Schizosaccharomyces pombe NIMA-related kinase, Fin1, regulates spindle formation and an affinity of Polo for the SPB

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The *Aspergillus nidulans* protein kinase NIMA regulates mitotic commitment, while the human and *Xenopus* equivalents influence centrosome function. Two recessive, temperature-sensitive mutations in the *Schizosaccharomyces pombe* NIMA homologue, Fin1, blocked spindle formation at 37°C. One of the two spindle pole bodies (SPBs) failed to nucleate microtubules. This phenotype was reduced by accelerating mitotic commitment through genetic inhibition of Wee1 or activation of either Cdc25 or Cdc2. Polo kinase (Plo1) normally associates with the SPB of mitotic, but not interphase cells. *cut12.s11* is a dominant mutation in an SPB component that both suppresses *cdc25* mutants and promotes Plo1 association with the interphase SPB. Both *cut12.s11* phenotypes were abolished by removing Fin1 function. Elevating Fin1 levels promoted Plo1 recruitment to the interphase SPB of wild-type cells and reduced the severity of the *cdc25.22* phenotype. These data are consistent with Fin1 regulating Plo1 function during mitotic commitment. The *fin1* mitotic commitment and spindle phenotypes resemble distinct *nimA* phenotypes in different systems and suggest that the function of this family of kinases may be conserved across species.
Keywords: cell cycle/mitosis/NIMA kinase/polo kinase/ *Schizosaccharomyces pombe*

Introduction

It is well established that the kinase activity of the $p34^{cdc2}$ cyclinB complex known as MPF regulates commitment to mitosis (Ohi and Gould, 1999). Once MPF has been activated, polo, aurora and NIMA-related kinases (Nrks) control most of the physical events of mitosis and cytokinesis (Nigg, 2001).

In interphase, Wee1 and related protein kinases phosphorylate and inhibit the $p34^{cdc2}$ catalytic subunit of MPF. This inhibitory phosphate is removed by the phosphatase Cdc25. The balance between Wee1- and Cdc25-related activities controls mitotic commitment (Ohi and Gould, 1999). An initial trigger level of MPF promotes further activation of Cdc25 to drive commitment to mitosis (Hoffmann *et al*., 1993), so that the activity of the bulk of Cdc25 is actually dependent upon $p34^{cdc2}$ activity

(Kovelman and Russell, 1996). While direct phosphorylation of Cdc25 by MPF does contribute to positive feedback loop activation of Cdc25 (Izumi and Maller, 1993), the loop still exists in *Xenopus* extracts from which both cdc2 and cdk2 have been depleted (Izumi and Maller, 1995). Polo kinases are thought to act in the feedback loop, because Plk neutralizing antibodies block mitotic commitment in non-transformed human cells (Lane and Nigg, 1996) and the *Xenopus* Plk, Plx, was purified as a Cdc25 activating kinase (Kumagai and Dunphy, 1996). Plx influences the rate of commitment to mitosis rather than mitotic commitment *per se* (Abrieu *et al*., 1998). Although supporting evidence is currently scant, Polo may also downregulate Wee1-related kinases. Wee1 and the related kinase Myt1 are both hyperphosphorylated upon mitotic commitment, and the *Saccharomyces cerevisiae* Plk, Cdc5p, binds and inhibits the activity of the Wee1 kinase, Swe1p (Tang *et al*., 1993; Honda *et al*., 1995; Mueller *et al*., 1995; Bartholomew *et al*., 2001).

The behaviour of the *Schizosaccharomyces pombe* polo kinase, Plo1, in *cut12.s11* mutants suggests that the association of polo kinase with the spindle pole may play a critical role in MPF activation. *cut12/stf1*[+] encodes an essential component of the spindle pole body (SPB). In the recessive *cut12.1* mutant only one of the two poles nucleates microtubules (Bridge *et al*., 1998). Dominant mutations in the same gene, called *stf* mutations (suppressor of twenty-five), enable cell division in the absence of Cdc25 (Hudson *et al*., 1990). Plo1 usually associates with mitotic, but not the interphase, SPBs (Bähler *et al*., 1998; Mulvihill *et al*., 1999). In *stf1.1* mutants (referred to here as *cut12.s11*), Plo1 associates with SPBs throughout the cell cycle (Mulvihill *et al*., 1999). Thus, the *cut12.s11* mutation confers mitotic behaviour to interphase Plo1.

In contrast to the conservation of function between species for polo- and aurora-related kinases, data do not currently suggest a similar conservation of function for the Nrks (reviewed in Nigg, 2001). *nimA* mutants were identified in a screen for conditional *Aspergillus nidulans* mutations that were unable to enter mitosis at the restrictive temperature (the never in mitosis phenotype; Morris, 1975). The *nimA* arrest point has all the features of interphase cells. The microtubules are cytoplasmic, spindles are absent and the chromosomes are not condensed (Oakley and Morris, 1983). Despite this apparent arrest before mitotic commitment, mitotic NIMA kinase activity is dependent upon prior activation of MPF (Ye *et al*., 1995).

The strength of the *A.nidulans* phenotype suggested that Nrks may play an important role in mitotic control in other systems. However, deletion of the *S.cerevisiae* and *S.pombe* Nrks suggested that this was not the case, as these cells survived (Jones and Rosamond, 1990; Krien *et al*., 1998). The functional data obtained to date for the

human NIMA-related protein, Nek2, suggest that it regulates centrosome function, rather than mitotic commitment. Nek2 associates with the centrosome. Overproduction of Nek2 in interphase cells drives the premature separation of the two centrosomes (Fry *et al.*, 1998). Similarly, Nek2B is required for the efficient centrosome maturation in *Xenopus* egg extracts (Fry *et al.*, 2000; Uto and Sagata, 2000). Although overproduction of *nimA* also blocks spindle formation (Osmani *et al.*, 1988a), the similarity of function between Nrks in different species remains in doubt (Nigg, 2001).

We have identified two temperature-sensitive, loss-of-function mutations in the *S.pombe* Nrk, *fin1+*, that could not form a mitotic spindle at 37°C. Genetic interactions suggested that Fin1 plays an important role in MPF activation during mitotic commitment. The recruitment of Plo1 to the interphase SPB of *cut12.s11* mutants required Fin1 activity, while increasing *fin1+* transcription mimicked the *cut12.s11* phenotype. These data are discussed in terms of a role for Fin1 in the MPF amplification feedback loop.

Results

Isolation of sad44.1

We have screened for genes that are required for correct architecture of the mitotic spindle (A.Grallert, R.A.Craven, F.E.Stevens and I.M.Hagan, unpublished). SPBs were labelled with a Cut12/GFP fusion protein (Bridge *et al.*, 1998) and conditional diploidizing mutants were isolated according to the methodology of Broek *et al.* (1991). Mutants that grew at 25°C but increased ploidy after one generation at 36°C were screened by DAPI and GFP fluorescence at 36°C to identify those in which spindle architecture was temperature sensitive. Immunofluorescence microscopy then confirmed the *sad⁻* (spindle architecture defective) phenotype. Here we focus on *sad44+*. The severity of the *sad44.1* spindle formation phenotype was reduced from 93 to 76% as the *cut12.NEGFP* allele was switched for a wild-type *cut12+* gene during outcrossing. Re-introduction of *cut12.NEGFP* to an outcrossed *sad44.1* mutation restored the spindle formation deficiency at 37°C to 93%.

sad44+ is allelic to fin1+

Screening a genomic library for sequences that could complement *sad44.1* temperature sensitivity identified three different plasmids that contained the *fin1+* gene. Crossing a *sad44.1* strain to a *fin1* deletion allele, *fin1.Δ* (Krien *et al.*, 1998), showed no recombination between the *ts⁻* of *sad44.1* and the *ura4+* of *fin1.Δ* in 81 tetrads. There was a single amino acid change from glycine to glutamic acid at position 188 of the *fin1+* open reading frame (ORF) in *sad44.1*. This residue lay within domain VIII (P + 1 loop) and is conserved in the majority of kinases. This established that *sad44.1* is allelic to *fin1* and so *sad44.1* will be referred to as *fin1.ts1* from here on.

