The EMBO Journal vol.13 no.24 pp.6099–6106, 1994

A role for Hsp90 in cell cycle control: Wee1 tyrosine kinase activity requires interaction with Hsp90

Rosa Aligue, Haleh Akhavan-Niak and Paul Russell[1]

Department of Molecular Biology, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, USA

[1]Corresponding author

Communicated by T.Hunter

Wee1 protein kinase regulates the length of G_2 phase by carrying out the inhibitory tyrosyl phosphorylation of Cdc2–cyclin B kinase. Mutations were isolated that suppressed the G_2 cell cycle arrest caused by overproduction of Wee1. One class of *swo* (*s*uppressor of *w*ee1 *o*verproduction) mutation, exemplified by *swo1-26*, also caused a temperature sensitive lethal phenotype in a *wee1*$^+$ background. The *swo1*$^+$ gene encodes a member of the Hsp90 family of stress proteins. Swo1 is essential for viability at all temperatures. Swo1 co-immunoprecipitates with Wee1, showing that the two proteins interact. The *swo1-26* mutant undergoes premature mitosis when grown at a semi-permissive temperature. These data strongly indicate that formation of active Wee1 tyrosine kinase requires interaction with Swo1, perhaps in a manner analogous to the previously demonstrated interaction between Hsp90 and v-*src* tyrosine kinase. These observations demonstrate a unexpected role for Hsp90 in cell cycle control.

Key words: cell cycle/heat shock protein/Hsp90/mitosis/Wee1

Introduction

Studies of the fission yeast *Schizosaccharomyces pombe* have uncovered many of the key elements that regulate the initiation of mitosis in eukaryotic organisms. Central to this control mechanism is the Cdc2–cyclin B protein kinase, which is directly responsible for activating events of mitosis (reviewed by Nurse, 1990; Murray, 1993; Dunphy, 1994). Cyclin B is destroyed at the end of each cycle, thus preparation for the next M phase involves the synthesis of new cyclin B and its association with Cdc2, the catalytic subunit of the protein kinase. The Cdc2–cyclin B kinase is maintained in a repressed state during interphase due to phosphorylation of the Cdc2 subunit on Tyr15 (Gould and Nurse, 1989). In fission yeast, the major Tyr15 kinase activity is provided by an ~107 kDa Wee1 protein kinase (Russell and Nurse, 1987b; Lundgren *et al.*, 1991; Parker *et al.*, 1992; McGowan and Russell, 1993), whereas the predominant activity that dephosphorylates Tyr15 is provided by Cdc25 protein phosphatase (Russell and Nurse, 1986; Dunphy and Kumagai, 1991; Gautier *et al.*, 1991; Millar *et al.*, 1991; Lee *et al.*, 1992). In *S.pombe*, the timing of mitosis is largely determined by the relative activities of Cdc25 phosphatase and Wee1 kinase.

A major aim of current investigations is to understand how the activities of Wee1 and Cdc25 are regulated. These investigations have identified two mechanisms regulating Wee1. The Nim1 kinase is the key component of one of these control processes. The gene encoding Nim1 was first identified as a high copy suppressor of *cdc25-22*, a temperature sensitive mutation of the gene encoding the Tyr15 phosphatase (Russell and Nurse, 1987a). Overexpression of *nim1*$^+$ caused premature initiation of mitosis, forcing cells to divide at half the size of the wild type cells. Genetic studies indicated that Nim1 probably acted as a negative regulator of Wee1, an idea consistent with the observation that *wee1*$^-$ mutations also suppressed *cdc25-22*. Subsequent biochemical experiments proved that Nim1 inactivated Wee1 via direct phosphorylation of the C-terminal catalytic domain (Coleman *et al.*, 1993; Parker *et al.*, 1993; Wu and Russell, 1993). A second mechanism that potentially regulates Wee1 was uncovered by adding purified *S.pombe* Wee1 kinase to lysates made from *Xenopus laevis* eggs (Tang *et al.*, 1993). It was noted that Wee1 became inactive in these lysates due to phosphorylations occurring in the N-terminal domain. M-phase lysates were most potent at inactivating Wee1. Recent studies have shown that endogenous Wee1 is negatively regulated by phosphorylation during mitosis in human cells (C.McGowan and P.Russell, submitted). These studies suggest that inhibition of Wee1 activity, perhaps via an indirect mechanism requiring Cdc2–cyclin B, might play an important role in promoting the G_2/M transition.

In this study we have identified a process involved in the positive regulation of Wee1. A genetic screen was designed that exploited the fact that overexpression of Wee1 causes cell cycle arrest in G_2 phase. We reasoned that recessive, extragenic suppressors of this phenotype would include genes that play important roles in the production of active Wee1 kinase. We report here that one such gene is *swo1*$^+$, which encodes an Hsp90 homolog. Our data strongly indicate that Swo1 is required for the formation of active Wee1 tyrosine kinase.

Results

Genetic screen for swo mutants

Plasmid pWAU-50 has a copy of the temperature sensitive *wee1-50* allele under the transcriptional control of the powerful and constitutive *adh1*$^+$ promoter (Russell and Nurse, 1987b). Strain VG2, having an integrated copy of pWAU-50, has a wee phenotype at 35°C, but undergoes cell cycle arrest when incubated at 25°C. The strategy of the *swo* (*s*uppression of *w*ee1 *o*verexpression) genetic screen was to select for mutations that suppressed the cdc

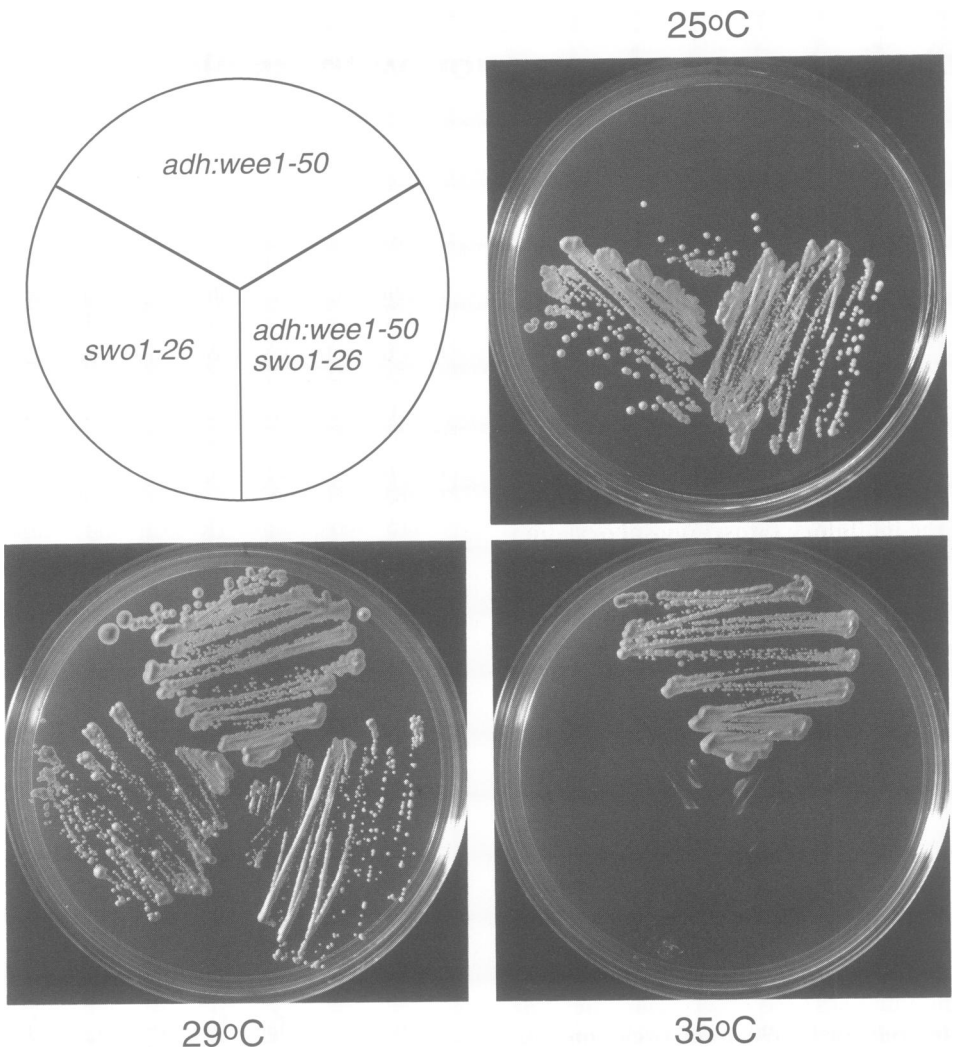

Fig. 1. The *swo1-26* mutation suppresses cell cycle arrest caused by overexpression of *wee1-50*. Cells of the genotypes *adh1:wee1-50 swo1*$^+$ (top), *adh1:wee1-50 swo1-26* (bottom right) and *wee1*$^+$ *swo1-26* (bottom left) were streaked on YES plates and incubated at 25, 29 and 35°C as indicated. The *adh1:wee1-50 swo1*$^+$ cells undergo cdc arrest at 25°C, this phenotype is suppressed by *swo1-26*. The *swo1-26* mutation imparts a temperature sensitive phenotype at 35°C.

