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# The protein phosphatase 2A B′-regulatory subunit par1p is implicated in regulation of the *S. pombe* septation initiation network

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**Abstract In order to identify regulators of the *Schizosaccharomyces pombe* septation initiation network (SIN), which signals the onset of cell division, we have isolated extragenic suppressors of mutations in the GTPase spg1p, which is a central element in this pathway. One of these encodes the protein phosphatase 2A (PP2A) B′-regulatory subunit par1p. Loss of par1p function rescues mutants in *cdc11*, *cdc7*, and *spg1*, but no other SIN mutants. Our data suggest that PP2A-par1p acts as a negative regulator of SIN signalling.** 

*Key words:* Cell cycle; Septation; Cytokinesis; Phosphatase; Kinase

## 1. Introduction

The fission yeast *Schizosaccharomyces pombe* grows by elongation at its tips, and divides by formation of a medially placed division septum. The position of the division site is defined at the onset of mitosis, probably by signals emanating from the centrally positioned nucleus, and results in the assembly of an actomyosin-based contractile ring at the cell cortex. At the end of mitosis, when nuclear separation has been completed, the onset of septation in fission yeast is signalled through the septation initiation network (SIN). This signal transduction network includes three protein kinases (cdc7p, sid1p, sid2p), and their associated proteins (cdc14p with sid1p and mob1p with sid2p). Signalling is mediated by the GTPase spg1p, which binds cdc7p. SIN mutants typically display an elongated, multinucleated phenotype, as the nuclear cycle and growth continue without cytokinesis. The GTPase is negatively regulated by the byr4p-cdc16p GAP complex, and mutants in both these genes display a multi-septated phenotype. These proteins are all located on the spindle pole body during mitosis, and the sid2p-mob1p protein kinase also associates with the medial ring during septation. The proteins sid4p [1] and cdc11p (Krapp et al., in press) are thought to provide a binding site on the spindle pole body for SIN proteins. The kinase plo1p is also required for septation, and functions upstream of the SIN [2], while mitotic levels of cdc2p activity are antagonistic to SIN signalling (for an in-depth review of the SIN see [3]).

To date, no SIN mutant encodes a phosphoprotein phosphatase. However, there is evidence that phosphatases play a role in controlling cytokinesis and septum formation in *S. pombe*. A null mutant of calcineurin-like phosphatase *ppb1* shows defects in cytokinesis, with an increased percentage of multi-compartmented cells, and a delay in septation [4]. There also appears to be a role for protein phosphatase 2A (PP2A) in controlling septation. PP2A is a heterotrimer comprised of a catalytic subunit (C), a scaffold (A) and a regulatory or targeting subunit (B or B′) [5]. In *S. pombe* the A subunit-encoding gene *paa1* is essential; null mutants fail to establish proper cell polarity [6]. A deletion of the gene for the major catalytic subunit, *ppa2*, is viable, but advanced into mitosis, and shows defects in septation [7]. The *pab1* gene, which encodes the B-type regulatory subunit, is not essential, but cells are both heat- and cold-sensitive, and sterile. They also display polarity and septation defects, and at high temperatures they accumulate as small, septated cells [6]. Increased expression of *pab1* is toxic, resulting in accumulation of septated cells, but does not alter cell length significantly. *S. pombe* has two genes encoding B′-regulatory subunits, which have been named *par1* and *par2*. At the protein level par1p is the major form of the B′ subunit. The double null mutant is viable, but is heat-, cold-, and stress-sensitive, and shows defects in septation and cleavage [8,9]. The double mutant of *ppb1* and *par1* is lethal [8], suggesting they have a common, essential target. In this study, we used pseudoreversion analysis of a heat-sensitive mutant in the *spg1* gene to identify new regulators of septum formation in fission yeast. We obtained a mutant that suppressed not only *spg1* heat-sensitive mutants, but also mutants in *cdc11* and *cdc7*. Cloning of the gene identifies it as *par1*. These data implicate PP2A-par1 in regulation of the SIN.

## 2. Materials and methods

### 2.1. General methods

Standard methods were used for manipulation of DNA [10] and

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fission yeast [11]. C-terminal tagging of the genomic *par1* locus with green fluorescent protein (GFP) was done by oligonucleotide-mediated integration according to the method of [12].

### 2.2. Cloning of par1

Attempts to clone *par1* by complementation of the *par1-35* mutant heat and cold sensitivity using a multicopy library were unsuccessful. Linkage to the *ade6* gene was noted in genetic crosses, so the gene was cloned by co-transfection of cosmids from the lower arm of chromosome III with a non-replicating plasmid carrying a selectable marker. Since the recipient strain carries the *ura4-D18* deletion, stable transformation to uracil prototrophy can only be obtained by non-homologous recombination, or by recombination between the plasmid and cosmid, followed by integration of the cosmid. After selection for uracil prototrophy, colonies were replicated to 19°C and 36°C to check for complementation of the *par1-35* mutation. Once a complementing cosmid (SPCC188) was identified, the open reading frame responsible was identified as *par1* by subcloning. Full details are described in [13].