Fin1 inactivation affects spindle formation and function

We were surprised to isolate the *fin1.ts1* mutation in a screen for *ts⁻* mutations, because the *fin1+* gene is not required for viability (Krien *et al.*, 1998). We therefore
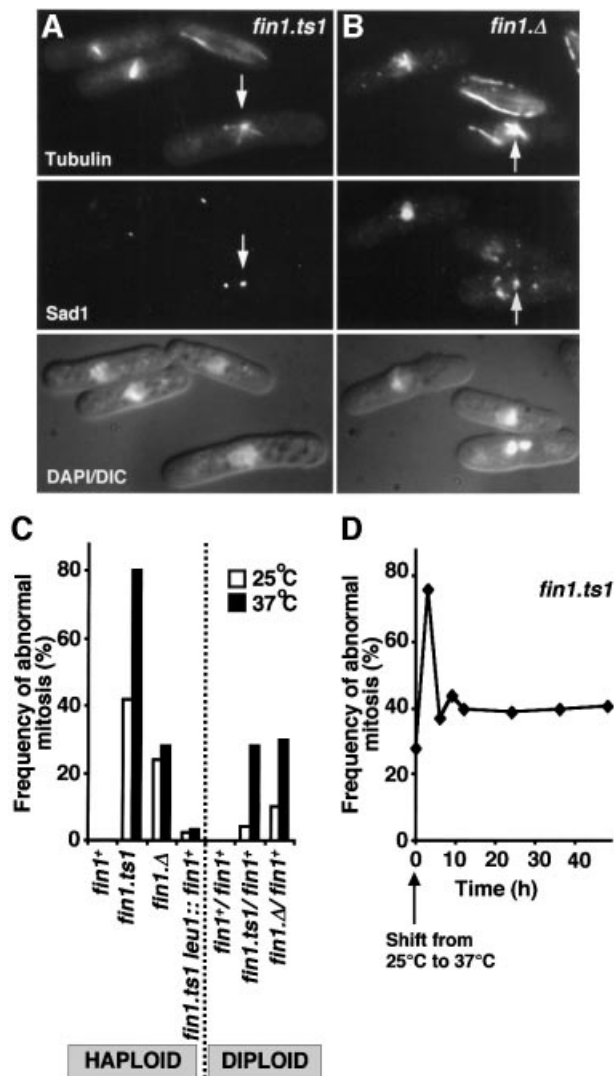
Fig. 1. *fin1* mutants have defects in spindle formation. The indicated strains were grown to early log phase in EMM2 at 25°C before the temperature of the culture was shifted to 37°C for 3 h. (A and B) Micrographs showing three images of the same field of cells shows from top to bottom microtubules, Sad1 and a combined DAPI DIC image. (**A**) *fin1.ts1* cells at 37°C; (**B**) *fin1.Δ* cells at 37°C. Arrows indicate Sad1 staining of a functional SPB that is nucleating far more microtubules than the other. (**C**) The proportion of mitoses that exhibit spindle formation defects at the indicated temperatures for the haploid and diploid strains is indicated. (**D**) Mitotic profiles were scored in *fin1.ts1* cells for 48 h after the temperature was changed from 25 to 37°C, and the proportion of those that had defective structure was calculated and plotted against time.

compared the phenotypes of the *fin1.ts1* and *fin1.Δ* strains after 3 h at 37°C. Both strains had mitotic defects (Figure 1A and B). In each case one spindle pole appeared to be nucleating a monopolar spindle (arrows in Figure 1A and B) whilst the other was either inactive or its nucleating capacity was greatly reduced. The defect was more severe in a *fin1.ts1* (76% defective; Figure 1A and C) than *fin1.Δ* strain (26% defective; Figure 1B and C) at 37°C.

Because complete removal of the *fin1+* gene from *fin1.Δ* cells did not affect viability, the severe phenotype of the *fin1.ts1* suggested that *fin1.ts1* was a dominant mutation. Unexpectedly however, the *fin1.ts1/fin1+* heterozygous

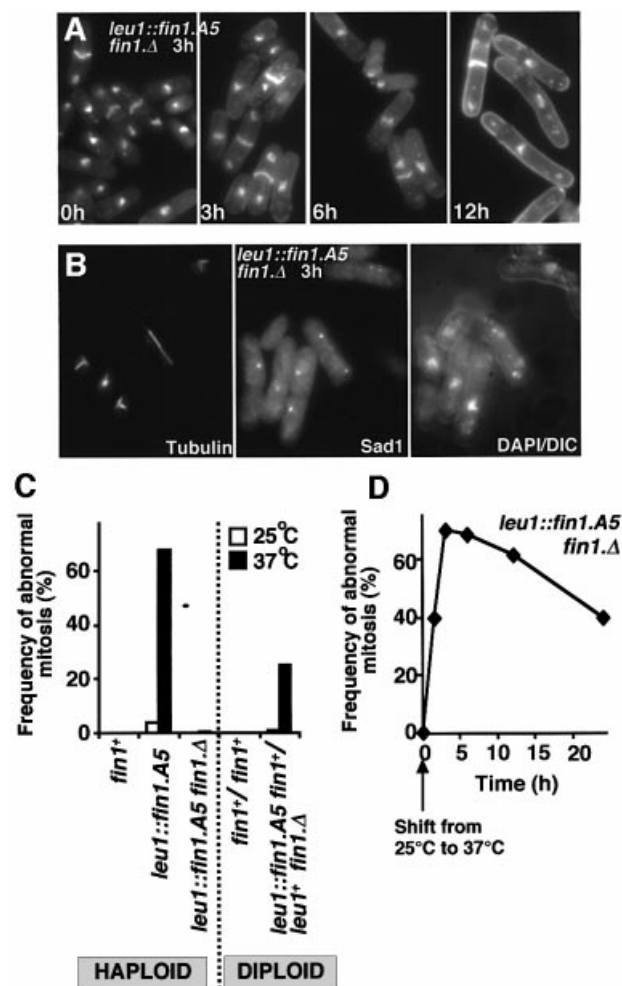**Table I.** The mitotic checkpoint is required for viability of *fin1* mutants

Strain	Viability at 25°C
mad2.Δ	++++++
mph1.Δ	++++++
Δbub1	++++++
fin1.ts1	++++
fin1.Δ	++++++
fin1.ts1 mad2.Δ	+
fin1.Δ mad2.Δ	++
fin1.ts1 mph1.Δ	Dead at all temperatures
fin1.Δ mph1.Δ	Dead at all temperatures
fin1.ts1 Δbub1	Dead at all temperatures
fin1.Δ Δbub1	Dead at all temperatures