arrest phenotype caused by overexpression of *wee1-50* in cells grown at 25°C. Approximately 10^7 VG2 cells were plated onto YES medium and incubated at 25°C. Approximately 500 colonies appeared, of which six were clearly inviable when incubated at 35°C. Mapping studies indicated that these mutations resided in two linkage groups: *swo1* (four alleles) and *swo2* (two alleles). The *swo1* and *swo2* mutations caused distinct phenotypes when outcrossed into a wild type (i.e. *wee1*$^+$) background. The *swo1* mutants exhibited a temperature sensitive lethal phenotype, resulting in the cessation of division when incubated at 35°C in liquid medium (Figures 1 and 2). In solid medium the phenotype was somewhat different, in that cells became swollen and eventually lysed. This might be due to different osmotic properties of liquid and solid medium. In contrast to *swo1*^{ts} mutations, *swo2* mutations did not cause lethality in a *wee1*$^+$ background. Instead, *swo2* mutants exhibited a wee phenotype at both 25 and 35°C, typical of *wee1*$^-$ loss-of-function mutations and dominant, activating mutations of *cdc2* such as *cdc2-1w* and *cdc2-3w* (Nurse, 1975; Thuriaux *et al.*, 1978). Genetic linkage experiments established that *swo2* mutations were located at the *cdc2* locus. Indeed, DNA sequence analysis revealed that the *swo2-2* mutation changed codon 67 from TGT encoding cysteine to TTT encoding phenylalanine. This mutation has been renamed *cdc2-4w* (MacNeill and Nurse, 1993). The same mutation has also been isolated as a extragenic suppressor of *cdc25-22* (S.A.MacNeill, P.Russell and P.Nurse, unpublished data). Interestingly, previous studies have shown that the *cdc2-3w* mutation changes codon 67 to TAT encoding tyrosine (Carr *et al.*, 1989). Earlier studies have shown that *cdc2-3w wee1-50* cells undergo mitotic catastrophe at 35°C (Russell and Nurse, 1987b) and the same is true for *cdc2-4w wee1-50* (MacNeill and Nurse, 1993).

The swo1$^+$ *gene encodes an Hsp90 homolog*

Genetic experiments demonstrated that the *swo1*^{ts} mutations were recessive and unlinked to *cdc2*$^+$, *cdc25*$^+$ or *cdc13*$^+$. Efforts were made to clone *swo1*$^+$ by rescue of *swo1-26* with an *S.pombe* genomic DNA library made in the plasmid pDW232. One of the plasmids which

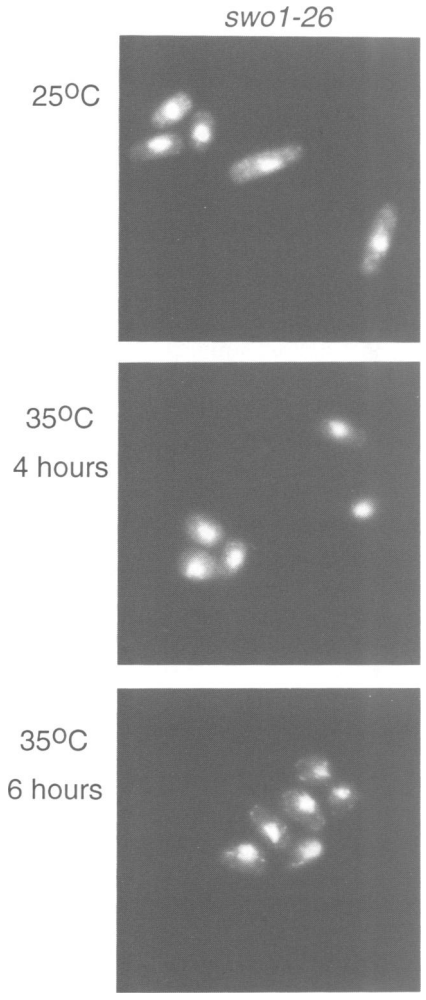

Fig. 2. Phenotype of *swo1-26* cells incubated at 35°C. Upon a temperature shift from 25°C to 35°C, *swo1-26* cells grown in liquid medium cease division and become ovoid.

rescued *swo1-26*, pDW232-48, contained an ~7.5 kb insert (Figure 3). A plasmid integration experiment demonstrated that pDW232-48 integrated at the *swo1*$^+$ locus. Transformation with various subclones indicated that the *swo1*$^+$ gene was located inside a 3.2 kb *Bam*HI−*Pst*I fragment (Figure 3). DNA sequence analysis of this region revealed an ~2.1 kb open reading frame encoding a 704 amino acid protein with a predicted molecular weight of ~81 kDa. (Figure 4). Homology searches revealed that Swo1 protein was highly homologous to proteins of the Hsp90 family (Lindquist and Craig, 1988). Swo1 was ~71% identical to *Saccharomyces cerevisiae* Hsp82 and ~60% identical to the human *HSP90* gene product (Borkovich *et al.*, 1989; Soeda *et al.*, 1989; Figure 5). Interestingly, previous studies have indicated that Hsp90 plays a positive role in the formation of active v-*src* tyrosine kinase, probably via direct interaction (Brugge, 1986; Xu and Lindquist, 1993). This suggested that Swo1 might have a similar role in the production of active Wee1 tyrosine kinase.

Swo1 is essential for growth at all temperatures

Hsp90 proteins were first identified as abundant cytosolic proteins that further increase in abundance in response to heat shock or growth at elevated temperatures (Lindquist

