Analysis of the *Schizosaccharomyces pombe* cyclin puc1: evidence for a role in cell cycle exit

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**Summary**

The *puc1* gene, encoding a G1-type cyclin from the fission yeast *Schizosaccharomyces pombe*, was originally isolated by complementation in the budding yeast *Saccharomyces cerevisiae*. Here, we report the molecular characterization of this gene and analyse its role in *S. pombe*. We fail to identify any function of this cyclin at the mitotic G1/S transition in *S. pombe*, but demonstrate that it does function in exit from the mitotic cycle. Expression of the *puc1* gene is increased during nitrogen starvation, and *puc1* affects the timing of sexual development in response to starvation. Overexpression of the *puc1* protein blocks sexual development, and rescues *pat1* cells, which would otherwise undergo a lethal meiosis. We conclude that *puc1* contributes to negative regulation of the timing of sexual development in fission yeast, and functions at the transition between cycling and non-cycling cells.

Key words: cell cycle, cyclin, meiosis, *puc1*, yeast

**Introduction**

Cyclin proteins were first identified in marine invertebrates because their levels varied through the cell cycle (Evans et al., 1983) and subsequently a large family of cyclin-sequence related proteins has been characterized in a variety of eukaryotic cell types (reviewed by Lew and Reed, 1992; Sherr, 1993; Xiong and Beach, 1991). This family comprises at least six separate classes: A, B, C, D, E and G1, which are distinguished by specific sequence characteristics although all share some homology in a 100 amino acid region known as the ‘cyclin box’ (reviewed by Lew and Reed, 1992; Xiong and Beach, 1991). The best characterized members of the family, the B-type cyclins, form a complex with the p34cdc2 protein kinase that acts at the G2/M transition (reviewed by Hunt, 1989; Maller, 1990; Nurse, 1990). Progression into mitosis in the eukaryotic cell cycle depends upon the activity of this complex, called MPF or maturation promoting factor. Periodic association with the mitotic B-cyclin is required for kinase activation; this is one of several levels of regulation affecting the activity of p34cdc2 throughout the cell cycle (reviewed by Niwa and Yanagida, 1988; Norbury and Nurse, 1992; Nurse, 1990; Reed, 1992), and this association has become a paradigm for the function of cyclin proteins. However, for some other members of the family, there is no evidence that their levels vary through the cell cycle, and a role in cell cycle progression has not been determined.

Genetic analysis in the budding yeast *Saccharomyces cerevisiae* and the fission yeast *Schizosaccharomyces pombe* is a powerful tool in studying these proteins. Comparisons between the two yeasts, which are evolutionarily highly diverged, have been helpful in identifying those components of cell cycle regulatory networks that are likely to be of general importance, and conserved in all eukaryotes (reviewed by Forsburg and Nurse, 1991a). Two major sequence classes of cyclins have been identified in the yeasts: the B cyclins, and G1 cyclins. It has been shown that the G2/M transition in *S. pombe* requires a single essential B-cyclin encoded by the *cdc13* gene (Booher and Beach, 1987, 1988; Hagan et al., 1988). The *cig2* gene encodes a non-essential B-cyclin with mitotic roles have also been identified in the budding yeast *S. cerevisiae* (Fitch et al., 1992; Ghiara et al., 1991; Surana et al., 1991), although in contrast to the fission yeast, there is substantial redundancy amongst them. *CLB5* and *CLB6*, also B-type cyclins, function in S phase (Epstein and Cross, 1992; Kuhne and Linder, 1993; Schwob and Nasmyth, 1993).

Commitment to the cell cycle in both *S. cerevisiae* and *S. pombe* occurs at START, near the G1/S transition (reviewed by Forsburg and Nurse, 1991a). Genetic analysis in both yeasts has implicated the p34cdc2 kinase in the regulation of the G1/S transition, regulating entry into the DNA replication phase of the cell cycle, as well as the G2/M transition. This G1/S function in higher cells appears to require, if not p34cdc2 itself, then a close relative (Blow and Nurse, 1990; D’Urso et al., 1990; Fang and Newport, 1991) and several cyclin sequence classes are hypothesized to act with the kinase at this first transition (Desai et al., 1992; Girard et al., 1991; Koff et al., 1992; Lees et al., 1992; Matsushime et al., 1991; Pagano et al., 1992; Roy et al., 1990; Sherr, 1993; Xiong et al., 1992). p34cdc2 activity at the G1/S transition in *S. cerevisiae* appears to be regulated by a cyclin/p34cdc2 complex (Cross and Blake, 1993;
Tyers et al., 1992, 1993; Wittenberg et al., 1990), in this case using one of a distinct class of so-called ‘G₁ cyclins’: the products of the CLN₁, CLN₂ and WHI₁ (also known as DAF₁ or CLN₃) genes (Cross, 1988; Hadwiger et al., 1989; Nash et al., 1988; Richardson et al., 1989; Sudbery et al., 1980). These G₁ cyclins are functionally redundant: deletion of all three causes a G₁ arrest, although the cells are viable with any one of the three intact (Cross, 1990; Richardson et al., 1989). Additional cyclins encoded by HCS₂₆ and CLG₁ have been identified in S. cerevisiae but their functions are still unknown (Matsumoto and Wickner, 1993; Ogas et al., 1991). In S. pombe, the non-essential B-type cyclin encoded by cig₁⁺ (Buñó et al., 1991) was reported to have a G₁ function and the mcs₂⁺ gene encodes an essential cyclin of a new sequence type (Molz and Beach, 1993). In an earlier report we described the initial characterization of the fission yeast cyclin puc₁⁺ (Forsburg and Nurse, 1991b), which is the closest relative of the distinct G₁-cyclin sequence class to be found outside of budding yeast.

The S. pombe puc₁⁺ gene was originally isolated as a cDNA that conferred alpha factor resistance to S. cerevisiae cells deficient in a single G₁ cyclin (WHI₁/CLN₃; Forsburg and Nurse, 1991b). In the absence of WHI₁/CLN₃, the cells are hypersensitive to the peptide mating pheromone alpha factor; their size is enlarged and they are delayed in G₁ (Cross, 1988; Nash et al., 1988; Tyers et al., 1993). Expression of the puc₁⁺ gene reverses all these phenotypes. The lethal arrest caused by deletion of CLN₁, CLN₂ and WHI₁/CLN₃ can also be efficiently rescued by expression of puc₁⁺ (Forsburg and Nurse, 1991b). The fission yeast cyclin puc₁ thus fulfils all the roles of a G₁ cyclin in the budding yeast. However, in our preliminary analysis, we found no evidence in fission yeast for a puc₁ function in the G₁ phase of the mitotic cell cycle. Rather than accelerating the cycle, overexpression of puc₁ was shown to delay cells with a G₂ DNA content, apparently by competing with the cdc₁₃ mitotic cyclin (Forsburg and Nurse, 1991b).