Fig. 2. Temperature-sensitive defects in spindle formation and cell cycle progression in *fin1.A5* cells. (A, B and D) *fin1.A5* cells were grown to early log phase in EMM2 at 25°C before the temperature of the culture was shifted to 37°C for the times indicated. (**A**) DAPI/ calcofluor staining of *fin1.A5* at 37°C. Note the cell elongation 12 h after the temperature shift. (**B**) Micrographs showing three images of the same field of cells 3 h after the temperature shift. From left to right; microtubules, Sad1 staining and a combined DAPI DIC image. (**C**) The proportion of mitoses that exhibit spindle formation defects at the indicated temperatures for the haploid and diploid strains is indicated. (**D**) Mitotic profiles were scored in *fin1.A5* cells at distinct time points for 24 h after the temperature shift, and the relative proportion of those that were defective was plotted against time.

diploid had the same degree of spindle formation defect as a *fin1.Δ/fin1+* diploid at 37°C (Figure 1C). Furthermore, integration of a wild-type copy of *fin1+* under the control of its own promoter into the *leu1* locus of a haploid *fin1.ts1* strain effectively abolished the spindle formation defect conferred by the *fin1.ts1* mutation (*fin1.ts1 leu1::fin1+*; Figure 1C). These data establish that, rather than being dominant, *fin1.ts1* was a recessive, loss-of-function mutation and raised the question of why a recessive point mutation conferred a more severe defect than deletion of the same gene. Monitoring spindle formation in *fin1.ts1* culture over 48 h showed that, although *fin1.ts1* cells had a severe defect in the first division at 37°C, they adapted to the loss of Fin1 function. After 6 h at the restrictive temperature the severity of spindle defect in *fin1.ts1* dropped towards the level seen in *fin1.Δ* (Figure 1D). We concluded that the severe phenotype of a *fin1.ts1* mutant is a transitory response to loss of Fin1 function. This implied that *fin1.Δ* haploids adapted to loss of Fin1 after the first division of a germinating spore.

As both *fin1.ts1* and *fin1.Δ* have constitutive spindle defects after adaptation, we asked whether the spindles in these surviving cells were still defective and whether the viability of these strains depended upon spindle checkpoint controls. The viability of both mutations at 25°C was greatly reduced when *mad2+* was deleted (He *et al.*, 1997) and neither mutant could grow at any temperature from 20 to 37°C if *mph1+* or *bub1+* was deleted (Bernard *et al.*, 1998; He *et al.*, 1998; Table I).

fin1.A5 delays mitotic commitment and blocks spindle formation

We were keen to establish whether the unusual transitory phenotype of *fin1.ts1* was unique to this allele or whether it was a general feature of recessive conditional *fin1* mutants. The *A.nidulans nimA5* mutation changes Tyr91 to Asp (Pu *et al.*, 1995). As this residue is conserved in all Nrks, we introduced the equivalent Y84N mutation in *fin1* (*fin1.A5*) and expressed the gene from its native promoter at the *leu1* locus in a *fin1.Δ* background (*leu1::fin1.A5 fin1.Δ*). *leu1::fin1.A5 fin1.Δ* cells entered mitosis, but arrested with the same spindle formation defect as *fin1.ts1* (Figure 2B and C). At 37°C a diploid *fin1.Δ leu1::fin1.A5/ fin1+ leu1+* strain had the same 26% deficiency in spindle structure as that of a *fin1.ts1/fin1+*

or a *fin1.Δ/fin1+* diploid (Figures 1C and 2C). The spindle defect of a haploid *leu1::fin1.A5* strain was suppressed by the inclusion of a wild-type gene at the *fin1+* locus (Figure 2C). Thus, like *fin1.ts1*, *fin1.A5* is a recessive, loss-of-function mutation. The reduction in the frequency of defective spindles upon prolonged incubation at 37°C of *fin1.A5* also resembled that seen in *fin1.ts1* (Figure 2D). There was a marked increase in cell length at commitment to mitosis during this extended incubation of *fin1.A5* cells at 37°C (Figure 2A; Table II). This cdc phenotype was recessive as *fin1.Δ leu1::fin1.A5/ fin1+ leu1+* heterozygous diploids divided at the same length as wild-type controls at 37°C (data not shown). While *fin1.Δ* cells also delay the timing of mitotic commitment (Table II; Krien *et al.*, 1998), *fin1.ts1* cells do not (Table II). We concluded that Fin1 was required for spindle formation and that the

Table II. Cell lengths upon commitment to mitosis

|	Length of dividing cell (μm), EMM2	Length of dividing cell (μm), YES
Wild type, 25°C	11.5 ± 1.1	12.8 ± 0.78
Wild type, 37°C, 3 h	12.6 ± 0.82	13.8 ± 1.44
fin1.Δ, 25°C	12.6 ± 1.3	12.3 ± 1.1
fin1.Δ, 37°C, 3 h	14.6 ± 0.98	13.5 ± 1.1
fin1.ts1, 25°C	11.9 ± 0.94	13.3 ± 1.7
fin1.ts1, 37°C, 3 h	13.0 ± 1.63	13.8 ± 1.4
leu1::fin1.A5 fin1.Δ, 25°C	13.4 ± 1.3	13.1 ± 0.98
leu1::fin1.A5 fin1.Δ, 37°C, 3 h	14.7 ± 0.88	13.3 ± 0.68
leu1::fin1.A5 fin1.Δ, 37°C, 6 h	15.5 ± 1.1	14.9 ± 3.2
leu1::fin1.A5 fin1.Δ, 37°C, 9 h	16.1 ± 2.4	18.3 ± 3.6
leu1::fin1.A5 fin1.Δ, 37°C, 12 h	17.4 ± 3.3	19.2 ± 3.9
wee1.6, 25°C	8.0 ± 0.67	8.4 ± 0.74
wee1.6, 37°C, 3 h	7.9 ± 0.84	8.1 ± 0.97
fin1.ts1 wee1.6, 25°C	11.1 ± 1.4	10.3 ± 1.7
fin1.ts1 wee1.6, 37°C, 3 h	9.9 ± 1.6	10.7 ± 1.04
pREP41fin1, no induction	11.5 ± 1.1	ND
pREP41fin1, 20 h induction	12.7 ± 1.1	ND
pREP1fin1, no induction	12.1 ± 1.9	ND
pREP1fin1, 20 h induction	13.4 ± 2.1	ND

ND, not determined.

fin1.A5 phenotype may have indicated a function in cell cycle control.

Defective spindle formation upon Fin1 overproduction

Krien *et al*. (1998) established that excessive *fin1+* transcription from the strong *nmt1+* promoter drove chromosome condensation in interphase cells, but their study did not address spindle architecture. We therefore monitored spindle architecture and chromosome morphology as the expression of multicopy plasmid borne *fin1+* was induced from either the strong (nmt1 in pREP1), intermediate (nmt41 in pREP41) or weak (nmt81 in pREP81) versions of the repressible *nmt1+* promoter (Maundrell, 1990; Basi *et al*., 1993). Spindle function was far more sensitive to increased expression of *fin1+* than interphase chromosome condensation. Monopolar (Figure 3A) and metaphase (Figure 3B) spindles accumulated upon induction from all three promoters (Figure 3C), while condensed chromosomes in interphase cells were only seen after expression from the strongest *nmt1* promoter (Figure 3C). Deletion of either *mad2+* or *bub1+* from the host cell abolished delay at the metaphase/anaphase transition arising from expression of pREP41fin1+ (Figure 4A) (He *et al*., 1997; Bernard *et al*., 1998). Many of the checkpoint-deficient anaphase cells were clearly elongating spindles despite considerable defects in chromatid separation (Figure 4B and C). This established that the accumulation of metaphase cells after induction of *fin1+* was due to altered spindle function, rather than an inability to regulate this transition *per se*. We concluded that spindle formation was more sensitive to elevation or depletion of Fin1 levels than chromosome condensation.

