild-type strains. *stf1-1 leu1-32* was transformed with pwee1.2. Transformed *stf1-1* strains also showed a delay in entering mitosis. Cells divided at approximately 2–3 times wild-type size and were similar in phenotype to wild-type strains transformed with the same plasmid.

Interaction between *stf1* and *cdr1,2*: Since alleles of *stf1* were isolated as suppressors of *cdr1-76 cdc25-22* and *cdr2-96 cdc25-22* double mutants, the ability of *stf1-1* to suppress defects in the *cdr* mutants was assessed (Table 7).

stf1-1 cdr1-76 and *stf1-1 cdr2-96* double mutants divided at somewhat smaller cell size than *cdr1-76* and *cdr2-96*, respectively, at 35°. When tested on minimal media lacking a nitrogen source *stf1-1* partially restored the wild type response to nitrogen starvation of *cdr2-96*.

Interaction between *stf1* and *cdc13*, *suc1* and *bws1* (*dis2*): No strong interactions were noted between *stf1-1* and mutant alleles of *suc1* and *cdc13*, however the terminal phenotype of *stf1-1 cdc13-117* was somewhat shorter than *cdc13-117* at 35° (Table 8).

TABLE 7

Interactions between *stf1* and *cdr1,2*

Strain	Mean cell length (in μm) 25°	35°
cdr1-76	16.1 ± 0.2	17.8 ± 0.3[a]
cdr1-76 stf1-1	16.3 ± 0.2	15.4 ± 0.2[a]
cdr2-40	14.8 ± 0.3	15.8 ± 0.4
cdr2-40 stf1-1	14.8 ± 0.3	16.2 ± 0.4
cdr2-96	16.8 ± 0.3	17.2 ± 0.3[b]
cdr2-96 stf1-1	16.1 ± 0.3	15.7 ± 0.2[b]

[a,b] Significantly different ($P < 0.005$).

To test whether *stf1-1* suppression of *cdc25-22* was reversed by $bws1^+$ overexpression, a *stf1-1 cd25-22 leu1-32* strain was transformed with pbws1-1. When tested at 35°, this strain was phenotypically cdc^-, indicating that overexpressed $bws1^+$ also reverses *stf1-1* suppression of *cdc25-22*. *stf1* is not allelic to *bws1*, however. No linkage could be detected between a $URA4^+$ marked *bws1* strain and *stf1* in a $cdc25^-$ genetic background.

DISCUSSION

Nature of the *stf1* phenotype: Mutant alleles of a new mitotic control gene, *stf1*, have been identified. These mutations can overcome the requirement for *cdc25* in mitotically cycling cells, suppressing several temperature-sensitive alleles and a *cdc25-disruption* strain at temperatures up to 35°. At 37°, $stf1^-$ strains exhibit a temperature-sensitive microcolony (growth) phenotype when $cdc25^+$ activity is not present. This effect is clearly due to *stf1-1* since it occurs in a *cdc25-disruption* strain (in which $cdc25^+$ activity is absent at all temperatures), while a *cdc2-3w cdc25-disruption* strain forms normal colonies at 37°. A similar effect is seen in diploids at 32°, where the $cdc25^-$ defect is more severe than in haploids. *stf1* may thus have a role in some aspect of growth in addition to its putative role in mitotic regulation. Other cell-cycle mutants have been described which exhibit pleiotropic phenotypes, including *suc1-disruption* and *nda3-K311* ($nda3^+$ encodes B-tubulin) of *S. pombe* and the *bimG11* ($bimG^+$ encodes a putative type 1 phosphatase) of *Aspergillus nidulans* (HAYLES, AVES and NURSE 1986; HIRAOKA, TODA and YANAGIDA 1984; DOONAN and MORRIS 1989). In fact, *cdc25* mutations exhibit a dual phenotype: as a cdc^- and as an allosuppressor, hypothesized to affect protein synthesis in some fashion (NURSE and THURIAUX 1984).