In this report we provide additional genetic and molecular characterization of the puc₁⁺ gene. The genomic copy of the puc₁⁺ gene has been isolated and disrupted in fission yeast, without effect on mitotic cell growth. The puc₁⁺ gene is expressed at a very low constitutive level in mitotic cells. We have combined the puc₁ deletion with mutations in other known cell cycle control genes, and found no evidence for synthetic interactions. In particular, we have combined a puc₁ deletion with a deletion of the B-type cyclin cig₁. Deletion of cig₁ was previously reported to cause G₁ delay (Buñó et al., 1991). There was no synthetic phenotype between Δcig₁ and Δpuc₁; moreover we find no G₁ or cell cycle phenotype associated with the single Δcig₁ mutation. We can therefore demonstrate no role for puc₁ in normal cell cycle progression.

We also present experiments suggesting that the fission yeast cyclin puc₁ functions in the exit from the mitotic cycle. Expression of the gene is strikingly induced when the cells are starved for nitrogen and exit the cycle. The structure of the puc₁⁺ message is similar to that of several genes required for sexual development, possibly defining a co-regulated family of genes. In the absence of puc₁, cells enter meiosis with accelerated timing. Overexpression of puc₁ blocks sexual development of haploids and diploids, and can suppress the lethal haploid meiosis entered by the puc₁⁺ mutant strain. These data all suggest that puc₁ has a role during exit from the cell cycle and entry into sexual development, in the transition from the cycling to the non-cycling state.

### MATERIALS AND METHODS

#### Strains, growth and media

Strains used are all congenic to the 972 hstrain of S. pombe. Growth conditions and strain manipulations were as described by Moreno et al. (1991). Transformation was carried out either by the lithium acetate method or by using a modified electroporation protocol derived from that described by Becker and Guarente (1991). Briefly, cells were grown in minimal medium to a cell density of \(5 \times 10^8\) particles/ml in ice-cold water, and resuspended in 1 M sorbitol at a concentration of \(1 \times 10^8\) cells/ml. DNA (<1 μg) was added and the cells were pulsed in a 0.2 cm cell in a Flowgen electroporator, with settings at 1500 V, 132 Ω and 40 μF. Cells were plated on selective medium.

Media were as described previously (Moreno et al., 1991) except where noted in the text.

#### Plasmids and DNA manipulations

DNA manipulations were carried out by standard protocols, using enzymes from Boehringer Mannheim and manufacturer’s recommended conditions.

The incomplete puc₁ cDNA clone, pSLF60, was subcloned for sequencing into pTZ18R, giving plasmid pSLF62. Plasmid pSLF71 containing the genomic clone of puc₁⁺ in pUR19 (Barbet et al., 1992) was subcloned using flanking PstI and BamHI sites into pTZ18R for sequencing to give pSLF75. pSLF76, with the smaller of two Δpuc₁::ura₄⁺ deletions, was constructed by ligating a 1.8 kb ura₄⁺ fragment flanked by EcoRI (blunted with Klenow) and SalI sites into pSLF75 cut with XhoI (blunted) and SalI. pSLF78, with the larger Δpuc₁::ura₄⁺ deletion, was derived from pSLF76 by first isolating the 6 kb SacI fragment spanning the ura₄⁺ gene and the upstream portion of the genomic clone, and ligating this into a SacI linker that was used to replace the entire upstream and coding region to the XbaI site at the 3’ end of the ORF. The fragments containing the gene disruptions were purified using sites in the flanking polylinker and transformed into yeast. The truncated allele puc₁ΔX88 was constructed by placing a NotI linker at the SalI site in the original puc₁⁺ clone and cloning this NotI fragment into pTZ18R and various expression vectors to give the following plasmids: UAS-ADH puc₁ΔX88 URA3 2μ (pSLF83), UAS-ADH puc₁ΔX88 TRPI 1ARS-CEN (pSLF81), Pmntl puc₁ΔX88 ars1 LEU2 (pSLF82) and Pmntl puc₁ΔX88 ars1 ura₄⁺ (pSLF95). The corresponding plasmids containing the full-length gene are (respectively) pSLF72, pSLF65, pSLF59 and pSLF86. Plasmid pSLF110 contains the puc₁ΔX88 construction under its own promoter in plasmid pUR18N (Barbet et al., 1992). The E. coli expression vector pSLF70 contains the puc₁⁺ cDNA, with sites modified as described below, cloned into the NclI and EcoRI sites of the vector pRK172 (Studier et al., 1990).

#### Oligo-directed mutagenesis

The internal Ndel site was deleted and a new site introduced at the methionine by using the Bio-Rad oligo-directed mutagenesis kit, and the following oligonucleotides: 5’-GTCATCCCCCATATGGTAG-3’ and 5’-TCATGGATCTGATGCTC-3’. The N-terminal truncation of puc₁⁺ was constructed into the genomic sequence environment using the oligonucleotide 5’-CTCACCTAAATACCCGTTCAT-ACTGGGTAGACAGGTATTTG-3’.
were grown at 30°C to early log phase (A600 = 0.2-0.5) in standard minimal medium, harvested, and washed 4 times in minimal medium without any nitrogen source. They were subsequently re-inoculated at the same density into minimal medium without nitrogen, and grown at 30°C. Timepoints were taken every 2 hours. The degree of starvation was assessed by monitoring the cells by eye and/or by flow cytometry. For glutamate timecourses, the cells were washed as described above and inoculated into minimal medium where the only nitrogen source was 1 g/l L-glutamate. The N-deficient media was supplemented with NH4Cl to the indicated concentration for the comparison study.

Hybridizations and probes
Southern hybridizations and colony screening using GeneScreen Plus were carried out as recommended by the manufacturer (DuPont). Probes for Southern blotting were prepared from the puc1 cDNA clone by random oligo priming with [32P]dATP using a Prime-It kit (Stratagene). RNA from elutriated cells was prepared by glass bead lysis in the presence of phenol, and was subsequently separated on a glyoxal gel (Maniatis et al., 1982). Other RNA gels were run in formaldehyde; transfer to GeneScreen Plus and probing was again according to manufacturers instructions. Where used, single-stranded formaldehyde; transfer to GeneScreen Plus and probing was again according to manufacturers instructions. Where used, single-stranded RNA was labelled by nick-translation with [32P]dATP using a Prime-It kit (Stratagene). Hybridizations and probes were carried out as recommended by the manufacturer (Dupont). Sequence determination was carried out using a Sequenase Kit (US Biochemicals) according to standard protocols. Fragments were subcloned into phagemids pTZ18R and pTZ19R (Pharmacia) and sequenced using priming in the presence of [32P]dATP, other NTPs and Klenow, cut with appropriate restriction enzymes and the labelled strand purified by denaturing acrylamide gel electrophoresis in 8 M urea.