Genetic interactions between fin1 mutants, and cut12/stf1 and mitotic regulators

Outcrossing the original *fin1.ts1 cut12.NEGFP* isolate indicated that the *cut12.NEGFP* allele enhanced the spindle formation deficiency conferred by *fin1.ts1*
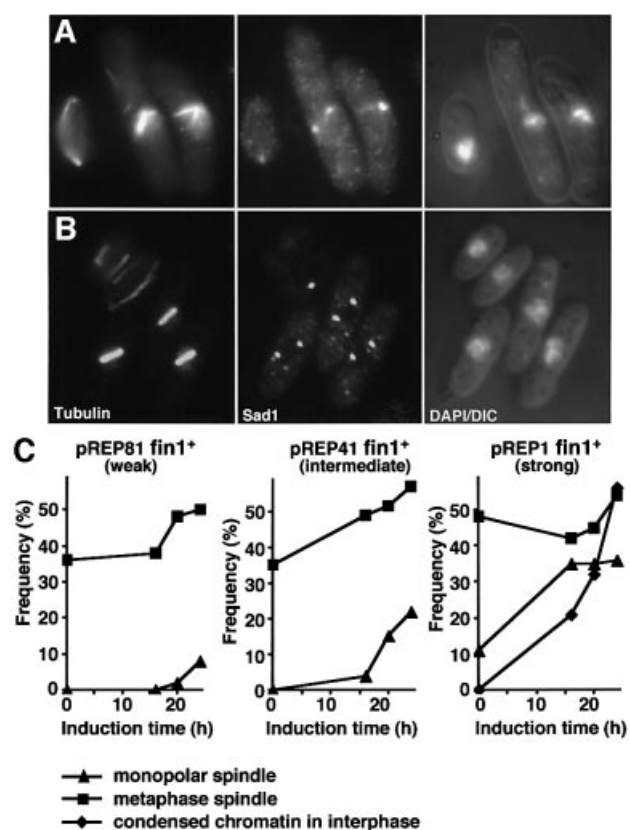
Fig. 3. *fin1+* overproduction induces defects in spindle function before it accumulates to a level that will induce chromosome condensation in interphase. Cells bearing pREP41fin1+ (*fin1+* controlled by the *nmt41* promoter) (A and C), pREP81fin1+ (*fin1+* controlled by the *nmt81* promoter) or pREP1fin1+ (*fin1+* controlled by the *nmt1* promoter) (C) were grown to early log phase in EMM2 + thiamine at 25°C before being washed and resuspended in thiamine-free EMM2 to induce gene expression. Cells were followed for 24 h. (**A** and **B**) Micrographs of pREP41fin1+ cells 16 h after induction. From left to right, microtubules, Sad1 and a combined DAPI DIC image. (**C**) The proportion of mitotic profiles that had either monopolar or metaphase spindles was plotted along with the proportion of total cells that had chromosome condensation in interphase.
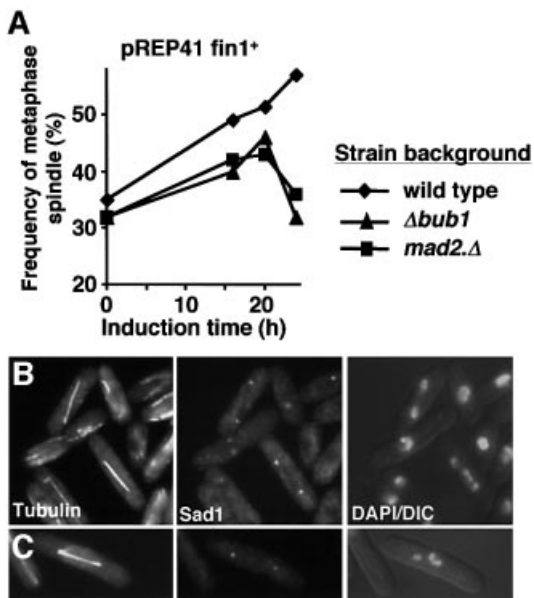
Fig. 4. Metaphase arrest arising from *fin1+* overproduction is due to an active spindle assembly checkpoint. Cultures of *mad2.Δ* or *Δbub1* strains containing pREP41fin1+ were grown to early log phase in EMM2 + thiamine at 25°C before being washed and resuspended in thiamine-free EMM2 to induce gene expression. Cells were followed for 24 h. (**A**) The proportion of mitotic cells that had metaphase spindles was scored for each strain. (B and C) Micrographs of *mad2.Δ* pREP41fin1+ (**B**) or *Δbub1* pREP41fin1+ (**C**) cells 20 h after induction. From left to right, microtubules, Sad1 and a combined DAPI DIC image.

(Figure 5A, B and D). As spindle formation is usually normal in *cut12.NEGFP* cells (Figure 5C), this interaction suggested a functional relationship between *fin1* and *cut12*. To investigate this possibility further, we looked for genetic interactions between *fin1* mutants and two different *cut12* alleles. *cut12.1* is a recessive temperature-sensitive mutation that confers a spindle formation defect at 36°C (Bridge *et al.*, 1998). This defect is very similar to the *fin1⁻* spindle defect. *cut12.1* reduces the restrictive temperature of *cdc25.22* from 30 to 20°C. *cut12.s11* is a dominant mutation that has normal spindle architecture but suppresses the mitotic commitment phenotypes of *cdc25.22* and *cdc25.Δ* (Hudson *et al.*, 1990; Bridge *et al.*, 1998).

Double mutants containing *cut12.1* and either *fin1.Δ* or *fin1.ts1* were dead at all temperatures (data not shown). In contrast, *cut12.s11* reduced the severity of the *fin1.ts1* spindle formation defect (Figure 5D, most apparent at 20°C). Thus, *cut12* loss-of-function mutations exacerbated the *fin1⁻* phenotype, while gain-of-function mutations reduced the requirement for Fin1. Overexpression of *fin1+* underscored the functional relationship between these two genes, as it both raised the restrictive temperature for *cut12.1* from 30 to 34°C (Figure 6A) and mimicked the effect of the *cut12.s11* in raising the restrictive temperature for *cdc25.22* cells (Figure 6B).