### 2.3. Microscopy

Cells were fixed, and stained with DAPI and Calcofluor, or TAT-1 as described [14,15]. To examine GFP-tagged proteins in living cells, TILLvisION software (v3.3; TILL Photonics GmbH) was used to analyse data captured with an IMAGO CCD camera mounted on an Olympus IX70 microscope. Images were assembled in Adobe Photoshop 5.5.

## 3. Results

### 3.1. Identification and cloning of par1

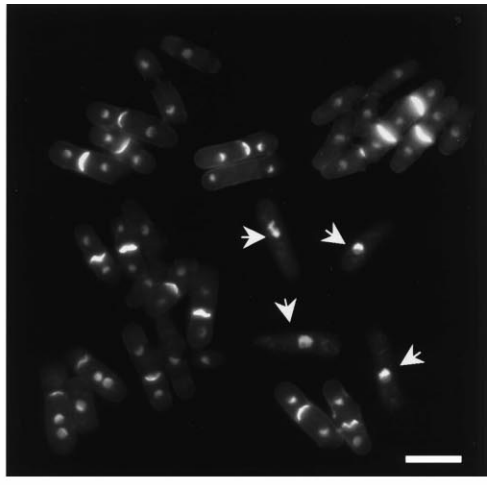
To identify effectors or regulators of signalling by the spg1p GTPase, we isolated suppressors of the heat-sensitive *spg1-W35* mutation. Spontaneously arising revertants able to grow at 36°C were back-crossed to wild-type and those containing extragenic suppressors that were either heat- or cold-sensitive per se were analysed further. Cloning of one of the genes (#35) showed that the mutant was an allele of *par1*. This allele was therefore named *par1-35*. The mutant is heat- and cold-sensitive, in agreement with previous studies of the *par1* null allele [8,9]. This mutant grows optimally at 25°C or 29°C. At 19°C or 36°C, colonies contain many dead cells, and are dark red on media containing phloxin B. Construction of a diploid with the genotype *spg1-B8/spg1-B8 par1-35/par1+* indicated that *par1-35* is recessive (not shown).

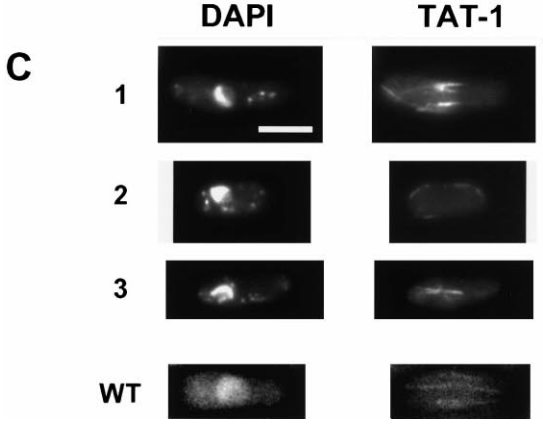
### 3.2. Loss of par1 function causes defects in nuclear positioning and placement of the division septum

Examination of *par1::ura4*$^+$ cells revealed that the percentage of cells with a division septum was increased over wild-type at all temperatures, though the effect was slightly en-

A

| | Percentage | | | |
|---|---|---|---|---|
| | Wildtype | Par1Δ 19°C | Par1Δ 29°C | Par1Δ 36°C |
| Total septation | 15 | 35 | 27.5 | 40 |
| Normally placed septum | 15 | 20.6 | 16.3 | 18.6 |
| Misplaced septum | 0 | 14.4 | 11.2 | 21.4 |
| Misplaced nucleus | 0 | 10.7 | 7.4 | 15.3 |
| Multi-compartmented | 0 | 13 | 1.5 | 4.1 |