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Antibody preparation
The puc1+ expression plasmid pSLF70 was transformed into E. coli strain BL21 DE3 (lysS). Cells were induced for 4 hours with 0.4 mM IPTG added at an A600 of 0.4. Cells were lysed in 50 mM Tris, pH 8.8, 1 mM EDTA, 50 mM NaCl, 1 mM DTT, 1 mM PMSF, 10% glycerol, and sonicated. Inclusion bodies were removed by centrifugation, washed in lysis buffer + 1% Triton X-100, and boiled and separated by SDS-PAGE; the puc1 band was cut out and crushed for the resulting crude fraction used to inoculate rabbits. Affinity purification of the antibody was carried out by blotting the bacterially produced protein and eluting into Tris glycine (Harlow and Lane, 1988).

Western blots
Crude yeast lysates were prepared essentially as described by Moreno et al. (1991) from 200 ml cultures grown to mid-exponential phase in HB15 buffer (60 mM B-glycerophosphate, 150 mM p-nitrophenyl phosphate, 25 mM MOPS, pH 7.2, 15 mM EGTA 15 mM MgCl2, 1 mM DTT, 0.1 mM Na- vanadate, 1 mM PMSF, 20 μg/ml leupeptin, 40 μg/ml aprotinin, 10 μg/ml pepstatin). For immunoprecipitations, each gel track contains the equivalent of 50 ml of starting culture. Additionally for immunoprecipitations, 5% dry milk was added to the buffer and samples were preclared with Protein A Sepharose beads before addition of antibody, incubated for 60 minutes, and collected with the addition of Protein A Sepharose beads (Pharmacia). Samples were washed six times with NP40 buffer (10 mM sodium phosphate, 1% nonidet P40, 100 mM NaCl, 0.2 mM EDTA, 50 mM sodium fluoride, 1 mM PMSF), and resuspended in 40 μl gel sample buffer. Proteins were separated on 12.5% gels and transferred to Immobilon P in 10 mM CAPS, pH 11, 10% MeOH. Detection, using anti-rabbit alkaline phosphatase antibody (Sigma) was essentially as described (Harlow and Lane, 1988).

Flow cytometry and cytology
Cells were fixed in ethanol and analysed using a Becton Dickinson FACScan as described (Sazer and Sherwood, 1990). Computer analysis was carried out using the program Lysis II. Fixed cells were rehydrated in PBS, stained with DAPI, and observed with an Axioskop microscope as described (Moreno et al., 1991).

Computer analysis
Analysis of gene structure was carried out using the GCG package and local programs on the Oxford University Molecular Biology Data Centre vax system.

RESULTS
Characterization of the puc1+ gene structure
The original isolate of the puc1+ gene was a cDNA in a S. cerevisiae expression vector (Forsburg and Nurse, 1991b). This lacked a poly-A sequence but contained an intact ORF. We screened the same cDNA library by colony hybridization to isolate additional puc1+ cDNAs, using the original cDNA as a probe. Only one candidate was found in approximately 40,000 clones screened; this poor representation in a cDNA bank suggested that the message is rare. The new cDNA encoded an incomplete gene; it contained a 5′ truncation relative to the original clone, which deleted the start codon and approximately 300 bp of sequence found in the original isolate, but did include an additional 85 bp and a poly-A sequence at the 3′ end. In the S. cerevisiae assay for alpha factor resistance, the new clone was non-functional (data not shown). We also screened a genomic library (kindly provided by A. M. Carr; Barbet et al., 1992) by hybridization and isolated several puc1+ clones. The sequence of the genomic clone and the surrounding region, as well as the site of the poly-A tail and the extent of both cDNA clones, are shown in Fig. 1. The puc1+ gene has no introns.

Approximately 1 kb of upstream DNA has been sequenced; this region is very A/T rich but there are no obvious regulatory motifs. There are at least four small, intact ORFs in this region, between 6 and 16 amino acid residues in length, indicated in Fig. 1. However, there are no consensus sequences for splicing and this upstream region is very large, so we conclude it is unlikely to be translated. As shown below, the size of the message is consistent with this conclusion. A search for the MCB motif, first identified in S. cerevisiae as a target for transcriptional regulation of genes required for DNA replication (ACGCGTNA; reviewed by Merrill et al., 1993), revealed no likely sites. In S. pombe, the MCB binding complex confers striking periodicity to transcription and is cdc10+-dependent (Lowndes et al., 1992; Kelly et al., 1993). Another group of S. cerevisiae genes expressed at START, including CLN1 and CLN2, uses a different sequence element, the SCB (CACGAAA; Andrews and Herskowitz, 1989; Breneden and Nasmyth, 1987), but this element has not been identified in fission yeast, and no such sequence is found in the puc1+ upstream region. Nor does puc1+ contain a TR box, which is found in fission yeast genes regulated by the ste11+ gene product in response to nitrogen limitation (TTCTTTGTTY; Sugimoto et al., 1993).
Thus no known transcription regulatory motif is found upstream of puc1*.

Construction of a truncated allele

The *S. cerevisiae* G1 cyclins all are characterized by C-terminal extensions that include a high proportion of prolines, serines, threonines and glutamates: so-called PEST sequences, which are thought to target the protein for destruction (Cross, 1988; Hadwiger et al., 1989; Nash et al., 1988; Rogers et al., 1986). Truncation of the WH1/CLN3 or CLN2 proteins removing these sequences creates dominant alleles, which have behaviour consistent with a hyperstable protein (Cross, 1988; Nash et al., 1988; Richardson et al., 1989; Tyers et al., 1992). The puc1* sequence lacks any such C-terminal extension, but there is a long N-terminal region that is rich in PEST residues (Fig. 1, and Forsburg and Nurse, 1991b), with a predicted PEST score of 11.55 (Rogers et al., 1986). We reasoned that if these sequences do act as a target for destruction, then deletion of them might lead to an allele with a similar phenotype to that seen with the truncated *S. cerevisiae* proteins: that is, intensifying the dosage-dependent effects seen if these sequences do act as a target for destruction, then deletion of them might lead to an allele with a similar phenotype to that seen with the truncated *S. cerevisiae* proteins: that is, intensifying the dosage-dependent effects seen