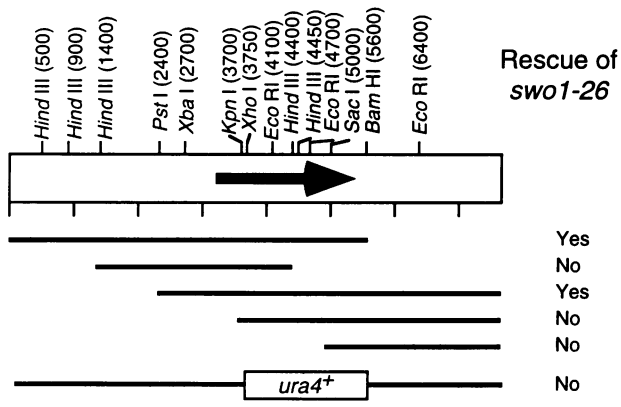

Fig. 3. Map and subclone analysis of ~7.5 kb genomic DNA fragment containing *swo1*$^+$. The arrow indicates the position of the *swo1*$^+$ open reading frame and the direction of transcription. Map of the *swo1::ura4*$^+$ construct used for gene disruption is also indicated.

```
CCA CCC CAA CCT CTA TCG CAA TCC AAA TAC CAA CCT TCG TCG ATC CGC ACT ATT CTT TGA   60
GTT CCA TGT CCT TCC ACC AAA AAC CAG GGT TAG GGT TTT GGA ATG TTC GAG TTG CTT TGT  120
TTA CAG AAT ATA AAC CAC CAT ACA CGT ACC CTC GCC ATC TAG CTC TCA AGT TAT TTA TAA  180
CCC ATT TCC CAC TAA GTT CCT TTG TTG AAC GCT TAA AAG ATT GTT CAT ACG TTT GAA CAT  240
ATT TAA AAT CAC GTT ATA GCT GAG AAG AAA ACC CCT TTT CAA GTA ATA AAA GTA TAT AAT  300
TAC TTA TTT TAC CCT TTA TAT TAC GAA CTG TAC GAC TTA ACC GGT TTT ATC TAT AAA TTC  360
CTT CAT TTT TCT TTG TTT TCG TTT TCA ATT TGA TTT CAT TAC AAA ATG TCG AAC ACA GAA  420
                                                M  S  N  T  E                    5
ACT TTC AAG TTT GAC TGG GAA ATC AGC CAG TTG ATG TCT TTA ATC ATC AAC ACT GTT TAT  480
 T  F  K  F  D  W  E  I  S  Q  L  M  S  L  I  I  N  T  V  Y                     25
TCT AAC AAG GAA ATT TTC CTT CGT GAG CTT ATT TCC AAC GCT TCC GAT GCC TTG GAC AAA  540
 S  N  K  E  I  F  L  R  E  L  I  S  N  A  S  D  A  L  D  K                     45
ATT CGT TAT CAA AGC CTT TCC GAT CCT CAT GCC TTA GAC GCT GAG AAG GAT CTT TTC ATT  600
 I  R  Y  Q  S  L  S  D  P  H  A  L  D  A  E  K  D  L  F  I                     65
CGC ATT ACT CCT GAC AAG GAG AAC AAA ATC CTT ACG ATT CGC GAT ACC GGT ATT GGT ATG  660
 R  I  T  P  D  K  E  N  K  I  L  T  I  R  D  T  G  I  G  M                     85
ACC AAG AAT GAC CTT ATC AAC AAC CTT GGT GTT ATC GCC AAG TCT GGT ACT AAG CAG TTT  720
 T  K  N  D  L  I  N  N  L  G  V  I  A  K  S  G  T  K  Q  F                    105
ATG GAA GCT GCT GCT TCT GGT GCT GAT ATC TCC ATG ATT GGT CAA TTC GGT GTT GGT TTC  780
 M  E  A  A  A  S  G  A  D  I  S  M  I  G  Q  F  G  V  G  F                    125
TAC TCT GCA TAC TTG GTT GCC GAC AAG GTC CAA GTC GTA AGT AAG CAT AAT GAC GAT GAG  840
 Y  S  A  Y  L  V  A  D  K  V  Q  V  V  S  K  H  N  D  D  E                    145
CAA TAC ATC TGG GAA TCC TCT GCT GGT GGT AGC TTC ACC GTC ACT TTG GAC ACC GAT GGA  900
 Q  Y  I  W  E  S  S  A  G  G  S  F  T  V  T  L  D  T  D  G                    165
CCT CGC TTG TTA CGT GGT ACC GAG ATT CGT CTC TTC ATG AAA GAA GAC CAA CTT CAA TAC  960
 P  R  L  L  R  G  T  E  I  R  L  F  M  K  E  D  Q  L  Q  Y                    185
CTC GAG GAA AAG ACT ATC AAA GAT ACC GTC AAG AAA CAT AGT GAG TTC ATC TCT TAC CCC 1020
 L  E  E  K  T  I  K  D  T  V  K  K  H  S  E  F  I  S  Y  P                    205
ATT CAA TTA GTT GTT ACC CGT GAG GTT GAG AAG GAG GTC CCT GAA GAA GAA GAG ACC GAA 1080
 I  Q  L  V  V  T  R  E  V  E  K  E  V  P  E  E  E  E  T  E                    225
GAA GTT AAG AAT GAA GAA GAC GAT AAG GCT CCT AAG ATT GAG GAG GTT GAT GAT GAA TCT 1140
 E  V  K  N  E  E  D  D  K  A  P  K  I  E  E  V  D  D  E  S                    245
GAG AAG AAG GAG AAG AAG ACC AAG AAG GTC AAG GAG ACC ACC ACT GAG ACT GAG GAG TTG 1200
 E  K  K  E  K  K  T  K  K  V  K  E  T  T  T  E  T  E  E  L                    265
AAC AAG ACC AAG CCC ATT TGG ACT CGC AAC CCC AGT GAA GTT ACC AAG GAA GAG TAT GCC 1260
 N  K  T  K  P  I  W  T  R  N  P  S  E  V  T  K  E  E  Y  A                    285
TCT TTC TAC AAG TCT TTG ACT AAC GAC TGG GAA GAC CAT TTG GCC GTC AAG CAC TTC AGC 1320
 S  F  Y  K  S  L  T  N  D  W  E  D  H  L  A  V  K  H  F  S                    305
GTT GAA GGT CAA TTG GAA TTC CGT GCC ATT CTT TTC GTC CCC CGC CGC GCT CCT ATG GAC 1380
 V  E  G  Q  L  E  F  R  A  I  L  F  V  P  R  R  A  P  M  D                    325
CTT TTT GAG GCC AAG CGT AAG AAG AAC AAC ATC AAG CTC TAT GTT CGC CGT GTT TTC ATT 1440
 L  F  E  A  K  R  K  K  N  N  I  K  L  Y  V  R  R  V  F  I                    345
ACC GAC GAC TGT GAA GAG TTG ATT CCT GAA TGG TTG GGT TTC ATT AAG GGT GTT GAT GAT 1500
 T  D  D  C  E  E  L  I  P  E  W  L  G  F  I  K  G  V  V  D                    365
TCT GAG GAC TTG CCC TTG AAC TTG TCT CGT GAG ATG CTT CAA CAA AAC AAG ATC ATG AAG 1560
 S  E  D  L  P  L  N  L  S  R  E  M  L  Q  Q  N  K  I  M  K                    385
GTT ATT CGT AAG AAC CTT GTC CGT CGT TGT CTT GAT ATG TTC AAC GAG ATT GCC GAG GAC 1620
 V  I  R  K  N  L  V  R  R  C  L  D  M  F  N  E  I  A  E  D                    405
AAG GAG AAC TTC AAG ACT TTC TAC GAT GCT TTC AGC AAG AAT TTG AAG CTT GGT ATC CAT 1680
 K  E  N  F  K  T  F  Y  D  A  F  S  K  N  L  K  L  G  I  H                    425
GAG GAT GCT GCT AAC CGC CCT GCT TTA GCC AAG CTT TTG CGT TAC AAC TCC CTT AAC TCC 1740
 E  D  A  A  N  R  P  A  L  A  K  L  L  R  Y  N  S  L  N  S                    445
CCT GAT GAT CTT ATC AGT CTT GAG GAC TAC ATC ACT AAG ATG CCC GAA CAT CAA AAG AAC 1800
 P  D  D  L  I  S  L  E  D  Y  I  T  K  M  P  E  H  Q  K  N                    465
ATT TAT TTC ATC ACT GGT GAA TCT AAG CAA GCC GTT GAG AAC TCT CCC TTC CTT GAA ATC 1860
 I  Y  F  I  T  G  E  S  K  Q  A  V  E  N  S  P  F  L  E  I                    485
TTC CGT GCC AAG AAG TTT GAT GTT CTC TTC ATG GTT GAC CCC ATT GAT GAG TAC GCT GTC 1920
 F  R  A  K  K  F  D  V  L  F  M  V  D  P  I  D  E  Y  A  V                    505
ACT CAA CTC AAG GAA TTC GAG GGC AAG AAA TTG GTT AAC ATT ACT AAG GAT GGT CTT GAG 1980
 T  Q  L  K  E  F  E  G  K  K  L  V  N  I  T  K  D  G  L  E                    525
TTG GAA GAG ACT GAC GAG GAG AAG GCT GCT CGC GAG AAG CTC GAA AAA GAG TAT GAG GAG 2040
 L  E  E  T  D  E  E  K  A  A  R  E  K  L  E  K  E  Y  E  E                    545
TTT GCT AAG CAA CTT AAG ACC ATT TTG GGT GAC AAG GTT GAA AAG GTT GTT GTT TCT AAC 2100
 F  A  K  Q  L  K  T  I  L  G  D  K  V  E  K  V  V  V  S  N                    565
AAG ATT GTT GGT TCT CCT TGC TTG TTA ACT ACC GGT CAA TAT GGT TGG TCT GCT AAC ATG 2160
 K  I  V  G  S  P  C  L  L  T  T  G  Q  Y  G  W  S  A  N  M                    585
GAA CGT ATC ATG AAG CTC AAG CCC TCT CGT GAT ACT TCT ATG AGT GCC TAT ATG TCT AGC 2220
 E  R  I  M  K  L  K  P  S  R  D  T  S  M  S  A  Y  M  S  S                    605
CGC AAG ACT TTT GAG ATT AAC CCC AAG TCT CCC ATC ATT GCT GAG CTC AAG AAG AAG GTT 2280
 R  K  T  F  E  I  N  P  K  S  P  I  I  A  E  L  K  K  K  V                    625
GAG GAG AAT GGT GCC GAA GAC CGC TCT GTG AAG GAT CTT GCT ACT ATT TTG TAT GAG ACC 2340
 E  E  N  G  A  E  D  R  S  V  K  D  L  A  T  I  L  Y  E  T                    645
GCC TTG TTG TCC TCT GGT TTC ACT CTT CAT GAC CCT AGT GCT TAT GCT CAA CGT ATC AAC 2400
 A  L  L  S  S  G  F  T  L  H  D  P  S  A  Y  A  Q  R  I  N                    665
CGG CTT ATT TCT CTT GGT CTT AGC ATT GAC GAA GAG GAG GAG GCT CCT ATT GAG GAA ATT 2460
 R  L  I  S  L  G  L  S  I  D  E  E  E  E  E  A  P  I  E  E  I                  685
TCT ACC GAA TCC GTC GCT GCT GAA AAC AAT GCT GAG AGC AAG ATG GAG GAA GTC GAT TAA 2520
 S  T  E  S  V  A  A  E  N  N  A  E  S  K  M  E  E  V  D  *                     704
ATC AAA TGC ATC TAA                                                            2535
```