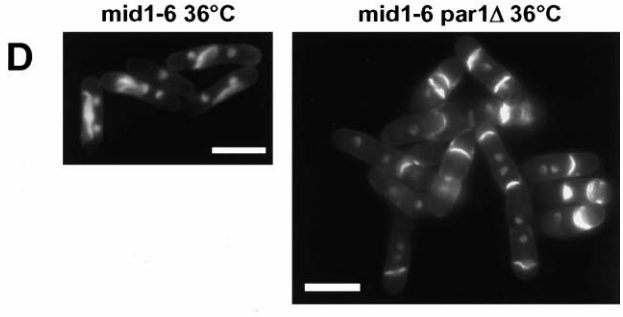




Fig. 1. Nuclei and division septa are misplaced in *par1::ura4*$^+$ cells. A: Wild-type or *par1::ura4*$^+$ cells were grown at 29°C, fixed, and stained with DAPI and Calcofluor. For *par1::ura4*$^+$, cells were also shifted to 19°C for 10 h, or 36°C for 5 h prior to fixation. The percentage of septated cells does not include multi-compartmented cells. B: DAPI–Calcofluor-stained image of *par1::ura4*$^+$ cells grown at 36°C. The arrowheads indicate cells with eccentrically positioned nuclei. The scale bar corresponds to 10 μm. C: *par1::ura4*$^+$ cells were fixed and stained with TAT-1 and DAPI. Microtubules in interphase cells with misplaced nuclei are shown (panels 1–3), with an interphase cell with a centrally placed nucleus for comparison (panels WT). D: The indicated mutants were grown at 25°C, then shifted to 36°C for 5 h, fixed and stained with DAPI and Calcofluor. Note that the septa in the *mid1-6 par1::ura4*$^+$ cells are at right angles to the long axis of the cell, while those in *mid1-6* are not.