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**Fig. 1.** Nucleotide sequence of the genomic clone of *puc1* and conceptual translation of the open reading frame. The open reading frame begins at position 1258. The positions of relevant restriction sites are boxed. The positions of the two cDNA clones (cDNA 1 from Forsburg and Nurse, 1991b) and the poly-A site are indicated. The ‘cyclin box’ region of protein homology to other cyclins is shown in bold type. The single-stranded probes used for northern blotting extend from position 1931 to 2161 (probe 1), and positions 521 to 771 (probe 2).
truncated \textit{puc1}^+ (\textit{puc1}\Delta88) was able to confer alpha-factor resistance (the screen used to isolate the original \textit{puc1}^+ cDNA; data not shown). Secondly, it was transformed into a strain deleted for all three G1 cyclins and kept alive by \textit{WHI1/CLN3} under control of UAS\textit{GAL}. We verified that \textit{puc1}\Delta88 rescued this strain and allowed growth on glucose medium nearly as well as full-length \textit{puc1}^+ (data not shown). The amino terminus of \textit{puc1} is therefore not required for cyclin function in the budding yeast assay. We next looked at the effect of this truncated cyclin in fission yeast.

First, \textit{puc1}\Delta88 was overexpressed in \textit{S. pombe}, under the thiamine-repressible promoter of the \textit{nmt1}^+ gene. Earlier we reported that overexpression of \textit{puc1}^+ causes a G2 delay in \textit{S. pombe}; cells divide at an elongated size, although they are still viable (Forsburg and Nurse, 1991b). This phenotype was slightly enhanced when cells overexpressed the \textit{puc1}\Delta88 allele; once again cells were still able to divide but were strikingly elongated (Fig. 2A,B,C). Flow cytometry analysis (Fig. 3A) showed a single peak as seen in the wild-type control, demonstrating that these cells were delayed with a 2C DNA content as was previously seen for overexpression of the full-length protein (Fig. 3A, and Forsburg and Nurse, 1991b). The phenotype presented itself a few hours earlier by plate assay than that seen with overexpression of the full-length protein (18 hours vs 24 hours). Thus when \textit{puc1}\Delta88 is strongly overproduced in fission yeast, there is a modest intensification of the phenotype associated with full-length \textit{puc1} overproduction.

In contrast, when the genomic copy of full-length \textit{puc1} or \textit{puc1}\Delta88 was present on a high copy plasmid under its own promoter, there was no visible phenotype in \textit{S. pombe} cells (data not shown). In this case, the upstream sequences and translation context were maintained in both clones, so levels of expression should be comparable. In \textit{S. cerevisiae}, the effect of even one or two extra copies of the PEST-truncation mutations \textit{WHI1-1} or \textit{CLN2-1} is dramatic: the cells are accelerated through G1 and divide at a noticeably smaller size. No equivalent effect on mitotic progression was observed in fission yeast with extra copies of \textit{puc1}\Delta88.

**Disruption of \textit{puc1}^+ has no mitotic phenotype**

Using the genomic clone, we were able to construct a disruption of the \textit{puc1}^+ gene. Two constructions were made using the \textit{ura4}^+ gene as a marker. One of them contained a short, 300 bp deletion within the conserved ‘cyclin box’ region between the \textit{SalI} and \textit{XhoI} sites; the other deletes the 945 bp region between the \textit{SalI} and \textit{XbaI} sites, which includes most of the open reading frame and the entire cyclin box (Fig. 1). Purified fragments containing these constructs were trans-
formed into a diploid strain of genotype k/lh+ ura4-D18/urate-4-
D18 leu1-32//leu1-32 ade6-M20/ade6-M216, which can be easily
maintained in the diploid state by the intragenic comple-
mentation of the two ade6 alleles. Transformants were
screened for stability and analysed by Southern blotting (data
not shown). The strains were sporulated, and viable ura4+
haploid clones were obtained. These were also tested by
Southern blot to confirm the structure of the disrupted allele.
Both deletion mutants were characterized, and were pheno-
typically indistinguishable from one another. There were no
visible phenotypes associated with the deletion of the puc1+
gene in either strain and normal cell growth was unaffected
(Fig. 2D). Again, this contrasts with observations in S. cere-
visiae, where cells deleted for the single G1 cyclin WHI1/CLN3
are viable, but enlarged in size and delayed in the G1 phase of
the cycle (Cross, 1988, 1990; Nash et al., 1988) although
deletion of CLN1 or CLN2 has less effect (Richardson et al.,
1989). Flow cytometry showed no change in the typical wild-
type profile of logarithmically growing cells (Fig. 3B).
Deletion 1 was also crossed into strains deficient in other cell
cycle control genes, including cdc2-33, cdc10-129, cdc13-117
and cdc25-22. There was no obvious synthetic phenotype asso-
ciated with any of the double mutants, either at the permissive
or restrictive temperatures. The puc1+ gene is thus non-
essential for normal mitotic progression and has no apparent
effect on G1.

Another fission yeast cyclin, encoded by the cig1+ gene, has
been reported to be a candidate for a G1 cyclin. Originally,
Buono et al. (1991) reported that, although deletion of cig1+
has no effect on cell size or growth rate, the distribution of the
population throughout the cycle is dramatically shifted towards
G1. For this reason, we were interested to see if there would be
any evidence for a functional overlap between these two
cyclins. We constructed a double mutant strain Δpuc1Δcig1
by tetrad dissection and carried out flow cytometry. The cell
cycle distribution was indistinguishable from wild type. Addi-
tionally we found no evidence for a G1 delay in the single
mutant Δcig1 (Fig. 3B). We confirmed this result independent of
flow cytometry by a hydroxyurea arrest. Hydroxyurea
blocks the cell cycle in late G1/early S. If the cells are largely
in G2 (as are wild-type cells), then they will undergo roughly
one doubling of cell number before reaching the block point
arresting in the hydroxyurea. If the cells are largely in G1, they
will reach the block point before undergoing division, and thus
the cell number will not increase as much. Once again, there
was no effect of a deletion of cig1 in this protocol when
compared to a wild-type strain (data not shown), and this
observation has been confirmed by Bueno et al. (1993). Thus
neither Δcig1 nor Δpuc1 has a discernible effect on G1 pro-
gression, whether alone or in combination.