Fig. 4. DNA sequence of *swo1*$^+$. The *swo1*$^+$ open reading frame encodes a 704 amino acid protein having a predicted molecular weight of ~81 kDa. The GenBank accession number of this sequence is L35550.

```
MPEETQTQDQ PMEEEEVETF AFQAEIAQLM SLIINTFYSN KEIFLRELIS     50
MA-------- ------GETF EFQAEITQLM SLIINTVYSN KEIFLRELIS     36
MS-------- -----NTETF KFDWEISQLM SLIINTVYSN KEIFLRELIS     37

NSSDALDKIR YESLTDPSKL DSGKELHINL IPNKQDRTLT IVDTGIGMTK    100
NASDALDKIR YQALSDPKQL ETEPDLFIRI TPKPEEKVLE IRDSGIGMTK     86
NASDALDKIR YQSLSDPHAL DAEKDLFIRI TPDKENKILT IRDTGIGMTK     87

ADLINNLGTI AKSGTKAFME ALQAGADISM IGQFGVGFYS AYLVAEKVTV    150
AELINNLGTI AKSGTKAFME ALSAGADVSM IGQFGVGFYS LFLVADRVQV    136
NDLINNLGVI AKSGTKQFME AAASGADISM IGQFGVGFYS AYLVADKVQV    137

ITKHNDDEQY AWESSAGGSF TVRTDTGEP- MGRGTKVILH LKEDQTEYLE    199
ISKNNEDEQY IWESNAGGSF TVTLDEVNER IGRGTVLRLF LKDDQLEYLE    186
VSKHNDDEQY IWESSAGGSF TVTLDTDGPR LLRGTEIRLF MKEDQLQYLE    187

ERRIKEIVKK HSQFIGYPIT LFVEKERDKE VSDDEAEEKE DKEEEKEKEE    249
EKRIKEVIKR HSEFVAYPIQ LLVTKEVEKE VPIPEEEKKD --EEKKDEDD    234
EKTIKDTVKK HSEFISYPIQ LVVTREVEKE VPEEEE--TE --EVKNEEDD    233

KESEDKPEIE DVGSDEEEEK KDGDKKKKKK -IKEKYIDQE ELNKTKPIWT    298
K----KPKLE EV--DEEEEE ----KKPKTK KVKEEVQELE ELNKTKPLWT    274
K----APKIE EV--DDESEK ----KEKKTK KVKETTTETE ELNKTKPIWT    273

RNPDDITNEE YGEFYKSLTN DWEDHLAVKH FSVEGQLEFR ALLFVPRRAP    348
RNPSDITQEE YNAFYKSISN DWEDPLYVKH FSVEGQLEFR AILFIPKRAP    324
RNPSEVTKEE YASFYKSLTN DWEDHLAVKH FSVEGQLEFR AILFVPRRAP    323

FDLFENRKKK NNIKLYVRRV FIMDNCEELI PEYLNFIRGV VDSEDLPLNI    398
FDLFESKKKK NNIKLYVRRV FITDEAEDLI PEWLSFVKGV VDSEDLPLNL    374
MDLFEAKRKK NNIKLYVRRV FITDDCEELI PEWLGFIKGV VDSEDLPLNL    373

SREMLQQSKI LKVIRKNLVK KCLELFTELA EDKENYKKFY EQFSKNIKLG    448
SREMLQQNKI MKVIRKNIVK KLIEAFNEIA EDSEQFDKFY SAFAKNIKLG    424
SREMLQQNKI MKVIRKNLVR RCLDMFNEIA EDKENFKTFY DAFSKNLKLG    423

IHEDSQNRKK LSELLRYYTS ASGDEMVSLK DYCTRMKENQ KHIYYITGET    498
VHEDTQNRAA LAKLLRYNST KSVDELTSLT DYVTRMPEHQ KNIYYITGES    474
IHEDAANRPA LAKLLRYNSL NSPDDLISLE DYITKMPEHQ KNIYFITGES    473

KDQVANSAFV ERLRKHGLEV IYMIEPIDEY CVQQLKEFEG KTLVSVTKEG    548
LKAVEKSPFL DALKAKNFEV LFLTDPIDEY AFTQLKEFEG KTLVDITKD-    523
KQAVENSPFL EIFRAKKFDV LFMVDPIDEY AVTQLKEFEG KKLVNITKDG    523

LELPEDEEEK KKQEEKKTKF ENLCKIMKDI LEKKVEKVVV SNRLVTSPCC    598
FELEETDEEK AEREKEIKEY EPLTKALKDI LGDQVEKVVV SYKLLDAPAA    573
LELEETDEEK AAREKLEKEY EEFAKQLKTI LGDKVEKVVV SNKIVGSPCL    573

IVTSTYGWTA NMERIMKAQA LRDNSTMGYM AAKKHLEINP DHSIIETLRQ    648
IRTGQFGWSA NMERIMKAQA LRDSSMSSYM SSKKTFEISP KSPIIIETKK    623
LTTGQYGWSA NMERIMKLKP SRDTSMSAYM SSRKTFEINP KSPIIAELKK    623

K-AEADKNDK SVKDLVILLY ETALLSSGFS LEDPQTHANR IYRMIKLGLG    697
RVDEGGAQDK TVKDLTNLLF ETALLTSGFS LEEPTSFASR INRLISLGLN    673
KVEENGAEDR SVKDLATILY ETALLSSGFT LHDPSAYAQR INRLISLGLS    673

IDEDDPT--- ADDTSAAVTE EMPP---LEG DDDTSRMEEV D            732
IDEDEETETA PEASTEAPVE EVP------- --ADTEMEEV D            705
IDEEEE---- ------APIE EISTESVAAE NNAESKMEEV D            704
```

Fig. 5. Swo1 is highly homologous to Hsp82 proteins. Swo1 (bottom) is aligned with a human HSP90 protein (top) and *S.cerevisiae* Hsp82 (middle). Identities between all three proteins are boxed. The *Schizosaccharomyces* and *Saccharomyces* proteins are ~71% identical, whereas the *Schizosaccharomyces* and human proteins are ~60% identical. EMBL accession numbers are X15183 for the human gene encoding HSP90 and M26044 for the *S.cerevisiae* gene encoding Hsp82.