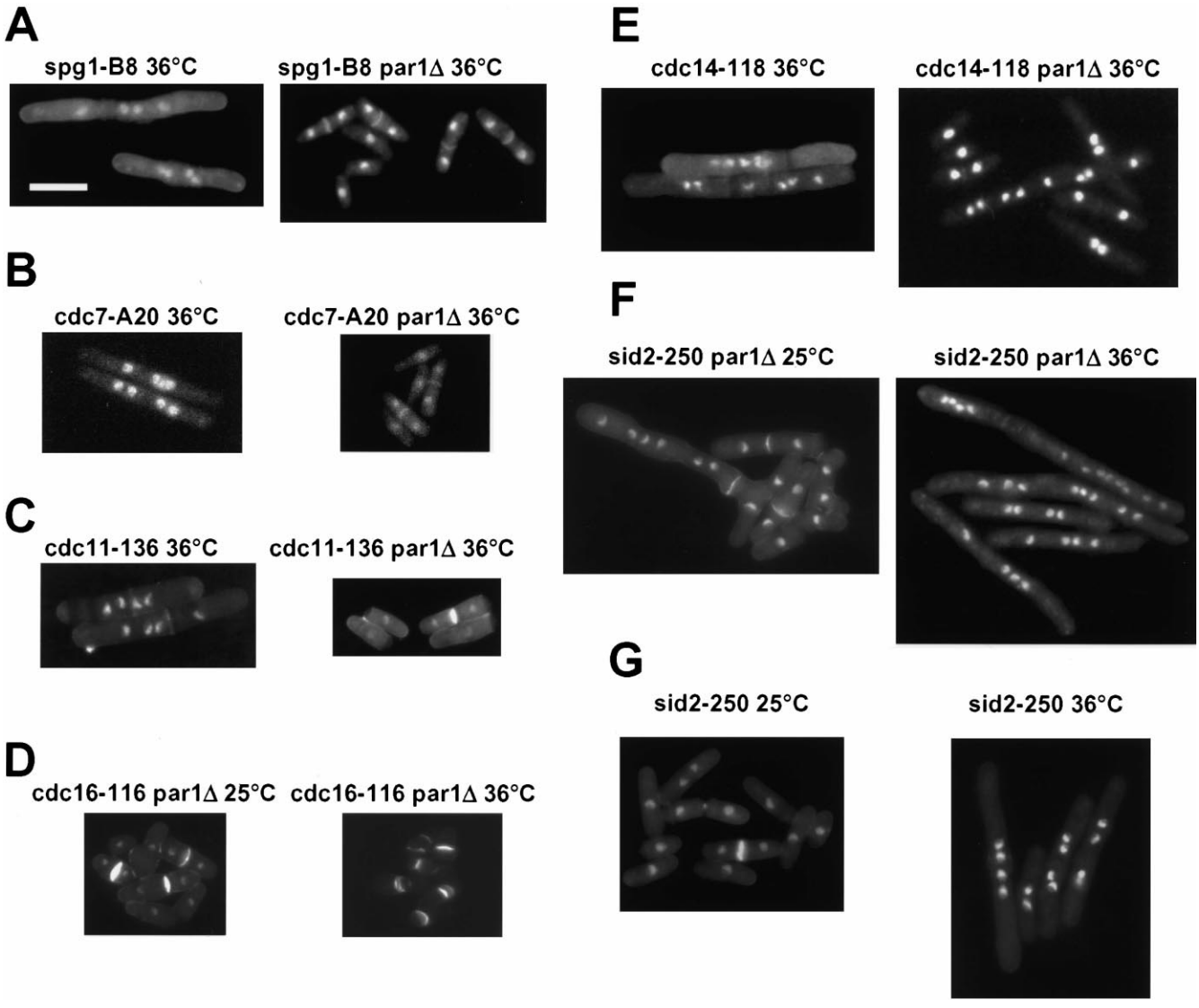



Fig. 2. Genetic interactions between SIN mutants and *par1::ura4*$^+$. The indicated strains were grown at 25°C, then shifted to 36°C for 5 h before fixation and staining with DAPI and Calcofluor. The scale bar in A represents 10 μm. Note that due to the intensity of the septa in D, the DAPI staining is not visible.

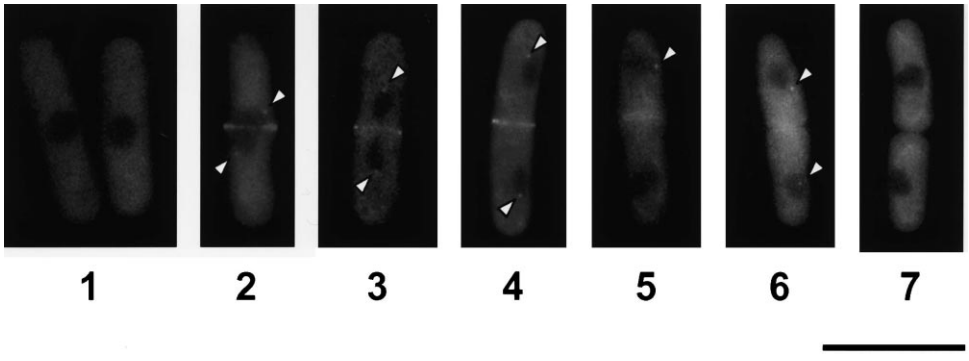



Fig. 3. Localisation of par1p-GFP. Living cells were photographed as described in Section 2. Panel 1 shows interphase cells. Panel 2 shows an early mitotic cell, panel 3 an early anaphase cell, panel 4 a late anaphase cell. The cell in panel 5 shows the occasionally observed double ring. Note that in this panel, only one of the two spindle pole bodies is in the plane of focus. Panel 6 shows a septating cell. Note the accumulation of par1p-GFP in the medial region, around the developing septum. Panel 7 shows a separating cell. The scale bar represents 10 μm. The arrowheads point to the position of the spindle pole bodies.