One possible explanation for the absence of a puc1 deletion
phenotype is that the fission yeast normally has a very short
G1, and a delay in this period of the cell cycle, unless pro-
nounced, may be undetectable. We accordingly characterized the
effects of puc1 deletion in a strain that already has an
extended G1 phase, to see if a further extension might be
apparent. The wee1-50 temperature-sensitive mutation accel-
ernates the G2/M transition, and this causes the cells to divide
at a smaller size. In order to pass the size control acting at
START, the cells must expand their G1 period (Nurse, 1975).
A double mutant Δpuc1::urate4+ wee1-50 was constructed and
the distribution of cells in the cycle was compared to a wee1-
50 strain during a timecourse following the shift to restrictive
temperature. There was no significant difference between
wee1-50 (Fig. 3C) and wee1-50 Δpuc1 (Fig. 3D) strains in the
percentage of cells with a 1C DNA content. Thus deletion of
puc1 has no apparent effect on G1 progression.

We used the Δpuc1::urate4 cross in strains to map the gene.
A Southern blot of S. pombe chromosomes separated by pulsed
field electrophoresis was probed with a cDNA fragment, and
puc1+ was localised to chromosome II. Pair-wise crosses were
carried out between the Δpuc1::urate4 allele and a variety of
markers on chromosome II. The Δpuc1::urate4 marker mapped
27 cM from his4-239 on the long arm of chromosome II (70
PD:3 NPD:46 TT). The met3-15 marker is centromere proximal to
his4-239 (Munz et al., 1989); since there was no more
significant linkage between Δpuc1::urate4+ and met3-15
(17:9:33) than between his4-239 and met3-15 (14:2:20), we
conclude that puc1+ is centromere distal to the his4 marker,
and defines a new locus. This assignment has subsequently
been confirmed by physical mapping (Hoheisel et al., 1993).

Protein analysis of puc1

We raised three polyclonal antibodies to bacterially expressed
puc1 protein to attempt to characterize its function more bio-
chemically. All three antibodies recognized both full-length
p40puc1 and the amino-terminal truncation p30puc1ΔAS8 when
overproduced by the heterologous nmt1 promoter in normally
growing cells (Fig. 4A, lanes 3 and 4). However, there was no
signal apparent in crude lysates from wild-type cells or in a
strain deleted for puc1, using standard alkaline phosphatase or
ECL methodologies (Fig. 4A, lanes 1 and 2, and data not
shown). Thus wild-type p40puc1 in logarithmically growing
cells is below the level of detection of these reagents. As shown
above, overproduction of p40puc1 or p30puc1ΔAS8 by the nmt1
promoter causes a cell cycle delay with a 2C DNA content
(Figs 2 and 3A). Previously, we suggested that this is due to
competition with the cdc13 mitotic cyclin, based on the
synthetic lethality seen when puc1 is overproduced in a cdc13-
117 mutant background (Forsburg and Nurse, 1991b). We
tested whether this was due to an interaction between p40puc1
and p34cdc2 under the unusual conditions of high ectopic
expression of puc1+. Four strains (wild type, Δpuc1, OP-puc1,
and OP-puc1ΔAS8) were grown to mid-exponential phase under
inducing conditions and harvested. Aliquots were immuno-
precipitated with either anti-p40puc1 or anti-p34cdc2 antibodies.
The immunoprecipitates were separated by gel electrophoresis
and western blotted for p40puc1. As shown in Fig. 4B, lanes 3
and 4, both full-length and truncated puc1 proteins can be
immunoprecipitated by the anti-p40puc1 antibody when they
are overproduced. There is no signal detected in the wild type
or the Δpuc1 lanes (1 and 2), or when the immunoprecipitation
is carried out using pre-immune serum (data not shown). In
Fig. 4C, the anti-p34cdc2 immunoprecipitates were blotted with
anti-p40puc1. A band is detected in lane 3, the OP-puc1ΔAS8
lane, but not in other lanes. Thus, when overproduced by a
powerful heterologous promoter, puc1ΔAS8 is co-precipitated
by anti-p34cdc2 antisera. The apparent association between
p34cdc2 and p30puc1ΔAS8 is consistent with our original proposal
that the synthetic lethality seen in the cdc13-117 background
was due to a competition between the cyclins when the puc1+
genome product was inappropriately expressed. As shown earlier,
the truncation has a somewhat enhanced phenotype relative to the full-length protein when overproduced (Fig. 2), and the presence of putative PEST sequences in the full-length protein may reduce protein stability; this could explain why full-length protein was not immunoprecipitated by anti-p34\(^{cdc2}\) antibody SF2. Alternatively, the deleted sequences could affect stability of the interaction. Because overexpression of the puc1 cyclin introduces a new phenotype, that of cell cycle delay, we are unwilling to conclude that this indicates that puc1 necessarily associates with p34\(^{cdc2}\) in a wild-type cell. Rather, we suspect that puc1 non-specifically associates with p34\(^{cdc2}\) when overproduced, thus delaying normal mitotic progression.

**puc1**\(^+\) is expressed when cells exit the cycle

In *S. cerevisiae*, both CLN1 and CLN2 are transcriptionally...
regulated through the cycle, with mRNA levels peaking during G1/S. We probed a blot of RNA from a synchronized culture (Fig. 5A) and found no significant variation in puc1+ mRNA expression; indeed, the signal of approximately 2.0 kb was very difficult to detect. If puc1+ were expressed specifically in G1, we reasoned that we might miss the window of puc1+ gene expression because the G1 phase in fission yeast is so short. Therefore we also probed a blot from a synchronous culture of cells with an extended G1, due to a wee1-50 mutation (as explained above). As shown in Fig. 5B, there is again no compelling evidence for any variation in expression. The nonspecific heterogeneity observed we conclude is an artefact of the low levels of the message, and long exposures required to visualize it. Previously we reported a 1.3 kb message (Forsburg and Nurse, 1991b) but this species is not consistently detected and when seen, is less abundant than the 2.0 kb species. With our earlier, shorter gels, the larger message reported here may have been distorted and masked by the ribosomal RNA that runs at approximately 1.8 to 2 kb. Subsequent experiments confirmed that the 2.0 kb message is indeed puc1-specific (see Fig. 6).

Given the low, constitutive levels of expression of the large RNA, we investigated whether under different growth conditions the message might be induced. Increased expression of the cyclin might indicate where it functions. We looked under conditions where cells exit the cycle. S. pombe cells require nitrogen starvation to arrest their cell cycle at START in order to undergo conjugation, which is followed immediately by sporulation (reviewed by Egeland et al., 1990). RNA was prepared from cells that were undergoing nitrogen arrest, to see whether under these conditions there was an increase in the levels of puc1+ message. Wild-type and Δpuc1 strains were grown to mid-exponential phase, washed, and re-inoculated into nitrogen-minus medium. During the following timecourse, we found that the large species of RNA was strongly induced in response to nitrogen starvation; no other signal was detected (Fig. 6). This message was undetectable in the disruption strain, indicating that it is puc1+-specific. Because the size of the message substantially exceeds the size of the ORF, we determined the extent of the message upstream by using a single-stranded probe from more than 500 bp upstream of the ATG (probe 2, Fig. 6). In this case, a transcript was detected in the deletion strain but its size was reduced, as expected if transcription terminates within the urad4+ region in this construct. Thus a large, puc1+-specific species of mRNA is specifically induced when cells are starved for nitrogen.