At 32°, diploids homozygous or heterozygous for $stf1^-$ were clearly rescued for the $cdc25^-$ first cycle arrest phenotype. At 30°, $stf1^-/+$ heterozygotes were generally intermediate in cell size between $stf1^-/stf1^-$ and +/+ homozygotes (when tested in a *cdc25-22/cdc25-22* genetic background). With respect to first

TABLE 8

Interactions between *stf1* and *cdc13*, *suc1*

Strain	Mean cell length (in μm) 25°	35°
cdc13-117	15.9 ± 0.3	cdc⁻
cdc13-117 stf1-1	15.8 ± 0.3	cdc⁻
suc1-210	16.3 ± 0.3	15.6 ± 0.4
suc-210 stf1-1	16.1 ± 0.3	15.9 ± 0.2

cycle cdc⁻ arrest and cell size (at the lower temperature), *stf1* mutations are incompletely dominant. Thus, *stf1⁻* alleles may encode for constitutively activated, altered function proteins. This is consistent with the relative frequency of *stf1* mutants obtained in the reversion analyses (3/816 revertants tested) being similar to the frequency of dominant alleles of *cdc2* obtained in a reversion analysis of *cdc25-22* alone (2/708; FANTES 1981). The frequency of these mutations is many times less than the frequency of presumed loss of function alleles of *wee1* (809/816). Alternatively, a genetic model accounting for the apparent incomplete dominance could be constructed based on partial loss of function (the rarity of *stf1⁻* mutations might suggest complete loss of function was lethal) and dosage dependence, however, no data is presently available to test this hypothesis.

Mechanism of *cdc25* suppression: In mitotically cycling *S. pombe* cells and cells germinated from spores, *cdc25* function is necessary to enter mitosis. At present, the biochemical action of *cdc25⁺* is unknown. It has been hypothesized that *cdc25* may act indirectly in the dephosphorylation of *cdc2*. Certainly, loss of phosphorylation of *cdc2* on both tyrosine and threonine accompanies entry into mitosis, and at the *cdc25* arrest point, *cdc2* is in a highly phosphorylated state (GOULD and NURSE 1989). *cdc2.Phe15*, a mutant in which *cdc2* can no longer be phosphorylated on tyrosine, does not require *cdc25⁺* activity (GOULD and NURSE 1989). This is consistent with *cdc25* normally functioning in the dephosphorylation of *cdc2* on tyrosine.

It is unclear, based solely on genetic evidence, how mutations in *stf1* bypass the requirement for *cdc25*, although it is very tempting to speculate that it is closely involved in the phosphorylation/dephosphorylation cycle of *cdc2* regulation. *stf1* does not regulate *cdc25⁺* activity since the *cdc25* disruption is rescued. *stf1* may transmit the *cdc25* signal to *cdc2*, *ie. stf1-1,2,3* being activated alleles acting downstream of *cdc25*. However, such mutants, if fully activated, might be expected to exhibit a wee phenotype, while *stf1⁻* mutants are indistinguishable from wild-type cells in size. Also, *stf1-1* is still sensitive to increased levels of *cdc25⁺* and *stf1* and *cdc2-3w* are clearly additive in the suppression of *cdc25-22*. Nevertheless, these data are consistent with *stf1⁻* being a partially activated allele of an element acting between *cdc25* and *cdc2*.

Reduction of *wee1* activity, through loss of function mutations or an increase in *cdr1* expression [*cdr1* is thought to negatively regulate *wee1* action (RUSSELL and NURSE 1987b, H. FEILOTTER, P. NURSE and P. G. YOUNG, personal communication)], is also sufficient to overcome loss of *cdc25⁺*. While *wee1* is believed to act through *cdc2*, its precise mode of action has yet to be determined (RUSSELL and NURSE 1987a). In some fashion, *wee1⁺* activity delays mitosis, possibly by inhibiting the assembly of the *cdc2*/cyclin complex, since *cdc2⁺* overexpression relieves the *cdr1⁻ cdc25-22* lethal interaction (H. FEILOTTER, P. NURSE and P. G. YOUNG, personal communication). While *wee1⁺* shows strong sequence similarity to known protein kinases, no evidence has yet been presented that indicates *wee1* activity affects the phosphorylation state of *cdc2*. Thus, the mechanism of *wee1⁻* suppression of *cdc25⁻* is not known.

stf1 does not appear to negatively regulate *wee1* activity. While *wee1-50* is believed to be a complete loss of function allele at 35° (RUSSELL and NURSE 1987a), *stf1-1* and *wee1-50* have an additive effect in rescuing *cdc25-22* at 35°. *stf1* probably does not function downstream of *wee1*. *stf1⁻* shows strong synergy with *cdc2-1w* in the rescue of *cdc25-22*, and *stf1-1* cells are still sensitive to overexpression of *wee1⁺*. Also, *stf1* mutants are phenotypically indistinguishable from wild type in size yet downregulation of the *wee1* inhibitory pathway sufficient to rescue defects in *cdc25* appears to result in a wee phenotype. Non-wee alleles of *wee1* able to suppress defects in *cdc25* have not been characterized, despite extensive screens (FANTES 1981; this work); in addition, *cdc2-1w*, a phenotypically wee allele that is believed to be related to *wee1* function, is only a weak suppressor of *cdc25ts* and does not suppress the *cdc25* disruption (RUSSELL and NURSE 1987a).