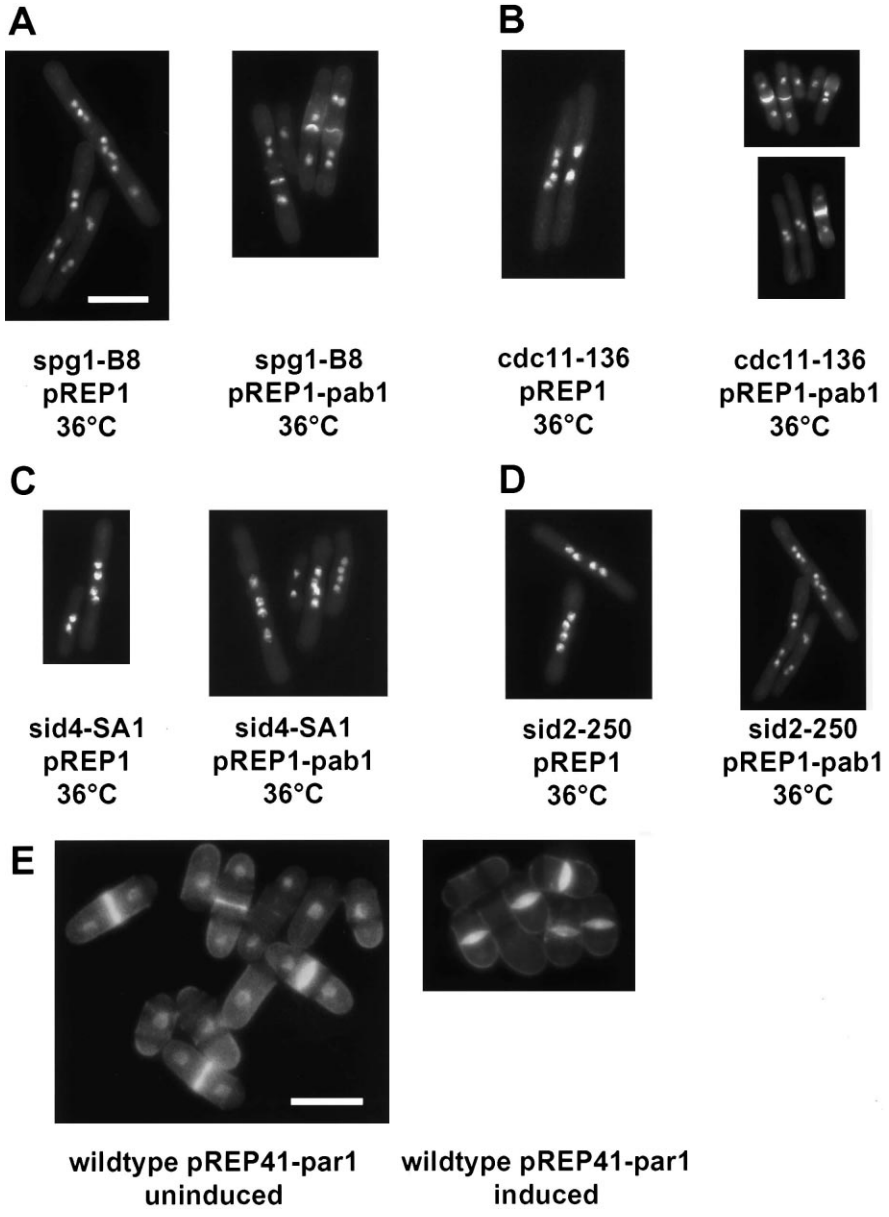



Fig. 4. Increased expression of pab1 can partially rescue some SIN mutants. A–D: The indicated SIN mutants carrying either empty pREP1 vector or pREP1-pab1 were grown at 25°C in the presence of thiamine. Expression was induced by washing out thiamine. After 15.5 h at 25°C, cells were shifted to 36°C for 5 h, before fixation and staining with DAPI and Calcofluor. E: Wild-type cells were transformed with pREP41-*par1*, and grown in the presence of thiamine. Expression was induced for 18 h at 25°C, and cells were fixed and stained as above. Note that due to the intensity of the septum staining the nuclei are not visible. The uninduced culture served as a control. The scale bar corresponds to 10 μm.

hanced at 19°C or 36°C compared with 29°C (Fig. 1A). Multicompartmented cells that had failed to cleave the division septum were also observed (Fig. 1A,B). Similar data were obtained for *par1-35* (not shown). Interestingly, the nucleus was not positioned centrally in a significant number of interphase *par1::ura4*$^+$ cells. The percentage of cells with misplaced nuclei was comparable to the percentage of cells with a misplaced division septum, and similar to the increase in the percentage of septated cells over that seen in wild-type (Fig. 1A). Since the nucleus is thought to provide spatial information for positioning the medial actomyosin contractile ring at the onset of mitosis [16], it seems likely that the misplaced interphase nuclei result in the formation of the misplaced septa. These are cleaved after a delay [16], which probably accounts for the increased percentage of septated cells in *par1::ura4*$^+$ cells. An increase in septation, but not a defect in nuclear positioning, was noted in a previous study of a par1Δ par2Δ double mutant [8,9].

The observation that the nucleus is not centrally placed in *par1* mutants is intriguing. Microtubules are important for positioning the fission yeast nucleus [17], and loss of PP2A function or B-regulatory subunits causes loss of cell polarity and cytoskeletal aberrations [6]. Interphase microtubules in *par1::ura4*$^+$ cells with an eccentrically placed nucleus appeared shorter (Fig. 1C, panels 1–3), while those in cells with a centrally placed nucleus appeared normal (Fig. 1C,

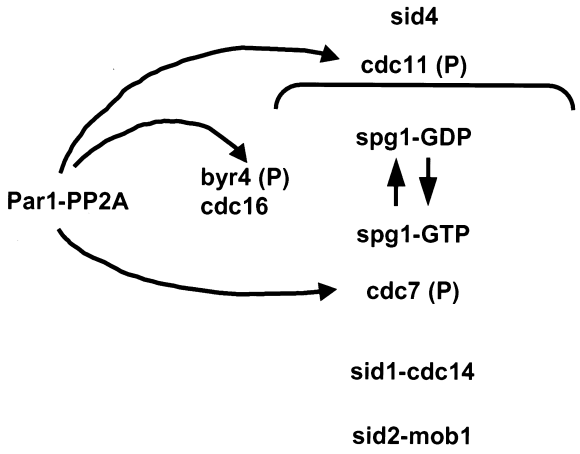



Fig. 5. Potential points of action of par1p. Schematic diagram of the *S. pombe* SIN indicating which components are known to be phosphoproteins (P) in *S. pombe* and potential sites of action of par1p-PP2A (arrows). Note that the order of action of SIN components remains speculative, and that the arrows between the two forms of spg1p indicate cycling between them. The bracket above spg1-GDP signifies that sid4p and cdc11p act as a scaffold for the other proteins on the spindle pole body.

panels WT). At present it is unclear whether the short microtubules are the cause of the mis-positioned nucleus, or result from it.