We found no evidence that this long transcript is spliced. Introns in fission yeast are usually small, often less than 100 bp, and have relatively conserved splice sequences (reviewed by Prabhala et al., 1992). The long upstream region of puc1+ contains four small open reading frames; three of these are rather distant from the presumed ORF and the fourth shows no consensus splice sites. The only message detected is significantly bigger than an expected spliced product (Fig. 6). These features of the puc1+ gene-induction by nitrogen starvation (long untranslated region, and small upstream open reading frames) are also found in three genes required for sexual development in fission yeast: ste11+ (Sugimoto et al., 1991); mei2+ (Shimoda et al., 1987); and esc1+ (Benten et al., 1993). The puc1+ gene may belong to an emerging family of genes with similar structures and patterns of expression, several of which are involved in sexual development.

The ste11+ gene encodes a transcriptional activator specifically required for sexual development, activating the expression of the mei2+ gene, amongst others. This activator functions through a highly conserved sequence (the TR box, TTCTTTGTTY; Sugimoto et al., 1991). No such sequence was found in puc1+ (Fig. 1). To determine whether absence of puc1+ affected the induction of ste11-regulated genes, we probed the nitrogen starvation timecourse blots with mei2+ (Shimoda et al., 1987). As shown in Fig. 6, mei2+ gene expression is induced some time after that of puc1+, and this induction is unaffected in a puc1 deletion strain. Additionally we found RNA from ste11+ strains in the absence of nitrogen, and detected puc1+ message but not mei2+ (data not shown, and Sugimoto et al., 1991); puc1+ induction therefore appears to operate independently of ste11+.

We attempted to detect protein under these conditions of nitrogen starvation, using our previously characterized antibodies. Using either alkaline phosphatase or ECL methods of western blotting, we were unable to detect a species corresponding to p40mei1 either in haploid or diploid strains undergoing nitrogen starvation (data not shown).
Diploid HC puc1 (containing the high copy plasmid pSLF110, with the truncated clone ura4-D18; puc1+)

Similar results were obtained using heterothallic a wild-type strain with the representative profiles are shown in Fig. 7. Four strains were compared: wild-type cells, the deletion strain, and a wild-type strain with the puc1+ (HC puc1) or puc1Δ88 (HC puc1Δ88) strain under its own promoter. Similar results were obtained using heterothallic puc1+ and Δpuc1 strains (data not shown). As discussed above, there is no visible cell cycle delay associated with high copy puc1 expression under its own promoter. All strains arrested in G1.

puc1 affects sexual development

Since the expression of the puc1+ gene is induced by N-starvation, we examined the behaviour of the deletion strain in conditions of starvation and sexual differentiation. Superficially, the strain behaved normally: that is, it seemed to show correct starvation response and was fertile, and spore viability was excellent. Therefore, we looked carefully at the physiology of the strain to see if there were any subtle phenotypes associated with the puc1 deletion. Wild-type and Δpuc1 strains were compared for their ability to arrest in G1 in response to nitrogen starvation (Fig. 7), and the timing of conjugation and sporulation were examined (Fig. 8).

First, homothallic haploid cells were starved for nitrogen and monitored by flow cytometry as they exited the cycle. Representative profiles are shown in Fig. 7. Four strains were compared: wild-type cells, the Δpuc1::ura4 deletion strain, and a wild-type strain with the puc1+ (HC puc1) or puc1Δ88 (HC puc1Δ88) strain under its own promoter on a high-copy plasmid. Similar results were obtained using heterothallic puc1+ and Δpuc1 strains (data not shown). As discussed above, there is no visible cell cycle delay associated with high copy puc1 expression under its own promoter. All strains arrested in G1.

Fig. 7. Flow cytometric analysis of starved cells. Homothallic strains were grown to mid-exponential phase, washed 4 times in minimal medium lacking nitrogen, and resuspended at the same density in N-minus medium. Aliquots were taken for flow cytometry at 0, 2, 4 and 6 hours, fixed in ethanol, and stained with propidium iodide. The following strains were compared: wild type h80 ade6-M210 leu1-32 ura4-D18; Δpuc1 h80 ade6-M210 leu1-32 ura4-D18 Δpuc1::ura4; HC puc1 h80 ade6-M210 leu1-32 ura4-D18 (containing the high copy plasmid pSLF71, with the genomic clone of puc1+ under its own promoter); HC-puc1Δ88 h80 ade6-M210 leu1-32 ura4-D18 (containing the high copy plasmid pSLF110, with the truncated clone puc1Δ88 under its own promoter).

Fig. 8. Comparison of sexual response in strains grown in limiting nitrogen. (A) Frequency of zygotes and ascis in strains grown in limiting glutamate, expressed as a function of Asso of the culture. Strains were pre-grown in minimal selective medium and inoculated at low density into glutamate medium (as described in Materials and Methods). Samples were counted for zygote and ascus formation hourly after overnight growth. The results of 7 different timecourses (diploids) and 3 different timecourses (homoathallic strains) are averaged. Strains: diploid Δpuc1 h+/h+ ade6-M210/ade6+ leu1-32/leu1+ Δpuc1::ura4+/Δpuc1::ura4+; diploid h+/h+ ade6-M210/ ade6+ leu1-32/leu1+. Diploids were maintained by complementation of the ade6 and leu1 markers. Homothallic strains: Δpuc1 h80 ade6-M210 leu1-32 ura4-D18 Δpuc1::ura4; wild type h80 ade6-M210 leu1-32 ura4-D18. (B) Growth of diploid strains in different concentrations of nitrogen. Strains were grown to mid-late exponential phase in selective medium, harvested, washed, and re-inoculated into minimal medium supplemented with the indicated amounts of ammonium chloride at a density of 5x10⁶ cells/ml. They were grown for 24 hours and then counted for frequency of tetranculate cells. The average of 6 experiments is presented in the graph. Strains: diploid Δpuc1 h+/h+ ade6-M210/ade6+ leu1-32/leu1+ Δpuc1::ura4+/Δpuc1::ura4+; diploid h+/h+ ade6-M210/ade6+ leu1-32/leu1+; diploid HC-puc1 h+/h+ ade6-M210/ade6+ leu1-32/leu1+ ura4-D18/ura4-D18 (containing the high copy plasmid pSLF71, with full-length puc1+ under its own promoter); diploid HC-puc1Δ88 h+/h+ ade6-M210/ade6+ leu1-32/leu1+ ura4-D18/ura4-D18 (containing the high copy plasmid pSLF110, with the truncated clone puc1Δ88 under its own promoter). Diploids were maintained by complementation of the ade6 and leu1 markers.
in response to nitrogen limitation as expected, but the kinetics differed slightly. The \(\Delta puc1\) strain arrested in G1 with somewhat accelerated kinetics relative to the wild type. Average values for the percentage of starving cells in G1, calculated from four separate timecourses, are shown in Table 1. We conclude that puc1 is not required for G1 arrest and may, in fact retard it. In the presence of the multi-copy plasmid containing \(puc1^+\) or \(puc1\Delta 88\) expressed under its own promoter, the cells were somewhat delayed in G2 and arrested more slowly (e.g. compare \(\Delta puc1\) to HC \(puc1\Delta 88\) in Fig. 7). In all cases, the cells became small as expected when starved for nitrogen. This suggests that in the presence of extra puc1, the strain is more resistant to the G1 arrest normally induced by nitrogen starvation.