Fig. 6. DNA sequence homology in the promoter regions of *swo1*$^+$ and a *Drosophila* hsp70 gene. Sequences corresponding to the heat shock consensus sequence GAANNTTC (nucleotides 99–111 in Figure 4) are indicated.

and Craig, 1988). Studies of the budding yeast *S.cerevisiae* have shown that Hsp90 function is essential for growth at all temperatures, but strains that are partially defective for Hsp90 function are particularly sensitive to growth at elevated temperatures (Borkovich *et al.*, 1989). Since Swo1 is an Hsp90 homolog, it was important to establish whether the temperature sensitive property of *swo1-26* cells was due to intrinsic conditional loss of function of *swo1-26* encoded protein at higher temperatures, as opposed to an increased requirement for Swo1 protein at elevated temperatures. To distinguish between these possibilities, one copy of *swo1*$^+$ was replaced with *swo1::ura4*$^+$ in a diploid strain (Figure 3). Random spore analysis showed that all of the viable haploid cells derived from this diploid were uracil auxotrophs, indicating that the *swo1::ura4*$^+$ mutation caused lethality. This was also true when spores were germinated at ~21°C.

Expression of swo1$^+$ *is increased in response to temperature elevation*

Examination of the DNA sequences upstream of *swo1*$^+$ open reading frame revealed potentially significant homology to the promoters of genes whose transcription is increased in response to temperature elevation (Pelham, 1982). One of the best studied examples is the *Drosophila* hsp70 gene (Amin *et al.*, 1988). This gene contains the heat shock consensus sequence NGAAN, beginning 26 nucleotides upstream of the TATA box. The crucial feature of the heat shock consensus sequence is multiple GAA blocks arranged in alternating orientations that are separated by two nucleotide intervals. As shown in Figure 6, the *swo1*$^+$ promoter region contains a perfect version of the heat shock consensus sequence beginning 28 nucleotides upstream of a TATA box. This homology suggested that *swo1*$^+$ expression was likely to be increased in response to temperature elevation.

In order to measure the level of Swo1 protein expression, we constructed a strain in which the genomic copy of *swo1*$^+$ was tagged at the C-terminus with the ha epitope (see Materials and methods). As shown in Figure 7, Swo1^{ha} was detected by immunoblotting as a ~90 kDa protein. Confirmation that this protein was indeed Swo1 was obtained using strains having a plasmid in which expression of an epitope-tagged copy of *swo1*$^+$ was under the control of the thiamine-repressible *nmt1* promoter. In cells grown under derepressing conditions, Swo1^{ha} was detected as a prominent ~90 kDa protein (Figure 7A). Swo1^{ha} was not detectable when this strain was grown in repressing medium.

The strain having the epitope-tagged genomic copy of *swo1*$^+$ was grown at 25°C and then shifted to 35°C. Samples were taken at regular intervals and analyzed by immunoblotting (Figure 7B). This analysis showed that the Swo1^{ha} protein signal increased continuously during the 2.0 h time course of the experiment. Overall there was an ~4-fold increase in Swo1^{ha} signal.

The swo1-26 mutation suppresses cell cycle arrest caused by overexpression of wee1$^+$

Having found that *swo1*$^+$ encodes an Hsp90 homolog, which might have some ability to function as a molecular chaperone (Jakob and Buchner, 1994), we tested the possibility that *swo1-26* would suppress cell cycle arrest

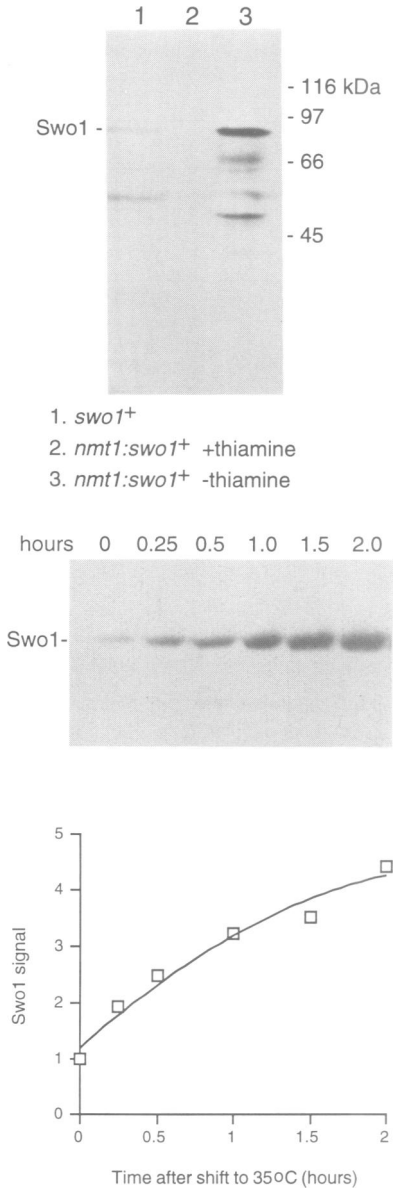

Fig. 7. The level of Swo1 protein is elevated at higher temperatures. (**A**) Extracts from strains expressing versions of Swo1 having the ha epitope at the C-terminus were subjected to SDS−PAGE and immunoblotting using an α-ha antibody (monoclonal 12CA5). Lane 1, cells having a single integrated copy of *swo1-ha*; lane 2, cells transformed with a plasmid having *nmt1:swo1-ha* and grown in repressing conditions (+thiamine); lane 3, cells transformed with a plasmid having *nmt1:swo1-ha* and grown in derepressing conditions (−thiamine). $Swo1^{ha}$ is detected as a ~90 kDa protein in lanes 1 and 3. (**B**) Cells having a single copy of swo1-ha were and grown in YES medium at 25°C were shifted to 37°C. Samples were taken for 2.5 h following the shift and subjected to SDS−PAGE and immunoblotting using an α-ha antibody. The level of Swo1 protein rose steadily during the 2.5 h time course.

caused by overexpression of wild type Wee1 protein. To address this question, *swo1-26* was crossed into a strain having an integrated copy of pREPWEE1(Enoch *et al.*, 1993). This plasmid has a copy of $wee1^+$ under the control of the thiamine-repressible *nmt1* promoter. In a strain background that is otherwise wild type, cells having $nmt1{:}wee1^+$ appear normal in medium containing thiamine but undergo cell cycle arrest when cultured in medium lacking thiamine. As shown in Figure 8, this level of $wee1^+$ overexpression is not lethal in a *swo1-26* background. This observation indicates that Swo1 protein function is required for normal activity of both $wee1^+$ and *wee1-50* gene products.

Partial loss of Swo1 activity advances mitosis in a wild type background

Finding that *swo1-26* suppressed the cell cycle arrest caused by the overproduction of wild type Wee1 indicated that Swo1 has a role in delaying the onset of mitosis in wild type cells. To investigate this possibility, the size at which *swo1-26* and wild type cells underwent mitosis and septation was measured under several different conditions. Grown in standard liquid YES medium at 25°C, the $swo1^+$ and *swo1-26* cells underwent division at nearly the same size (14.0 ± μm and 13.2 ± 0.9 μm, respectively; Table I). At 29°C the *swo1-26* cells grew well but had a semi-wee phenotype, dividing at 9.8 ± 0.8 μm compared with 14.1 ± 0.5 μm for $swo1^+$ cells. These data indicate that partial loss of Swo1 activity advances the onset of mitosis, consistent with a model in which Swo1 is required for Wee1 activity.

In vivo physical interactions involving Wee1 and Swo1

Having evidence indicating that Swo1 is required for the formation of active Wee1, we next inquired whether this function involved close physical interactions between the two proteins. Lysates from three strains transformed with a plasmid having an epitope-tagged version of $swo1^+$ expressed from the *nmt1* promoter were incubated with α-Wee1 antibodies. One strain was VG2 (*adh1:wee1-50*), the second had a copy of $wee1^+$ expressed from the *nmt1* promoter ($nmt1{:}wee1^+$), and the third was a wild type strain. The immunoprecipitations were carried out under non-denaturing conditions in order to preserve inter-molecular protein interactions. The antibodies and precipitating proteins were then recovered using protein A, electrophoresed by SDS−PAGE and then immunoblotted using α-ha antibodies to detect $Swo1^{ha}$. As shown in Figure 9, $Swo1^{ha}$ was readily detected in immunoprecipitates from cells that were co-overexpressing Wee1 and Swo1 (lanes 4 and 6). $Swo1^{ha}$ was not detected in cells that did not overexpress Wee1, consistent with the extreme paucity of Wee1 protein in wild type cells.