Mutants in *pom1* and *mid1* both have defects in positioning the division septum, and their products co-operate to define the position of the contractile ring [18–20]. We therefore examined the phenotypes of the double mutants *par1-35 mid1-6* and *par1-35 pom1::ura4*$^+$. At 36°C, *par1-35 mid1-6* cells exhibited multiple septa, but these were mostly at right angles to the long axis of the cell, compared to the random angles in a *mid1-6* single mutant (Fig. 1D). These data indicate that loss of par1p function can rescue the septum orientation defect of *mid1-6*. The residual multi-compartmented phenotype at 36°C resembles that of the single *par1* mutant [8,9]. No additive effects were observed in the double mutant with *pom1*, suggesting that *par1-35* may lie in the same pathway as *pom1* (not shown). Mid1p is a phosphoprotein [20], and is thought to be regulated by the protein kinase plo1p [18]. It is possible that the loss of PP2A-par1p allows the residual activity of the mutant mid1p to direct septation orthogonally to the long axis of the cell.

### 3.3. Genetic interactions between par1 and the SIN

Since *par1-35* was isolated as a suppressor of a mutant in *spg1*, we examined the effect of *par1* mutation on other SIN mutants. First, we found that the double mutant *par1-35 spg1-B8* was also capable of division at 36°C, indicating that *par1-35* could suppress multiple alleles of *spg1* (Fig. 2A). Next, *par1-35* was crossed to a strain in which the *spg1* null allele is rescued by increased expression of *cdc7* [21]. No viable progeny carrying the *spg1::ura4*$^+$ null without the presence of the *cdc7* expression plasmid were obtained at any temperature, indicating that *par1-35* cannot bypass the requirement for *spg1*. Crosses to other SIN mutants indicated that the double mutants of *par1-35* with either *cdc7-A20* or *cdc11-136* were capable of division at 36°C (Fig. 2B,C), while the mutant *cdc11-123 par1-35* was not (not shown). In contrast, the double mutant *sid2-250 par1-35* had a dramatically reduced non-permissive temperature, showing defects in septation and a branched morphology at 25°C (Fig. 2F), which are not seen in the single *sid2-250* mutant (Fig. 2G). A similar result was obtained for the double mutant of *par1::ura4*$^+$ with *mob1-R4* (not shown). No additive effect or rescue was observed in the double mutants of *par1-35* with either *cdc14-118* (Fig. 2E) or *sid1-239* (not shown). The double mutant *cdc16-116 par1-35* grew poorly at all temperatures, indicating a strong additive effect with *par1::ura4*$^+$. At 36°C cells were multiseptate (Fig. 2D), and resembled *cdc16-116*, while at 25°C, they displayed an aberrant morphology, and an increased number of septated and multi-compartmented cells were present (Fig. 2D). Similar results were obtained when examining the interactions between the *par1::ura4*$^+$ null allele, and the SIN, suggesting that *par1-35* phenocopies a null allele (not shown).

### 3.4. Localisation of par1p

To localise par1p, the protein was tagged by addition of GFP to the C-terminus at the chromosomal locus. The cells appeared wild-type, indicating that the tagged protein is functional. Examination of living cells indicated that in interphase a cytoplasmic fluorescence was observed, and the protein was excluded from the nucleus (Fig. 3, panel 1). In early mitotic cells, the protein was observed on the spindle pole bodies and the medial ring, though the spindle pole body staining was faint (Fig. 3, panel 2). In anaphase, par1p-GFP was visible on both poles of the mitotic spindle, and on the contractile ring (Fig. 3, panels 3, 4). In cells where the nuclei were undergoing the spindle pole body-directed reorientation at the end of mitosis [22], par1p-GFP was sometimes seen as a double ring (Fig. 3, panel 5). In septating cells the protein was seen on the spindle pole body, and was also concentrated in the medial region of the cell around the division septum, though it was not seen on the leading edge of the developing division septum (Fig. 3, panel 6). In separating cells, par1p-GFP was no longer present on the spindle pole body (Fig. 3, panel 7). This pattern of localisation differs from that observed previously [9], most notably in the finding that par1p is present on the medial ring and spindle pole body during mitosis. The reason for this discrepancy is unclear, but the most likely explanation is that the HA epitope tag used in the previous study is poorly preserved on the ring and spindle pole body after fixation.