The suppression of *cdc25.22* following increased expression of *fin1+* suggested that Fin1 may positively regulate commitment to mitosis. If this were the case, then independently promoting mitotic commitment should reduce the requirement for Fin1. Commitment to mitosis
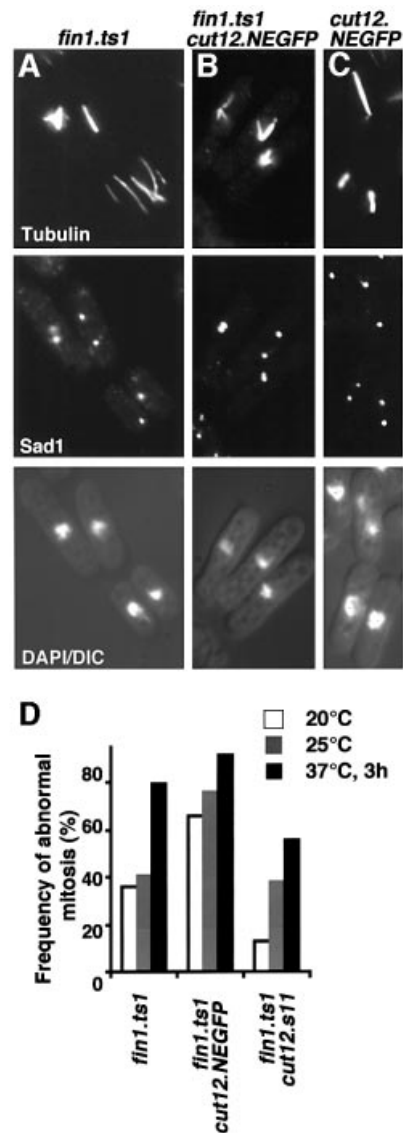
Fig. 5. The spindle formation defect of *fin1.ts1* is exacerbated by *cut12.NEGFP* and suppressed by *cut12.s11*. (A–C) Cells were grown to mid-log phase in EMM2 at 25°C before processing for immunofluorescence microscopy. Micrographs showing three images of the same field of cells from top to bottom microtubules, Sad1 staining and a combined DAPI DIC image. (**A**) *fin1.ts1*; (**B**) *fin1.ts1 cut12.NEGFP*; (**C**) *cut12.NEGFP*. (**D**) The proportion of mitotic cells that had defects in spindle morphology at different temperatures for the indicated strains. Cells were grown overnight to mid-log phase at the indicated temperatures with the exception of 37°C cells, which were first grown at 25°C overnight to early log phase before the temperature was changed to 37°C for 3 h. For each strain >200 mitotic cells were counted.

can be accelerated by abolishing the function of the mitotic inhibitor Wee1 or by mutations in *cdc2* that make the protein somewhat independent of regulation by either Wee1 or Cdc25 (Thuriaux *et al.*, 1978; Fantes, 1979; Russell and Nurse, 1987). When *wee1.6*, *wee1.50*, *cdc2.1w* or *cdc2.3w* were introduced into *fin1.ts1* cells, the severity of the spindle formation defect was reduced (Figure 7A). Cdc25 is regulated, in part, by regulating the translation of *cdc25+* message (Daga and Jimenez, 1999). Deletion of a control element in the 5′ untranslated region (UTR) of *cdc25+* generates a hyperactive allele, *cdc25.d1*, that provides yet another way of accelerating commitment to mitosis (Daga and Jimenez, 1999). Expression of *cdc25.d1*
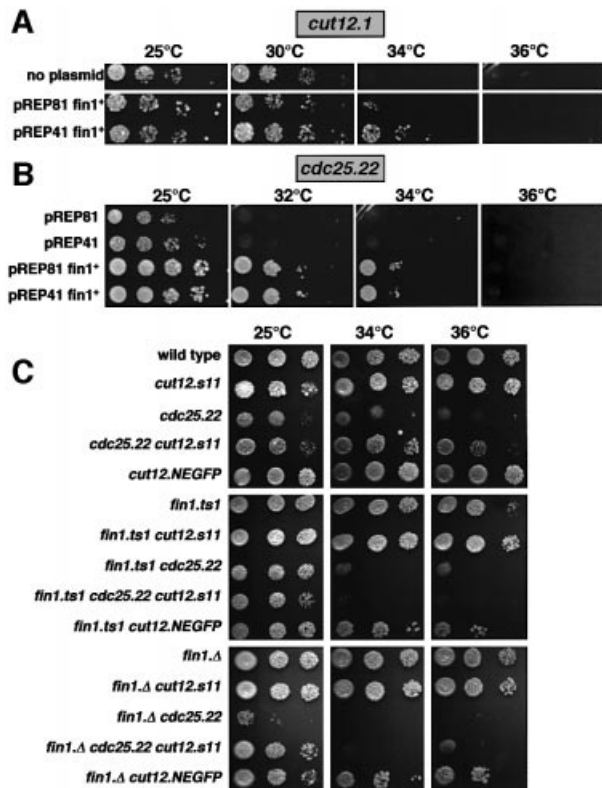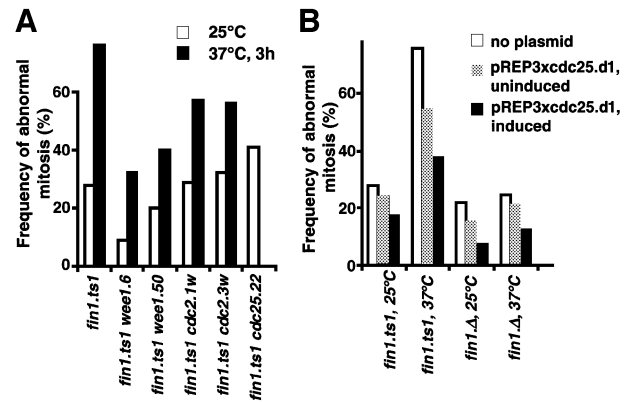
Fig. 6. Increasing *fin1*+ gene dosage raises the restrictive temperature of *cut12.1* and *cdc25.22*, while loss of *fin1* function abolishes the ability of *cut12.s11* to suppress *cdc25.22*. Five-fold dilution series of strains were spotted onto EMM2 and the plates were incubated at the temperatures indicated. (**A**) *cut12.1* bearing pREP41fin1+, pREP81fin1+ or no plasmid; (**B**) *cdc25.22* bearing empty vectors, pREP41fin1+ or pREP81fin1+. (**C**) The indicated strains without any plasmids.

Fig. 7. Acceleration of mitotic commitment reduces the spindle defect of *fin1* mutants. (**A**) Cells were grown overnight to mid-log phase at 25°C (no shading) or were at 25°C overnight to early log phase before the temperature was changed to 37°C for 3 h (black). For each strain in (A) and (B), >200 mitotic cells were counted. (**B**) *fin1.ts1* cells containing pREP3xcdc25.d1 were grown to mid-log phase in EMM2 containing thiamine, washed in thiamine-free medium, resuspended at a lower density and 13 h later the culture was split in two and the temperature of one half was shifted to 37°C (37°C) for 3 h whilst the other was maintained at 25°C (25°C). The bar chart shows the proportion of mitotic cells that had defects in spindle morphology. In all strains the expression of the *cdc25.d1* allele reduced the severity of the spindle defect and the strength of suppression correlated with the strength of expression.
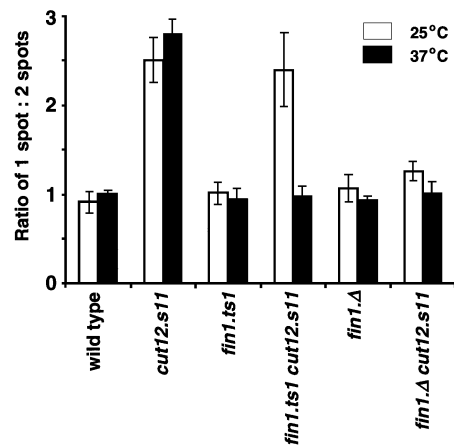
Fig. 8. Premature recruitment of Plo1 to the SPB of *cut12.s11* cells depends upon Fin1 function. Cells were grown to mid-log phase in EMM2 at 25°C and either immediately processed for anti-Plo1 immunofluorescence, or processed 3 h after the temperature of the culture had been changed to 37°C. The ratio of cells with one spot of Plo1 SPB staining versus the number of cells with two spots of Plo1 SPB staining was scored for the strains indicated on the graph (see text for details). The graph is the result of four independent experiments, and 200 cells were counted for each strain in each experiment.

again reduced the extent of the spindle formation defect conferred by *fin1.ts1* (Figure 7B).