A number of different models could be proposed regarding the action of *stf1*. On balance, we favor one in which *stf1* functions downstream of *cdc25* or bypasses the requirements for *cdc25* via an alternative pathway acting upon *cdc2*. We are presently undertaking the cloning and molecular characterization of *stf1* to define its molecular properties.

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LITERATURE CITED

BEACH, D., and P. NURSE, 1981 High frequency transformation of the fission yeast *Schizosaccharomyces pombe*. Nature **290:** 140–142.

BOOHER, R., and D. BEACH, 1987 Interaction between *cdc13*$^+$ and *cdc2*$^+$ in the control of mitosis in fission yeast; dissociation of the G_1 and G_2 roles of the *cdc2* protein kinase. EMBO J. **6:** 3441–3447.

BOOHER, R., and D. BEACH, 1988 Involvement of *cdc13*$^+$ in mitotic control in *Schizosaccharomyces pombe*: possible interaction of the gene product with microtubules. EMBO J. **7:** 2321–2327.

BOOHER, R., and D. BEACH, 1989 Involvement of a type 1 phosphatase encoded by *bws1*$^+$ in fission yeast mitotic control. Cell **57:** 1009–1016.

BOOHER, R., C. ALPHA, J. HYAMS and D. BEACH, 1989 The fission yeast *cdc2/cdc13/suc1* protein kinase: regulation of catalytic activity and nuclear localization. Cell **58:** 485–497.

BRIZUELA, L., G. DRAETTA and D. BEACH, 1987 p13^{suc1} acts in the fission yeast cell division cycle as a component of the p34^{cdc2} protein kinase. EMBO J. **6:** 3507–3514.

CREANOR, J., and J. M. MITCHISON, 1982 Patterns of protein during the cell cycle of the fission yeast *Schizosaccharomyces pombe*. J. Cell Sci. **58:** 263–285.

DOONAN, J. H., and N. R. MORRIS, 1989 The bimG gene of *Aspergillus nidulans*, required for completion of anaphase, encodes a homolog of mammalian phosphoprotein phosphatase 1. Cell **57:** 987–996.

DRAETTA, G., F. LUCA, J. WESTENDORF, L. BRIZUELA, J. RUDERMAN and D. BEACH, 1989 *cdc2* protein kinase is complexed with both cyclin A and B: evidence for proteolytic inactivation of MPF. Cell **56:** 829–838.

DUCOMMUN, B., G. DRAETTA, P. G. YOUNG and D. BEACH, 1990 Fission yeast *cdc25* is a cell-cycle regulated protein. Biochem. Biophys. Res. Commun. **167:** 301–309.

EVANS, T., E. ROSENTHAL, J. YOUNGBLOOM, D. DISTEL and T. HUNT, 1983 Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cell division. Cell **33:** 389–396.

FANTES, P. A., 1979 Epistatic gene interactions in the control of division in fission yeast. Nature **279:** 428–430.

FANTES, P. A., 1981 Isolation of cell size mutants of a fission yeast by a new selective method: characterization of mutants and implications for division control mechanisms. J. Bacteriol. **146:** 746–754.

GOEBL, M., and B. BYERS, 1988 Cyclin in fission yeast. Cell **54:** 738–740.

GOULD, K. L., and P. NURSE, 1989 Tyrosine phosphorylation of the fission yeast *cdc2*$^+$ protein kinase regulates entry into mitosis. Nature **342:** 39–45.

GUTZ, H., H. HESLOT, U. LEUPOLD and N. LOPRIENO, 1974 *Schizosaccharomyces pombe*, pp. 395–446 in *Handbook of Genetics 1*, edited by R. C. KING. Plenum Press, New York.

HAYLES, J., S. AVES and P. NURSE, 1986 *suc1* is an essential gene involved in both the cell cycle and growth in fission yeast. EMBO J. **5:** 3373–3379.