Next, we analysed the timing of conjugation and sporulation. Timecourses were carried out in which the cells were grown in minimal medium supplemented with glutamate as the sole nitrogen source. Glutamate is a poor nitrogen source relative to ammonia. As available nitrogen in the culture is exhausted, homothallic strains begin to conjugate and diploid strains undergo meiosis. These events were monitored microscopically and the average number of zygotes from several experiments is presented as a function of cell density in Fig. 8A. The absence of puc1 had little effect on the formation of zygotes in the homothallic strain. However, reproducibly, there were a larger number of meiotic (tetranucleate) cells at earlier timepoints in the \(\Delta puc1\) diploid strain than in the wild-type diploid strain. That is, with each division under such conditions, there appeared to be a greater probability that a \(\Delta puc1\) diploid strain entered meiosis. This suggests that the cells in the absence of puc1 are somewhat more sensitive to the depletion of nitrogen. In order to specifically examine sensitivity to nitrogen limitation, we looked at the sexual responses of a number of strains in media of different nitrogen concentrations.

Table 1. Average distribution of cells with a 1C DNA content during nitrogen starvation

<table>
<thead>
<tr>
<th>Hours - N</th>
<th>Wild-type h(^{100})</th>
<th>(\Delta puc1) h(^{100})</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>45</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>58</td>
<td>72</td>
</tr>
</tbody>
</table>

The percentage of cells with a 1C DNA content was determined by applying gates to each series of flow cytometry histograms using the Lysis II/ Becton Dickinson software. Data were averaged from four separate nitrogen starvation series comparing wild type to \(\Delta puc1\) strains at 0, 2, 4, and 6 hours without nitrogen.

Fig. 9. OP-puc1 rescues \(puc1^{19}\). A strain of genotype \(pat1-114\) \(ura4-D18\) \(leu1-32\) h\(^{-}\) was transformed with plasmid pSLF59 expressing \(puc1^+\) under control of the \(nmt1^+\) promoter. The promoter was induced for 16 hours at permissive temperature in minimal medium lacking thiamine before the culture was shifted to the restrictive temperature of 32°C for 20 hours. Cells were harvested and fixed in ethanol, stained in DAPI, and photographed. (A) \(puc1^{19}\) without plasmid. All the cells have entered meiosis. (B) \(pat1-114\) strain overexpressing \(puc1^+\). Note the number of non-miotic cells. This strain forms colonies at the restrictive temperature. Bar, 10 \(\mu m\).
cells are still apparent so this rescue, while sufficient to allow colony formation, does not completely suppress the pat1<sup>ts</sup> phenotype. This rescue of pat1<sup>ts</sup> by puc1<sup>ts</sup> is again consistent with a model in which puc1<sup>ts</sup> acts negatively upon some aspect controlling the exit from the cell cycle and delays entry into sexual development.

**DISCUSSION**

In this report we describe our characterization of the puc1 cyclin gene product. A genomic puc1<sup>+</sup> clone has been isolated; the gene contains no introns, and has no obvious regulatory motifs in the upstream region. The gene has been mapped, and defines a new locus 27 cM centromere distal from his<sup>4+</sup> on chromosome II. A disruption has no mitotic phenotype; in particular, there is no detectable delay in G<sub>1</sub> progression. There is no evidence for a synthetic phenotype when Apc1<sup>1</sup>:<sup>ura4</sup> is combined with cdc2-33, cdc10-129, cdc13-117, cdc25-22 or wee1-50. Additionally, there is no synthetic phenotype associated with deletion of the cig1<sup>ts</sup> cyclin, which we show has no G<sub>1</sub> phenotype. This suggests one of two possibilities: either puc1<sup>+</sup> is a redundant gene product, and additional gene functions can replace it, or it is not required for the mitotic cell cycle under the growth conditions that we have examined. These possibilities are not mutually exclusive.

We also describe subtle physiological effects on the process of sexual development in the presence and absence of puc1. In strains deleted for puc1<sup>+</sup>, conjugation is unaffected but there is a modest, reproducible acceleration of meiosis in diploids. Moderate overexpression of puc1<sup>+</sup> on a high copy plasmid delays both conjugation and meiosis although it has no apparent effect on mitosis. In addition, strong overproduction of puc1<sup>+</sup> suppresses the lethality of the pat1<sup>ts</sup> mutant, which otherwise enters meiosis from a haploid state.

Even given the possibility that puc1<sup>+</sup> is redundant, there is some evidence that it is not functioning in the mitotic G<sub>1</sub>/S transition in fission yeast in the same way as its homologues function in budding yeast. First, the puc1<sup>+</sup> gene is expressed at a very low, constitutive level in mitotic cells and expression is strikingly induced as cells exit the cycle. In budding yeast, CLN1 and CLN2 messages are expressed in a cell cycle-dependent fashion (Cross and Tinkelenberg, 1991; Dirick and Nasmyth, 1991; Nasmyth and Dirick, 1991; Ogas et al., 1991; Wittenberg et al., 1990) and cell cycle exit in response to mating pheromone represses their expression (Wittenberg et al., 1990). The WHI1/CLN3 gene is expressed at a constitutive level (Nash et al., 1988; Tyers et al., 1992, 1993; Wittenberg et al., 1990) but mating pheromone only modestly affects message levels (Nash et al., 1988; Wittenberg et al., 1990). However, none of these genes has been examined in conditions of nutrient limitation. Second, there is no G<sub>1</sub> delay detectable as a consequence of deletion of puc1<sup>+</sup> in fission yeast, either in wild-type cells, or in wee1-50 mutant cells. Deletion of WHI1/CLN3 in budding yeast does affect G<sub>1</sub> progression: the cells are delayed in G<sub>1</sub> and divide at an enlarged size relative to wild type as expected from its apparent role as a dosage-dependent inducer of START (Cross, 1988; Nash et al., 1988; Tyers et al., 1993). This is despite the fact that WHI1/CLN3 protein is expressed at low levels and difficult to detect immunologically (Cross and Blake, 1993; Nash et al., 1988; Tyers et al., 1992, 1993). However, there is little phenotype associated with deletion of either of the much more actively expressed CLN1 or CLN2 genes (Richardson et al., 1989).