### Fin1 function is required for the premature recruitment of Plo1 to the SPB by cut12.s11

The genetic interactions in the previous section suggested that the functions of Fin1 and Cut12 may be related: both molecules are required for spindle formation; accentuated function in either gene reduces the defects arising from deficiencies in the other; simultaneously reducing the function of both kills the cell; and manipulation of either can suppress the *cdc25.22* mutation. We therefore asked whether the suppression of *cdc25.22* by *cut12.s11* was dependent upon Fin1 function, and found that it was. Introduction of either *fin1.ts1* or *fin1.Δ* to a *cut12.s11 cdc25.22* mutant background abolished the growth of *cdc25.22* above 30°C that had been conferred by the *cut12.s11* mutation (Figure 6C).

Polo kinase is proposed to act during the MPF-amplifying feedback loop that activates Cdc25 and downregulates Wee1. *cut12.s11* may bypass *cdc25.22* by influencing polo kinase function in this feedback loop. There is no discrete localization of the *S.pombe* polo kinase, Plo1, during interphase; upon mitotic commitment it is recruited to the SPB (Bähler *et al.*, 1998; Mulvihill *et al.*, 1999). Strong Plo1 staining is seen until anaphase. In *cut12.s11* mutant cells, strong Plo1 SPB staining is seen in

interphase as well as mitotic cells (Mulvihill *et al.*, 1999). This premature recruitment of Plo1 to the SPB is apparent when scoring the number of single versus double dots of Plo1 staining. Single dots represent interphase or early mitotic SPBs, while two dots represent the SPBs at a range of stages of mitosis. Strong association of Plo1 with the SPB is the earliest mitotic event recorded to date (Mulvihill *et al.*, 1999). This strong association persists through to the start of anaphase when the staining intensity

reduces. Plo1 associates with a single SPB structure for the same amount of time as it takes to proceed from this stage to anaphase. This means that the ratio of cells with a single spot of Plo1 to those with two spots is around 1 in a wild-type strain (Figure 8). In a *cut12.s11* mutant this value increases to 2.8 because the increase in the association of Plo1 with the interphase SPB increases the number of single spots.

We were able to use the one to two spot assay to ask whether Fin1 was also required for the premature recruitment of Plo1 to the interphase SPB in *cut12.s11*. The ratio of one versus two spots of Plo1 staining was reduced from the *cut12.s11* level of 2.8 to the wild-type level of 0.92 at both 25°C and 37°C when *fin1⁺* was deleted (Figure 8). More significantly, the temperature sensitivity in Fin1 function conferred by the *fin1.ts1* mutation was mirrored by temperature-dependent association of Plo1 with the interphase SPB in *cut12.s11 fin1.ts1* cells (Figure 8). It associated with the SPB at 25°C when Fin1 was working, but did not associate at 37°C when Fin1 was defective.

As Fin1 was required for spindle formation (Figures 1 and 2), it was possible that compromising Fin1 function in the previous cycle led to an inherited change in SPB structure that affected Plo1 recruitment in the subsequent cell cycle. In this case the altered behaviour of the *cut12.s11* SPB in a *fin1⁻* background was a secondary consequence of the role of Fin1 in mitosis rather than a reflection of a genuine function for Fin1 during interphase. We therefore assessed the effect of Fin1 function upon the association of Plo1 with the SPB of mutant cells that had been returned to the cell cycle following G_1 arrest at the permissive temperature. In order to avoid the confusion in the analysis arising from the normal association of Plo1 with the SPB that accompanies mitotic commitment, cells were stopped from progressing through the cell cycle beyond S phase by inclusion of the drug hydroxyurea (HU). This assessment therefore addresses Plo1 recruitment to the SPB independently of mitotic defects in the previous, or current, cell cycles.

cut12.s11 and *cut12.s11 fin1.ts1* cells that had been arrested in G_1 phase at 25°C by removal of a nitrogen source for 12 h cells were resuspended in nitrogen-rich medium containing HU and incubated at the 25°C for 3 h. The temperature of the culture was shifted to 37°C to inactivate Fin1 at this 3-h time point and the culture was monitored for a further 2 h. The frequency of cells with a single spot of Plo1 on the nucleus for *cut12.s11* cells is represented by light blue diamonds in all experiments shown in Figure 9, while *cut12.s11 fin1.ts1* cells with a single spot of Plo1 on the nucleus are represented by red triangles. The grey shading represents a period at 25°C, and the white background a period at 37°C. At 25°C, in either strain, ~80% of the cells had Plo1 on the SPB (Figure 9A). The number of cells with Plo1 on the interphase SPB dramatically dropped in the *fin1.ts1 cut12.s11* double mutant (red line) after the temperature of the culture was shifted to 37°C, but remained largely unaffected in the single *cut12.s11* mutant (light blue line). For further controls see Supplementary data available at *The EMBO Journal* Online.

The experiment was repeated with both mutants but this time the temperature was increased at the start of the experiment ($t = 0$) rather than at the 3 h time point
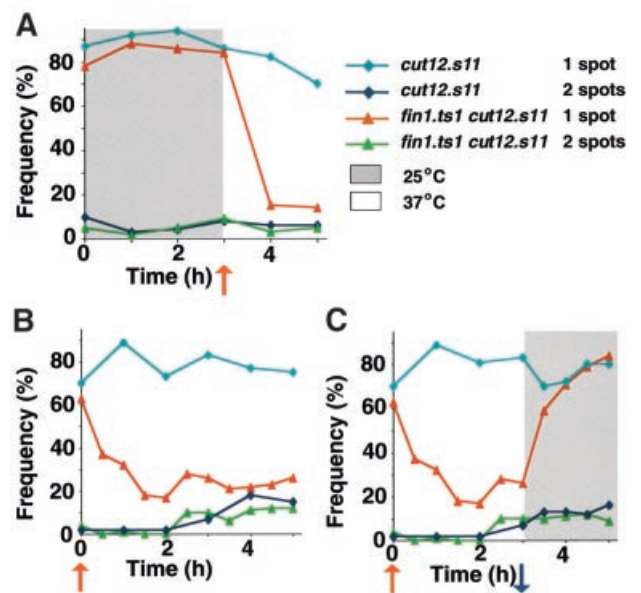
Fig. 9. Plo1 association with the SPB in a *cut12.s11* mutant is dependent upon Fin1 activity. (**A**) Cells were grown to mid-log phase in EMM2 at 25°C before being washed three times and resuspended in nitrogen-free EMM2 at 25°C. Twelve hours later cells were pelleted once more and resuspended in EMM2 + HU. The nitrogen-free medium induced G_1 phase arrest, whereas the nitrogen-rich medium induced cells to return to the cell cycle, where the HU then induced cell cycle arrest in S phase. The areas of the graphs with a white background indicate the periods at 37°C, whilst the grey background indicates periods at 25°C. The point of temperature shifts are indicated by arrows below the *x*-axes (blue for a shift down to 25°C, red for a shift up to 37°C). The number of spots of Plo1 on the nucleus was scored for each strain as indicated in the key. *cut12.s11* mutants show a normal checkpoint arrest in HU (data not shown). Note that premature recruitment of Plo1 in *stf1.1* does not result in septation. G_1 cells were resuspended in nitrogen-rich EMM2 medium containing HU at 25°C for 3 h and then the temperature was shifted to 37°C. (B and C) G_1 cells were resuspended nitrogen-rich EMM2 medium containing HU at 37°C and incubated for a further 3 h at 37°C. The culture was then split in two. One half was maintained at 37°C (**B**), while the temperature of the other was dropped to 25°C (**C**). Each half was incubated at its respective temperature for a further 2 h.