Wee1 protein is unstable in swo1-26 mutant cells

Our studies suggested that Swo1 might be required for the formation of active Wee1 protein. To investigate this, immunoblots of lysates from $adh1{:}wee1\text{-}50\ swo1^+$ and *adh1:wee1-50 swo1-26* cells were probed with α-Wee1 antibodies (Figure 10). The level of Wee1 protein was reduced ~5- to 10-fold in *swo1-26* compared with $swo1^+$ cells. This suggests that Wee1 protein is abnormally unstable in *swo1-26* cells, which could account in part for the rescue of *wee1-50* and $wee1^+$ overexpression by *swo1-26*. Longer exposures of this immunblot showed that the level of Wee1 protein in the *adh1:wee1-50 swo1-26* cells was still much higher than in wild type cells (data not shown), consistent with estimations of a 50- to 100-fold overproduction of Wee1 in *adh1:wee1-50* cells.

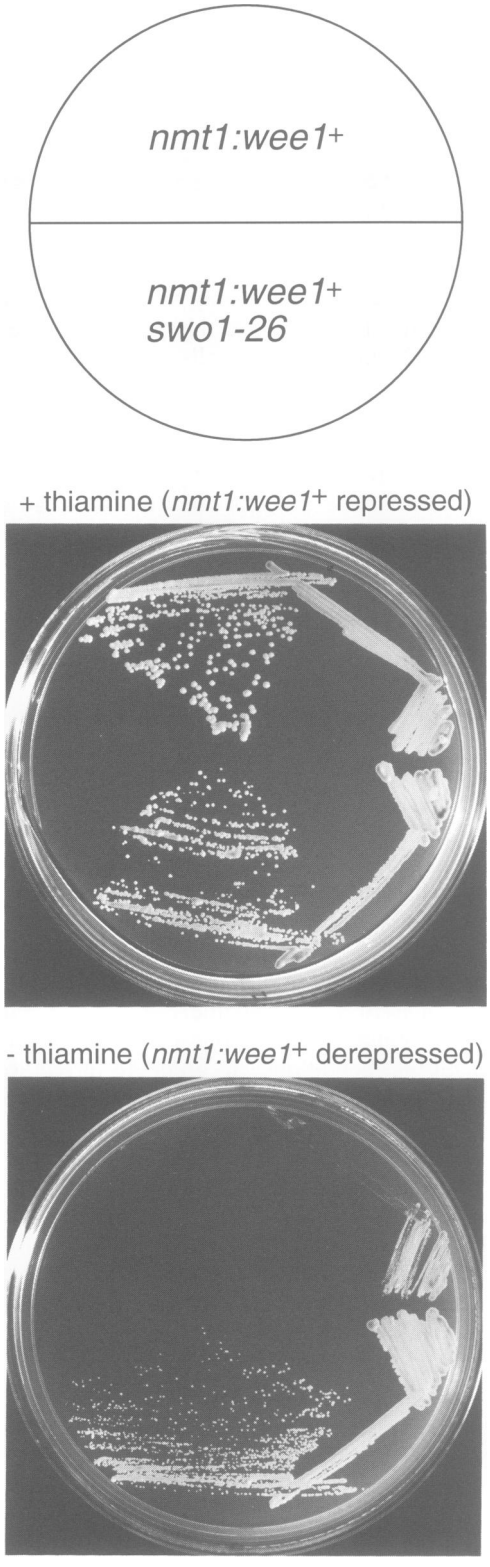

Fig. 8. The *swo1-26* mutation suppresses cell cycle arrest caused by overexpression of *wee1*$^+$. Cells of the genotypes *nmt1:wee1*$^+$ *swo1*$^+$ (top) and *nmt1:wee1*$^+$ *swo1-26* were streaked on EMM plates with or without thiamine as indicated and then incubated at 25°C. In medium lacking thiamine, *nmt1:wee1*$^+$ *swo1*$^+$ underwent cell cycle arrest due to overproduction of Wee1; this phenotype was suppressed by *swo1-26*.

Table I. Cells having the *swo1-26* mutation undergo cell division at a reduced cell size when grown at intermediate temperatures in YES medium

	Size of cell (μm)	
	25°C	29°C
swo1$^+$	14.0 ± 0.5	14.1 ± 0.5
swo1-26	13.2 ± 0.9	9.8 ± 0.8

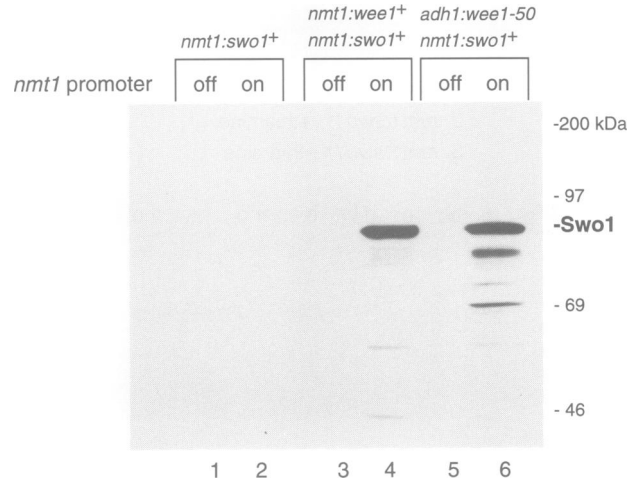

Fig. 9. Co-immunoprecipitation of Swo1 and Wee1 proteins. Cells having the genotype *wee1*$^+$, *nmt1:wee1*$^+$ or *adh1:wee1-50* were transformed with a plasmid having *nmt1:swo1-ha* and grown under conditions in which the *nmt1* promoter is repressed (off) or derepressed (on). Wee1 protein was isolated by immunoprecipitation using α-Wee1 antibody. Immunocomplexes were subjected to SDS–PAGE and immunoblotting using an α-ha antibody. Swo1^{ha} was detected in samples in which Wee1 protein was overproduced (lanes 4 and 6).

Discussion

The key conclusion to emerge from these studies is that the formation of active Wee1 protein kinase requires an interaction with Swo1, an Hsp90 homolog. This conclusion is supported by several different types of evidence. (i) Wild type Swo1 activity is required for the cell cycle arrest phenotype caused by overexpression of both *wee1-50* and *wee1*$^+$. (ii) The *swo1-26* mutation causes a semi-wee phenotype in a *wee1*$^+$ background, indicative of advancement of mitosis that is most probably due to partial loss of Wee1 activity. (iii) The abundance of Wee1 protein in a strain that overexpresses *wee1-50* is greatly reduced in a *swo1-26* background. (iv) An *in vivo* interaction involving Wee1 and Swo1 is detected by co-immunoprecipitation. These findings are most consistent with the conclusion that the production of active Wee1 requires a physical interaction with Swo1.

This is not the only evidence indicating that Hsp90 proteins are required for formation of active protein tyrosine kinases. The first suggestion of this phenomena came from the observation that newly synthesized cytoplasmic forms of v-*src* tyrosine kinase are found in a protein complex that includes Hsp90 (Brugge, 1986). This form of v-*src* tyrosine kinase has low activity compared with that found in the plasma membrane which has

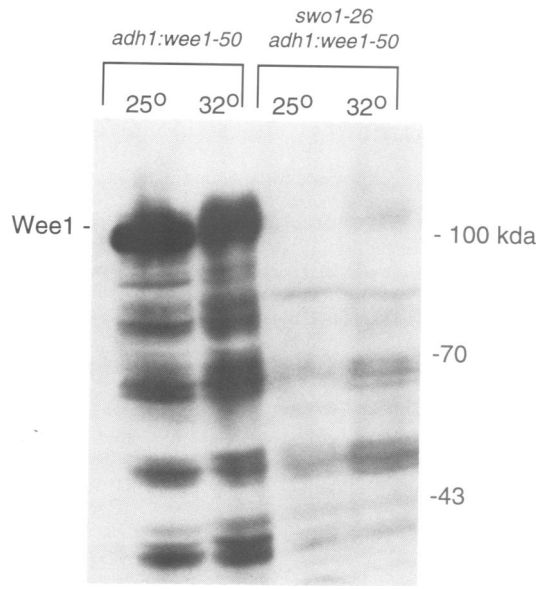

Fig. 10. Wee1 protein levels are greatly reduced in *swo1-26* cells. Lysates from *swo1*$^{+}$ *adh:wee1-50* and *swo1-26 adh:wee1-50* cells grown at 25°C or 32°C were subjected to SDS−PAGE and immunoblotting with α-Wee1 antibody. Full-length (~107 kDa) and presumptive breakdown products of Wee1 are readily detected only in the *swo1*$^{+}$ strain background.

disassociated from Hsp90. It was more recently shown that mutations that reduce Hsp90 levels in *S.cerevisiae* relieve the toxicity caused by high levels of v-*src* expression (Xu and Lindquist, 1993). Reduced Hsp90 levels also reduced the level of v-*src* protein as detected by immunoblotting. These observations strongly suggested that the interaction between pp60$^{v\text{-}src}$ and Hsp90 is part of the normal process by which newly translated v-*src* protein product is converted to an active tyrosine kinase. More recently, a mutation of a *Drosophila* Hsp90 gene was discovered as an enhancer of *sevenless*, which encodes a receptor tyrosine kinase (Cutforth and Rubin, 1994). The mechanism by which partial loss of Hsp90 activity enhance *sevenless* mutations is unknown, but it is reasonable to speculate that it involves a direct interaction between Hsp90 and Sevenless.