### 3.5. Increased expression of pab1 rescues spg1

PP2A is a heterotrimer comprised of a catalytic subunit (C), a scaffold (A) and a regulatory or targeting subunit (B or B′) (reviewed by [5]), and it has been suggested that the B and B′ subunits compete for binding to the core A-C dimer [23,24]. Previous studies have shown that par1p is the major B′ subunit in fission yeast [9]. Loss of par1p would therefore favour accumulation of PP2A with mostly B, rather than B′ subunits bound to the core dimer. To test whether increased expression of the B subunit pab1p could also rescue the septation defect of *spg1*, expression of *pab1* was induced from the *nmt1* promoter, and cells were shifted to 36°C. Cells were able to septate, indicating that elevated expression of pab1p can permit a heat-sensitive mutant of *spg1* to septate (Fig. 4A). However, multinucleate cell compartments were still present, indicating an imperfect rescue. A similar result was obtained upon ex-

pression in a *cdc11-136* mutant (Fig. 4B). Since prolonged overexpression of pab1p is toxic [6], long-term colony formation could not be assessed. We were unable to test *cdc7-A20* in this assay, as the allele is very leaky in minimal medium at 36°C. In contrast, increased expression in other SIN mutants, such as *sid4-SA1* (Fig. 4C), *sid2-250* (Fig. 4D), and *sid1-239* (not shown), did not produce any septation. Thus, the spectrum of SIN mutants in which increased expression of pab1 will induce septation is similar to that which is rescued by *par1::ura4*$^+$.

### 3.6. Increased expression of par1 causes cells to divide at a reduced size

Expression of par1 from the full-strength *nmt1* promoter is toxic [8]. Induction of *par1* expression in a wild-type background from the thiamine-regulated medium-strength *nmt1-41* promoter [25] is tolerated, and the length of septated cells was reduced from approximately 15 μm to 8 μm, suggesting that cells were advanced into mitosis (Fig. 4E). None of the SIN mutants was rescued by increased expression of *par1* (data not shown).

## 4. Discussion

We have identified a mutant of *par1* in a genetic screen for regulators and effectors of SIN signalling. Since a null allele of *par1* rescues mutants that reduce the efficiency of SIN signalling, this implies that PP2A-par1p acts as a negative regulator of SIN signalling. This is also consistent with the additive effect with the *cdc16-116 par1-35* mutant at the permissive temperature for *cdc16-116*. The rescue of mutants in *spg1*, *cdc7*, and *cdc11* suggests that PP2A-par1p acts as a negative regulator of these proteins. Analysis of the interaction between null alleles of *par1*, *par2* and the SIN by others [26] has also shown that a *par1* null mutant will rescue the lethality of *spg1* thermosensitive, but not null mutants. The mutant *cdc7-A20* can be rescued by *par1::ura4*$^+$ (this study), while *cdc7-24* cannot [26]. This may be because *cdc7-A20* retains more residual activity than *cdc7-24* [27]. The ability to rescue mutants in *cdc11* correlates with the presence of the mutant protein on the spindle pole body [13], suggesting that PP2A-par1p may regulate cdc11p on the spindle pole body. The potential points of action of PP2A-par1p in the SIN are indicated in Fig. 5.

Protein kinases of the ndr/DBF2 family, of which sid2p is a member, are negatively regulated by PP2A [28], so it is surprising that we found a strong negative interaction between par1 mutants and both *sid2* and *mob1*. It is possible that PP2A acts at multiple levels in the SIN, playing both a negative and a positive role in regulating SIN signalling. Alternatively, altering the balance of PP2A regulatory subunits through removal of par1p may increase the concentration of a form of PP2A that is inhibitory for the sid2p-mob1p complex.

*Saccharomyces cerevisiae* Cdc15p, which is the orthologue of *S. pombe* cdc7p, is known to be regulated negatively by phosphorylation, and to be activated by the phosphatase Cdc14p [29,30]. However, flp1p/clp1p, the fission yeast orthologue of Cdc14p, seems to regulate cell cycle progression differently to Cdc14p [31,32], and though it interacts genetically with the SIN [31,32], the basis for this is unclear.

Par1p-GFP is found in the cytoplasm, consistent with the previous localisation of par1p [9] and the PP2A catalytic subunit ppa2p [7]. However, in contrast to the previous study of par1p, we found that during mitosis the protein is present both on the spindle pole bodies and on the medial ring, and is concentrated in the medial region of the cell during septum formation. These data are consistent with a role in regulation of the SIN, whose key elements are located on the spindle pole body and medial ring during mitosis.