(Figure 9B and C). Each culture was then split into two after 3 h; one half was maintained at 37°C while the other half was returned to 25°C. The temperature shift to 37°C at time 0 caused Plo1 to leave the interphase SPBs in the double mutant *cut12.s11 fin1.ts1* (Figure 9B, red), but not single *cut12.s11* (Figure 9B, light blue) mutant. Plo1 reassociated with the SPBs, from which it had been excluded by the temperature shift at time 0, when Fin1 function was restored in the double mutant culture by returning the temperature to 25°C (Figure 9C, red).

We concluded that Fin1 was required for both *cut12.s11* suppression of *cdc25.22* and the premature association of Plo1 with the SPB of interphase *cut12.s11* cells. Moreover, the data show that the association of Plo1 with the SPB was dynamic and indicated that Fin1 could influence interphase events (i.e. it could function in interphase as well as during mitosis).

Overproduction of *fin1⁺* promoted the association of Plo1 with the interphase SPB of wild-type cells

We next asked whether the effect of Fin1 upon the recruitment of Plo1 to the SPB was a specific feature of the

cut12.s11 mutant background, or whether Fin1 could influence the SPB association of Plo1 in wild-type cells. We reasoned that increasing Fin1 levels might recruit Plo1 to the interphase SPB. _fin1+_ expression was induced from the _nmt81_ and _nmt41_ promoters of pREP81 and pREP41 (Basi _et al._, 1993) and we monitored Plo1 association with the SPB. Again, to rule out any confusion arising from the association of Plo1 that accompanies normal commitment to mitosis, HU was added to a portion of each culture 5 h before the time point indicated as 'HU arrest' on the graphs in Figure 10A. The very low level of basal transcription from the _nmt81_ promoter (Forsburg, 1993) forced Plo1 to associate with interphase SPBs before expression was induced. Induction of transcription enhanced this association and the basal transcription from the _nmt41_ promoter of pREP41 was sufficient to drive maximal association of Plo1 with the SPB (Figure 10A and B) of cells that had the interphase microtubule configuration of an S-phase-arrested culture (Figure 10C).

Discussion

We have described two mutations in the non-essential _fin1+_ gene that conferred a temperature-sensitive inability to form a mitotic spindle. One of these alleles resembled the null allele in displaying the additional phenotype of cell cycle delay. These two recessive, conditional mutations conferred a much more severe spindle formation defect than that arising from complete loss of the gene. The strong phenotype in the conditional mutants is probably an initial response to the loss of Fin1 function, after which the cells adapt, because the severity of the defect in both alleles declined towards _fin1.Δ_ levels with prolonged incubation at 37°C. The mechanism by which cells adapt to the loss of function is unclear at present; however, it is unlikely to arise from the accumulation of suppressor mutations in the strain as the same suppressor would have to arise spontaneously in at least 40% of the population within one generation. It is most likely, therefore, that the organization of control networks enables cells to adapt to losing Fin1 function. Future genetic screens will identify how cells compensate for the loss of Fin1.

Despite the recessive nature of the _fin1.Δ_, _fin1.ts1_ and _fin1.A5_ mutations, heterozygous diploids which combine any of these alleles with a wild-type allele exhibited a 26% deficiency in spindle formation at 37°C. The greater sensitivity of diploids to a reduction in _fin1+_ gene dosage may reflect the greater challenges faced by the diploid spindle. Differences in the demands of chromosome segregation between haploid and diploid spindles are highlighted by the fact that haploid cells lacking the spindle checkpoint protein Bub1 grow without any apparent difficulty, whereas diploid _Δbub1_ cells rely heavily upon this gene for survival (Bernard _et al._, 1998). For _fin1+_, these challenges would be greater at 37°C than 25°C as _fin1.Δ fin1+_ heterozygotes have essentially normal spindle function at the lower temperature. Sensitivity to gene dosage may be a common feature for Nrks in different systems, as the number of wild-type _nimA_ nuclei in _nimAΔ/nimA_ heterokaryons is disproportionately high (Ye _et al._, 1998).
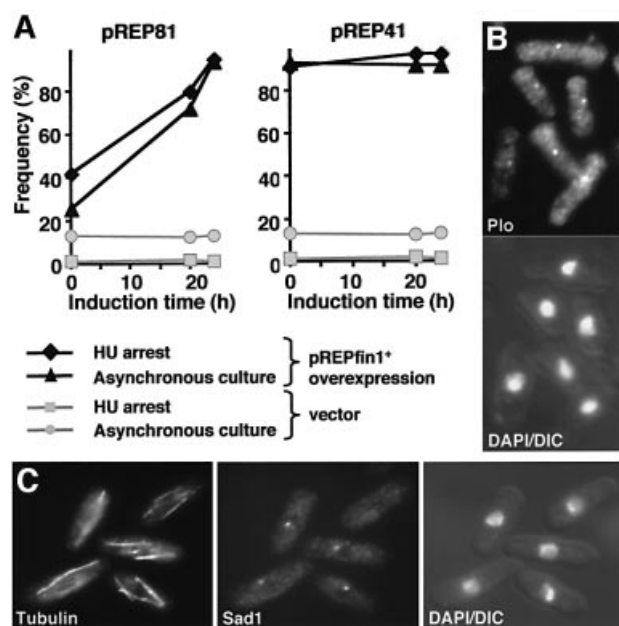
Fig. 10. Modest increases in _fin1+_ transcription recruits Plo1 to the interphase SPB of wild-type cells. (**A**) Cells bearing pREP81fin1+ or pREP41fin1+ were grown to early log phase in EMM2 + thiamine at 25°C before being washed and resuspended in EMM2 to induce gene expression. HU (10 mM) was added to a portion of the culture 5 h before each time point indicated on the graph, and this portion was then processed for immunofluorescence microscopy to detect Plo1 5 h later at the time shown on the graph. Thus, where indicated, the values indicated by a black diamond or a grey square on each graph are for an S phase arrested population. The percentage of total cells with any Plo1 staining is shown on the graph. Wild-type cells arrest in S phase without any Plo1 on the SPB (grey squares), while increasing expression of _fin1+_ promotes association of Plo1 with the SPB. (**B**) Micrographs from the uninduced (_t_ = 0) pREP41fin1+ strain showing a Plo1 localization (top) and a DAPI DIC image (bottom). (**C**) Micrographs from the pREP41fin1+ culture in (B) showing, from left to right, microtubules, Sad1 and a combined DAPI DIC image. No mitotic cells were seen in 200 cells counted.