It is important to understand that most protein tyrosine kinases do not appear to require interactions with Hsp90. In the *S.cerevisiae* studies mentioned above, it was also shown that the activity of pp160$^{v\text{-}abl}$ and pp60$^{c\text{-}src}$ tyrosine kinases were unaffected by reduction of Hsp90 levels (Xu and Lindquist, 1993). Thus, although Swo1 must have essential functions that are unconnected to Wee1, because Swo1 is essential for viability, whereas Wee1 is not, it is unlikely that Swo1 is generally required for the activity of a large number of protein kinases in *S.pombe*. At present there is no obvious common denominator that discriminates between tyrosine kinases that interact with Hsp90 and those that do not. In fact, Wee1 is extremely different from the v-*src* and *sevenless* kinases, having closer sequence homology to serine/threonine kinases than to tyrosine kinases. Wee1 is also predominantly a nuclear protein (R.Aligue and P.Russell, manuscript in preparation), whereas v-*src* kinase is attached to the plasma membrane and *sevenless* gene product is a transmembrane receptor tyrosine kinase.

The *in vivo* cellular functions of Hsp90 proteins are beginning to be understood. At the most basic level, Hsp90 proteins appear to be essential for viability at all temperatures. In *S.cerevisiae*, two genes encode Hsp90 isotypes. Disruption of either gene compromised the ability to grow at elevated temperatures, whereas simultaneous disruption of both genes was lethal (Borkovich *et al.*, 1989). The data presented here show that a single Hsp90-encoding gene is essential for viability in *S.pombe*. This conclusion is supported both by a gene disruption experiment and the conditional lethal phenotype of the *swo1*^{ts} mutants. Recent studies have suggested that Hsp90 possibly acts as ATP-independent molecular chaperone involved in protein folding (Jakob and Buchner, 1994). Roughly stoichiometric amounts of Hsp90 had a moderate ability to prevent the aggregation of unfolded citrate synthetase during refolding (Wiech *et al.*, 1992). Similar data were obtained in refolding studies involving MyoD (Shaknovich *et al.*, 1992).

Hsp90 is a very abundant cytosolic protein in eukaryotic cells. *In vivo* it has been found to interact with several proteins, including calmodulin, actin, tubulin, several kinases and steroid receptors (Lindquist and Craig, 1988). Although in most cases the physiological significance of these interactions remains to be determined, there is convincing evidence that Hsp90 plays a crucial role in the function of steroid receptors (Bohen and Yamamoto, 1993). Steroid receptors function to regulate transcription in response to extracellular signals, they do this by shuttling between the cytoplasm and the nucleus. Hsp90 appears to bind newly synthesized receptors and thereby makes them competent to bind steroids. Steroid binding leads to disassociation of Hsp90 and receptor dimerization. Thus, Hsp90 is physically associated with inactive forms of the receptor, but it is required for receptor activation.

The shuttling of steroid receptors between the nucleus and cytoplasm suggests a possible explanation for a paradox involving Wee1. In human cells, immunofluorescence studies have shown that Wee1 is predominantly a nuclear protein, whereas its substrate, Cdc2−cyclin B, is predominantly cytoplasmic (C.McGowan and P.Russell, submitted). Recent studies have shown that the single *WEE1* gene that has been discovered in humans accounts for >90% of the Cdc2−cyclin B directed tyrosine kinase activity that can be measured in HeLa cell lysates (C.McGowan and P.Russell, submitted). Thus the paradox of the different intracellular localizations of Wee1 and Cdc2−cyclin B probably cannot be explained by suggesting the existence of other Wee1-like enzymes. The most likely explanation involves nuclear/cytosolic shuttling of either Wee1 or Cdc2−cyclin B. Preliminary studies have shown that Wee1 rapidly leaks from the nucleus during standard cell fractionation procedures, showing that Wee1 is not tethered in the nucleus (C.McGowan and P.Russell, submitted). This leaves open the possibility that Wee1 shuttles from the nucleus to phosphorylate Cdc2−cyclin B in the cytoplasm. One might further speculate that Wee1 could be subject to negative regulation while in the cytoplasm and require an interaction with Swo1 in order to be effectively transported back into the nucleus. In *S.pombe* the situation is a little different, in that Cdc2−cyclin B has been reported to be exclusively nuclear (Alfa *et al.*, 1989, 1990). It will be

important to determine whether Wee1 shuttles between the nucleus and cytoplasm in fission yeast, as this could be an important feature of the mitotic control process.

Materials and methods

General genetic and physiological methods

Schizsaccharomyces pombe strains used in this study were all isogenic derivatives of wild type 972 h^- (Mitchison, 1970). Unless indicated all were *leu1-32* and *ura4-D18*. YES and EMM2 media were used in growing *S.pombe* cells (Moreno *et al.*, 1991). Cell size measurements were performed using an eyepiece micrometer at 1500× magnification.

Screen for swo mutants

S.pombe genetic methods and media have been described (Moreno *et al.*, 1991). Strain VG2 [*adh1:wee1-50(ura4*$^+$) wee1-50 leu1-32 ura4-D18 h^-], having a copy of pWAU-50 integrated at the *wee1-50* locus, has also been previously described. Log phase VG2 cells grown at 35°C were plated on 85 mm YES plates at a density of ~10^5 cells per plate. The plates were incubated at 25°C for 7 days and then ~500 colonies were picked and patched onto YES plates and incubated at 25°C. Six colonies that were unable to grow at 35°C were identified by replica plating. The six mutant strains were crossed to a *leu1-32 ura4-D18* h^+ strain to generate both mating types of *swo* mutants in *wee1*$^+$ and *adh1:wee1-50(ura4*$^+$) wee1-50 backgrounds. Genetic linkage experiments established that the six mutants identified two linkage groups: *swo1* (alleles 12, 21, 25 and 26) and *swo2* (alleles 2 and 3). Alleles of *swo1* were found to cause a temperature sensitive lethal phenotype at 35°C, whereas *swo1* alleles caused a wee phenotype at all temperatures. Mutants *swo1-26* and *swo2-3* were crossed to *cdc2-33*, *cdc13-117* and *cdc25-22* strains. Wild type recombinants were obtained in all crosses except *swo2-3*×*cdc2-33*.

Plasmid and strain constructions

The *swo1* open reading frame was amplified by PCR from an *S.pombe* cDNA library (Fikes *et al.*, 1990), using the primers 5′-CAC*CATATG*T-CGAACACACAGAAACT incorporating an *Nde*I site (italicized) and 5′-TAT*GCGGCCGCC*ATCGACTTCCTCCATCTT incorporating a *Not*I site (italicized). PCR amplification generated a 2.2 kb DNA fragment that was cleaved with *Nde*I and *Not*I and cloned into a modified form of pREP1 (Maundrell, 1993). This form of pREP1 adds two copies of the ha epitope followed by six consecutive histidine residues to the C-terminus of proteins encoded by genes clones as *Nde*I−*Not*I fragments. The resulting plasmid, pREP1HaH:swo1, was transformed into *leu1-32* strains. The epitope-tagged version of swo1 was subsequently mobilized as a 2.3 kb *Nde*I−*Bam*HI fragment into a *ura4*$^+$ containing plasmid and then integration at the *swo1*$^+$ locus was directed by digesting the plasmid at the *Xho*I site located in the *swo1*$^+$ open reading frame. This generated a chromosomal copy of epitope-tagged *swo1*$^+$ whose expression was driven by the *swo1*$^+$ promoter.