Thirdly, strong overexpression of puc1<sup>+</sup> under a heterologous promoter in S. pombe leads to a G<sub>2</sub> delay, which is exacerbated by the amino-terminal truncation puc1<sup>ΔN88</sup>. Moderate overproduction under its own promoter has no effect. In contrast, overproduction of the budding yeast G<sub>1</sub> cyclins in S. cerevisiae leads to acceleration of mitotic G<sub>1</sub> and division at a smaller size, again consistent with their role as dosage-dependent inducers of START (Cross, 1988; Hadwiger et al., 1989; Nash et al., 1988; Richardson et al., 1989; Tyers et al., 1992, 1993). In all these comparisons, puc1<sup>+</sup> acts differently in fission yeast than do its homologues in the budding yeast, and has no detectable effect upon G<sub>1</sub> progression in the normal mitotic cell cycle.

There is always the chance that a G<sub>1</sub> cyclin function is masked by redundancy and of course we cannot exclude this possibility for puc1. However, apart from its sequence similarity to the budding yeast proteins, we have no experimental evidence that links puc1 to the mitotic cycle of normally cycling fission yeast cells. Indeed, the very low level of puc1<sup>+</sup> gene expression argues against such a role. Our analysis points instead to a function outside ‘normal’ mitotic progression, in the transition between cycling and non-cycling cells. One could argue that the apparent negative regulation of sexual development by puc1 could reflect that, under limiting nitrogen conditions, puc1 is induced and actually does have a G<sub>1</sub>-cyclin-like function in promoting START and the mitotic cycle. In the absence of puc1, the cells are more prone to leave the cell cycle at START and undergo a developmental pathway. Such a model would be consistent with our data. But it posits a role for puc1 specifically in the transition from one state to another. It still does not support a conclusion that puc1 functions in the normal mitotic cycle.

It is of interest in this context that puc1<sup>+</sup> was isolated in a budding yeast screen by its ability to confer mating pheromone resistance on the cells, thus preventing them from initiating sexual development. This delay of sexual response is exactly analogous to its effect when overproduced in the fission yeast. Of heterologous cyclins examined (Tyers et al., 1993), only puc1<sup>+</sup> (Forsburg and Nurse, 1991b) and a truncated form of cig1<sup>+</sup> (Tyers et al., 1993) have been shown to confer alpha factor resistance to budding yeast cells. While the pheromone response pathway in fission yeast is known to affect mating functions rather than cell cycle progression (reviewed by Egel et al., 1990; Hayles and Nurse, 1992), the possibility that it also reinforces the G<sub>1</sub> arrest caused by nutrient limitation cannot be excluded. There is an extensive similarity of the MAP-kinase like pathway that responds to pheromone in both budding and fission yeasts (reviewed by Errede and Levin, 1993, Nielsen, 1993), and in budding yeast the path terminates with the G<sub>1</sub> cyclins. An alternative model to that described above is that puc1 functions in the pheromone response pathway of fission yeast (which is itself nutrient regulated), and the differences between puc1 and the cerevisiae cyclins reflect differences between pheromone response in these two organisms. This would be consistent with the similarity of gene structure we identified between puc1 and other genes involved in sexual development. It will be of interest in the future to determine whether there are synthetic interactions between Δpuc1 and...
other genes thought to be involved in the nutrient response, including cdr1+ and nim1+ (Feilotter et al., 1991; Young and Fantes, 1987), cdr2+ (Young and Fantes, 1987), cys1+ (adenylate cyclase; Yamawaki, et al., 1989; Young et al., 1989), or spkl+ (Toda et al., 1992). Preliminary results constructing Δspkl and Δcys1 double mutants with Δpuc1 show no obvious interactions (S. L. Forsburg and P. Nurse, unpublished results).

At the moment, there is no strong candidate for a G1-specific cyclin in fission yeast completely analogous to the G1 cyclins in budding yeast. We do not infer that S. pombe goes through its cycle without any S. cerevisiae-like G1 cyclin activity (although that remains a formal possibility), only that no such gene product has yet been clearly identified. As reported here and recently confirmed by Bueno et al. (1993), the G1 delay associated with a deletion of the B cyclin cgl1+ has not been reproduced. Of the currently known fission yeast cyclins, none has an apparent G1 cyclin function. In the budding yeast, the reproducibly, of the currently known fission yeast cyclins, none has associated with a deletion of the B cyclin cgl1+ (1989). The yeast SWI4 protein contains a

transduction and differentiation. cell cycle but more generally in the interface between signal budding yeasts and higher cells may function not only in the mitotic sense, there is a clear relationship between the two yeasts. It signals for cell cycle exit to developmental decisions. In this aspect of signal transduction to developmental decisions. In this sense, there is a clear relationship between the two yeasts. It seems likely that the the wide variety of cyclins isolated in yeasts and higher cells may function not only in the mitotic cell cycle but more generally in the interface between signal transduction and differentiation.

We thank H. Okayama for communicating results prior to publication, S. Moreno for the weel-50 blot, A. M. Carr for the genomic library, M. Yamamoto for the Δste11 and Δcys1 strains, P. Russell for the Δcig1 strain, and K. P. M. Labib for the chromosome blot. Liz Cowe of the Oxford University Molecular Biology Data Centre kindly assisted with PEST analysis. We especially thank Tom Kelly, Dick McIntosh, and members of the Cell Cycle Group for their generous advice and encouragement throughout the course of this work. S.L.F. was supported by postdoctoral fellowships from the Helen Hay Whitney Foundation and NATO. P.N. was a Royal Society Research Professor. This work was supported by the ICRF and the MRC. The nucleotide sequence of the genomic clone of puc1+ has been submitted to the GenEMBL database under accession number X74451.

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(Rceived 25 October 1993 - Accepted 2 December 1993)