HAYLES, J., D. BEACH, D. DURKACZ and P. NURSE, 1986 The fission yeast cell cycle control gene *cdc2*: isolation of a sequence *suc1* that suppresses *cdc2* mutant function. Mol. Gen. Genet. **202:** 291–293.

HINDLEY, J., and G. PHEAR, 1984 Sequence of the cell division gene *cdc2* from *Schizosaccharomyces pombe*; patterns of splicing and homology to protein kinases. Gene **31:** 129–134.

HIRAOKA, Y., T. TODA and M. YANAGIDA, 1984 The *nda3* gene of fission yeast encodes B-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. Cell **39:** 349–358.

LEUPOLD, U., 1958 Die Vererbung von Homothallie und Heterothallie bei *Schizosaccharomyces pombe*. C. R. Lab. Carlsberg Ser. Physiol. **24:** 381–480.

MITCHISON, J. M., 1970 Physiological and cytological methods for *Schizosaccharomyces pombe*. Methods Cell Physiol. **4:** 131–165.

MOLZ, L., R. BOOHER, P. G. YOUNG and D. BEACH, 1989 *cdc2* and the regulation of mitosis: six interacting *mcs* genes. Genetics **122:** 773–782.

MORENO, S., P. NURSE and RUSSELL, 1990 Regulation of mitosis by cyclic accumulation of p80^{cdc25} mitotic inducer in fission yeast. Nature **344:** 549–552.

NASMYTH, K., and P. NURSE, 1981 Cell division cycle mutants altered in DNA replication and mitosis in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **182:** 119–124.

NURSE, P., 1975 Genetic control of cell size at cell division in yeast. Nature **256:** 547–551.

NURSE, P., 1990 Universal control mechanism regulating onset of M-phase. Nature **344:** 503–508.

NURSE, P., and Y. BISSETT, 1981 Gene required in G_1 for commitment to cell cycle and in G_2 for control of mitosis in fission yeast. Nature **292:** 558–560.

NURSE, P., and P. THURIAUX, 1980 Regulatory genes controlling mitosis in the fission yeast *Schizosaccharomyces pombe*. Genetics **96:** 627–637.

NURSE, P., and P. THURIAUX, 1984 Temperature sensitive allosuppressor mutants of the fission yeast *Schizosaccharomyces pombe* influence cell cycle control over mitosis. Mol. Gen. Genet. **196:** 332–338.

NURSE, P., P. THURIAUX and K. NASMYTH, 1976 Genetic control of the cell division cycle in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **146:** 167–178.

OGDEN, J., and P. FANTES, 1986 Isolation of a novel type of mutation in the mitotic control of *Schizosaccharomyces pombe* whose phenotypic expression is dependent on the genetic background and nutritional environment. Curr. Genet **10:** 509–514.

OHKURA, H., N. KINOSHITA, S. MIYATANI, T. TODA and M. YANAGIDA, 1989 The fission yeast *dis2*$^+$ gene required for chromosome disjoining encodes one of two putative type 1 protein phosphatases. Cell **57:** 997–1007.

RUSSELL, P., and P. NURSE, 1986 *cdc25*$^+$ functions as an inducer in the mitotic control of fission yeast. Cell **45:** 145–153.

RUSSELL, P., and P. NURSE, 1987a Negative regulation of mitosis by *wee1*$^+$, a gene encoding a protein kinase homolog. Cell **49:** 559–567.

RUSSELL, P., and P. NURSE, 1987b The mitotic inducer *nim1* functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. Cell **49:** 569–576.

SIMANIS, V., and P. NURSE, 1986 The cell cycle control gene *cdc2* of fission yeast encodes a protein kinase potentially regulated by phosphorylation. Cell **45:** 261–268.

SOLOMON, M., R. BOOHER, M. KIRSCHNER and D. BEACH, 1988 Cyclin in fission yeast. Cell **54:** 738–740.

THURIAUX, P., P. NURSE and B. CARTER, 1978 Mutants altered in the control coordinating cell division with cell growth in the fission yeast *Schizosaccharomyces pombe*. Mol. Gen. Genet. **161:** 215–220.

YOUNG, P. G., and P. A. FANTES, 1984 Changed division response mutants function as allosuppressors, p. 221–228 in *Growth, Cancer, and the Cell Cycle*, edited by P. SKEHAN and S. J. FREIDMAN, Humana Press, Clifton, N.J.

YOUNG, P. G., and P. A. FANTES, 1987 *Schizosaccharomyces pombe* mutants affected in their division response to starvation. J. Cell Sci. **88:** 295–304.

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