To date, using two-hybrid analysis, we have been unable to detect any interaction between par1p and any component of the SIN. This may be because the interactions are transient, or because they require the presence of the PP2A core dimer. Since increased expression of the B subunit of PP2A also allows *spg1* and *cdc11* mutants to septate, it seems likely that the rescue of these mutants by mutation of *par1* is in part due to an alteration of the balance of B and B-type subunits associated with the PP2A core dimer. How this balance is used to regulate the SIN and septum positioning will be the subject of future studies.

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## References

[1] Chang, L. and Gould, K.L. (2000) Proc. Natl. Acad. Sci. USA 97, 5249–5254.
[2] Tanaka, K., Petersen, J., MacIver, F., Mulvihill, D.P., Glover, D.M. and Hagan, I.M. (2001) EMBO J. 20, 1259–1270.
[3] McCollum, D. and Gould, K.L. (2001) Trends Cell Biol. 11, 89–95.
[4] Yoshida, T., Toda, T. and Yanagida, M. (1994) J. Cell Sci. 107, 1725–1735.
[5] Mayer-Jaekel, R.E. and Hemmings, B.A. (1994) Trends Cell Biol. 4, 287–291.
[6] Kinoshita, K., Nemoto, T., Nabeshima, K., Kondoh, H., Niwa, H. and Yanagida, M. (1996) Genes Cells 1, 29–45.
[7] Kinoshita, N., Yamano, H., Niwa, H., Yoshida, T. and Yanagida, M. (1993) Genes Dev. 7, 1059–1071.
[8] Tanabe, O., Hirata, D., Usui, H., Nishito, Y., Miyakawa, T., Igarashi, K. and Takeda, M. (2001) Genes Cells 6, 455–473.
[9] Jiang, W. and Hallberg, R.L. (2000) Genetics 154, 1025–1038.
[10] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Handbook, 2nd eds., Cold Spring Harbor Laborator Press, Cold Spring Harbor, NY.
[11] Moreno, S., Klar, A. and Nurse, P. (1991) Methods Enzymol. 194, 795–823.
[12] Bahler, J. et al. (1998) Yeast 14, 943–951.
[13] Krapp, A., Schmidt, S., Cano, E. and Simanis, V. (2001) Curr. Biol. 11, 1559–1568.
[14] Balasubramanian, M.K., McCollum, D. and Gould, K.L. (1997) Methods Enzymol. 283, 494–506.
[15] Hagan, I.M. and Hyams, J.S. (1988) J. Cell Sci. 89, 343–357.
[16] Tran, P.T., Doye, V., Chang, F. and Inoue, S. (2000) Biol. Bull. 199, 205–206.
[17] Tran, P.T., Marsh, L., Doye, V., Inoue, S. and Chang, F. (2001) J. Cell Biol. 153, 397–411.
[18] Bahler, J., Steever, A.B., Wheatley, S., Wang, Y., Pringle, J.R., Gould, K.L. and McCollum, D. (1998) J. Cell Biol. 143, 1603–1616.
[19] Chang, F., Woollard, A. and Nurse, P. (1996) J. Cell Sci. 109, 131–142.
[20] Sohrmann, M., Fankhauser, C., Brodbeck, C. and Simanis, V. (1996) Genes Dev. 10, 2707–2719.

[21] Schmidt, S., Sohrmann, M., Hofmann, K., Woollard, A. and Simanis, V. (1997) Genes Dev. 11, 1519–1534.
[22] Hagan, I. and Yanagida, M. (1997) J. Cell Sci. 110, 1851–1866.
[23] Shu, Y., Yang, H., Hallberg, E. and Hallberg, R. (1997) Mol. Cell. Biol. 17, 3242–3253.
[24] Evans, D.R. and Hemmings, B.A. (2000) Mol. Gen. Genet. 264, 425–432.
[25] Basi, G., Schmid, E. and Maundrell, K. (1993) Gene 123, 131–136.
[26] Jiang, W. and Hallberg, R.L. (2001) Genetics 158, 1413–1429.
[27] Fournier, N., Cerutti, L., Beltraminelli, N., Salimova, E. and Simanis, V. (2001) Arch. Microbiol. 175, 62–69.
[28] Millward, T.A., Hess, D. and Hemmings, B.A. (1999) J. Biol. Chem. 274, 33847–33850.
[29] Menssen, R., Neutzer, A. and Seufert, W. (2001) Curr. Biol. 11, 345–350.
[30] Jaspersen, S.L. and Morgan, D.O. (2000) Curr. Biol. 10, 614–618.
[31] Cueille, N., Salimova, E., Esteban, V., Blanco, M., Moreno, S., Bueno, A. and Simanis, V. (2001) J. Cell Sci. (in press).
[32] Trautmann, S., Wolfe, B.A., Jorgensen, P., Tyers, M., Gould, K.L. and McCollum, D. (2001) Curr. Biol. 11, 931–940.