Immunoprecipitation and immunoblot analysis

For immunoprecipitation, *adh:wee1-50 nmt:swo1*$^+$ cells were grown in selective medium to early log phase (OD_{600} = 0.5–0.6), collected by centrifugation and washed with ice-cold TBS. Cell pellets of 50 OD units of cells were resuspended in 1 ml of lysis buffer consisting of 1% Nonidet P-40, 50 mM NaF, 10 mM sodium pyrophosphate, 250 mM NaCl, 10% glycerol, 2 mM PMSF, aprotinin (5 μg/μl), pepstatin (5 μg/μl), leupeptin (5 mg/μl) and 50 mM Tris−HCl, pH 7.2. Soluble proteins were extracted by vortexing with glass beads for 5 min at 4°C. Lysates were clarified by centrifugation for 15 min at 14 000 *g*. Wee1 antibody was bound to protein A−Sepharose (PAS) by mixing 60 μl of α-Wee1 serum to 1 ml of 10% PAS in RIPA buffer (137 mM NaCl, 10% glycerol, 0.1% SDS, 0.5% Sodium deoxycholate, 1% Triton X-100, 2 mM EDTA, 20 mM Tris−HCl, pH 7.4) Cell lysates and α-wee1-PAS were mixed for 3 h at 4°C. After incubation, samples were washed three times with RIPA buffer. Immunoprecipitated proteins were extracted from the beads by solubilization in 2× SDS sample buffer (6% SDS, 10% glycerol, 4% mercaptoethanol and 125 mM Tris−HCl, pH 6.8) at 100°C for 5 min.

For immunoblotting, proteins were resolved on 5–15% SDS−polyacrylamide gel and transferred to nitrocellulose filters using a semi-dry blotting apparatus. The nitrocellulose was blocked in TBS buffer (20 mM Tris pH 8.0, 150 mM NaCl and 0.3% Tween) containing 5% dried milk and incubated with α-ha antibody (12CA5, 1:2000 dilution) or α-Wee1 affinity-purified antibody (1:500 dilution) in 3% dried milk TBS. After washing with TBS, the blot was incubated in HRP-conjugated goat α-mouse or α-rabbit antibody. Immunodetection was performed using an enhanced chemiluminescence (ECL) system (Amersham). Signals were quantitated using a Molecular Dynamics Densitometer.

Acknowledgements

Technical assistance was provided by Autumn Chapman, who sequenced *swo1*$^+$ and created the *swo1::ura4*$^+$ gene disruption. Ian Wilson and Gail Feiser kindly provided 12CA5 antibody. Avelino Bueno discovered that *swo2-3* was identical to *cdc2-4w*. Sergio Moreno provided pREPWEE1. Tony Carr provided the pDW232 library. We thank Ricardo Basco, Clare McGowan, Kazuhiro Shiozaki and Lin Wu for advice and support. R.Aligue was supported by fellowships from EMBO and MEC/Spain. This work was supported by the NIH.

References

Alfa,C.E., Booher,R., Beach,D. and Hyams,J.S. (1989) *J. Cell Sci.*, Suppl., **12**, 9–19.
Alfa,C.E., Ducommun,B., Beach,D. and Hyams,J.S. (1990) *Nature*, **347**, 680–682.
Amin,J., Ananthan,J. and Voellmy,R. (1988) *Mol. Cell. Biol.*, **8**, 3761–3769.
Bohen,S.P. and Yamamoto,K.R. (1993) *Proc. Natl Acad. Sci. USA*, **90**, 11424–11428.
Borkovich,K.A., Farrelly,F.W., Finkelstein,D.B., Taulien,J. and Lindquist,S. (1989) *Mol. Cell. Biol.*, **9**, 3919–3930.
Brugge,J.S. (1986) *Curr. Top. Microbiol. Immunol.*, **123**, 1–22.
Carr,A.M., MacNeill,S.A., Hayles,J. and Nurse,P. (1989) *Mol. Gen. Genet.*, **218**, 41–49.
Coleman,T.R., Tang,Z. and Dunphy,W.G. (1993) *Cell*, **72**, 919–929.
Cutforth,T. and Rubin,G.M. (1994) *Cell*, **77**, 1027–1036.
Dunphy,W.G. (1994) *Trends Cell Biol.*, **4**, 202–207.
Dunphy,W.G. and Kumagai,A. (1991) *Cell*, **67**, 189–196.
Enoch,T., Carr,A.M. and Nurse,P. (1993) *Genes Dev.*, **6**, 2035–2046.
Fikes,J.D., Becker,D.M., Winston,F. and Guarente,L. (1990) *Nature*, **346**, 291–294.
Gautier,J., Solomon,M.J., Booher,R.N., Bazan,J.F. and Kirschner,M.W. (1991) *Cell*, **67**, 197–211.
Gould,K.L. and Nurse,P. (1989) *Nature*, **342**, 39–45.
Jakob,U. and Buchner,J. (1994) *Trends Biochem. Sci.*, **19**, 205–211.
Lee,M.S., Ogg,S., Xu,M., Parker,L.L., Donoghue,D.J., Maller,J.L. and Piwnica-Worms,H. (1992) *Mol. Biol. Cell*, **3**, 73–84.
Lindquist,S. and Craig,E.A. (1988) *Annu. Rev. Genet.*, **22**, 631–677.
Lundgren,K., Walworth,N., Booher,R., Dembski,M., Kirschner,M. and Beach,D. (1991) *Cell*, **64**, 1111–1122.
MacNeill,S.A. and Nurse,P. (1993) *Mol. Gen. Genet.*, **236**, 415–426.
McGowan,C.H. and Russell,P. (1993) *EMBO J.*, **12**, 75–85.
Maundrell,K. (1993) *Gene*, **123**, 127–130.
Millar,J.B.A., McGowan,C.H., Lenaers,G., Jones,R. and Russell,P. (1991) *EMBO J.*, **10**, 4301–4309.
Mitchison,J.M. (1970) *Methods Cell Physiol.*, **4**, 131–146.
Moreno,S., Klar,A. and Nurse,P. (1991) *Methods Enzymol.*, **194**, 795–823.
Murray,A.W. (1993) *Curr. Biol.*, **3**, 291–293.
Nurse,P. (1975) *Nature*, **256**, 547–551.
Nurse,P. (1990) *Nature*, **344**, 503–508.
Parker,L.L., Atherton-Fessler,S. and Piwnica-Worms,H. (1992) *Proc. Natl Acad. Sci. USA*, **89**, 2917–2921.
Parker,L.L., Walter,S.A., Young,P.G. and Piwnica-Worms,H. (1993) *Nature*, **363**, 736–738.
Pelham,H.R.B. (1982) *Trends Genet.*, **1**, 31–35.
Russell,P. and Nurse,P. (1986) *Cell*, **45**, 145–153.
Russell,P. and Nurse,P. (1987a) *Cell*, **49**, 569–576.
Russell,P. and Nurse,P. (1987b) *Cell*, **49**, 559–567.
Shaknovich,R., Shue,G. and Kohtz,D.S. (1992) *Mol. Cell. Biol.*, **12**, 5059–5068.
Soeda,E., Yokoyama,K., Yamazaki,M., Akaogi,K., Miwa,T. and Imai,T. (1989) *Nucleic Acids Res.*, **17**, 7108.
Tang,Z., Coleman,T.R. and Dunphy,W.G. (1993) *EMBO J.*, **12**, 3426–3427.
Thuriaux,P., Nurse,P. and Carter,B. (1978) *Mol. Gen. Genet.*, **161**, 215–220.
Wiech,H., Buchner,J., Zimmermann,R. and Jakob,U. (1992) *Nature*, **358**, 169–170.
Wu,L. and Russell,P. (1993) *Nature*, **363**, 738–741.
Xu,Y. and Lindquist,S. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 7074–7078.

Received on September 7, 1994; revised on October 13, 1994