Fin1 and spindle formation

The nucleation capacity of one of the two SPBs was compromised in _fin1.ts1_ mutants at the restrictive temperature. A similar phenotype has been reported in _A.nidulans_ when the _nimA.5_ mutation was combined with a mutation in the APC component BIME (Osmani _et al._, 1988b, 1991; Peters _et al._, 1996). The _bimE7_ mutation increased the level of NIMA protein (Ye _et al._, 1998) and so, presumably, increased NIMA activity at a critical location, or above a critical threshold, to enable mitotic commitment but not spindle formation. Spindle formation defects are also seen upon overproduction of _nimA_ (Osmani _et al._, 1988a). The shared spindle phenotype raises the possibility that NIMA and Fin1 perform similar functions in each system. If this is the case then the complete cell cycle arrest of _nimA5_ mutants suggests that _A.nidulans_ has a greater dependency upon Nrk function than _S.pombe_.

Related function for Fin1 and Cut12?

Genetic manipulations established a strong link between Fin1 and the SPB component Cut12. The recessive _cut12.1_ mutation blocks spindle formation in a manner that is indistinguishable from the _fin1−_ spindle formation defect,

while dominant mutations in the same gene, such as *cut12.s11*, suppress *cdc25.22* (Hudson *et al.*, 1990; Bridge *et al.*, 1998). Combining *cut12.1* with *fin1.ts1* or *fin1.Δ* was lethal, while *cut12.s11* reduced the severity of the *fin1⁻* spindle formation. Conversely, increasing *fin1⁺* expression reduced the severity of the *cut12.1* defect (although it could not suppress *cut12Δ*; data not shown). Furthermore, increasing *fin1⁺* expression mimicked the phenotype of *cut12.s11* in both raising the restrictive temperature of *cdc25.22* and in promoting the premature recruitment of Plo1 to the interphase SPB.

How could cut12.s11 or overproduction of fin1⁺ suppress Cdc25?

The only requirement for Cdc25 is to counteract Wee1 as inhibition of Wee1 suppresses *cdc25.22* and *cdc25.Δ* mutants (Fantes *et al.*, 1979; Russell and Nurse, 1986). Wee1 inhibition during mitotic commitment correlates with increased phosphorylation of the protein (Tang *et al.*, 1993; Honda *et al.*, 1995; Mueller *et al.*, 1995). This phosphorylation is probably achieved by a feedback loop kinase(s) which is triggered by the initial activation of MPF (Hoffmann *et al.*, 1993). Considerable data suggest that polo kinases act as a Cdc25 feedback loop kinase (Nigg, 2001). While the analysis of Wee1 feedback loop kinase is less extensive, the budding yeast polo kinase, Cdc5p, does bind and inhibit the Wee1 homologue Swe1p, suggesting that polo kinases could both inhibit Wee1 and activate Cdc25 during mitotic commitment (Bartholomew *et al.*, 2001). If this were the case, manipulations that activate feedback loop kinases such as polo would suppress *cdc25* mutations, because they would inhibit Wee1 without the need for the priming activation of MPF, which is dependent upon Cdc25.

Both the *cut12.s11* mutation and *fin1⁺* overexpression suppress *cdc25.22* and promote the premature recruitment of Plo1 to the interphase SPB. As Plo1 is usually only found on mitotic SPBs (Bähler *et al.*, 1998; Mulvihill *et al.*, 1999), these two manipulations result in mitotic behaviour of Plo1 in interphase cells. As mitotic behaviour of Plo1 could be indicative of an activate feedback loop. These two manipulations may therefore suppress *cdc25.22* by inappropriately activating the feedback loop. Fin1 and Cut12 are likely to act in a common pathway, because Fin1 is required for both *cut12.s11* phenotypes. Inactivating Fin1 blocks the suppression of *cdc25.22* by *cut12.s11*, and stops Plo1 from associating with the interphase SPB in *cut12.s11* mutants. Given that Fin1 associates with the SPB from late G_2 through to the end of mitosis (Krien *et al.*, 2002) it is possible that these two proteins physically interact. However, we have been unable to detect an interaction in a yeast two-hybrid assay (data not shown).

A number of the phenotypes are consistent with a function for Fin1 in feedback loop control of mitotic commitment: *fin1⁺* is not an essential gene. In the absence of *fin1⁺*, cells rely upon the spindle assembly checkpoint to survive. The checkpoint may halt mitosis long enough for MPF activity to accumulate to permit full mitotic commitment; *fin1.Δ* cells transit the G_2/M boundary more slowly than wild-type cells (Krien *et al.*, 1998). This is reminiscent of the consequences of depleting Plx from *Xenopus* extracts—the rate of mitotic commitment is slow, but cells eventually enter mitosis (Abrieu *et al.*,
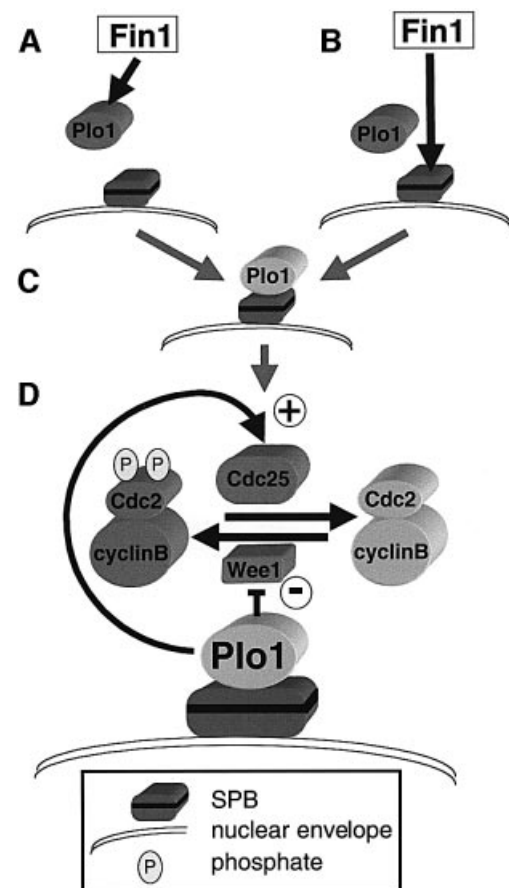
Fig. 11. Fin1, Plo1 and mitotic commitment. Fin1 either modifies the Plo1/regulatory subunit (**A**) or it modifies the SPB (**B**) to promote Plo1 association with the SPB (**C**). (**D**) SPB associated Plo1 promotes entry into mitosis by activating Cdc25 and inhibiting Wee1. P represents phosphate on Y15 and T14 of Cdc2.

1998); *wee1*, *cdc25* or *cdc2* mutants, which accelerate commitment to mitosis by reducing the reliance upon correct activation of the mitotic switch, also reduce the spindle formation defect of *fin1* mutants.

The bypass of *cdc25.22* when Plo1 is prematurely recruited to the SPB suggests that association of Plo1 with the SPB may be a critical step in regulating mitotic commitment. As Fin1 can influence this step, it may regulate the affinity of Plo1 for the SPB (Figure 11A). Alternatively Fin1 may modify the SPB to promote Plo1 recruitment (Figure 11B), perhaps by modifying Cut12 function. Either way Plo1 is brought into a context in which it promotes MPF activation (Figure 11C). Because polo kinase regulates many events that are likely to be associated with the SPB (such as APC activation and cytokinesis; Nigg, 2001), it may bind to different SPB components to regulate different mitotic events. The step that we postulate here could be one of several means by which Plo1 associates with the mitotic SPB. The nature of the interaction between Fin1 and Plo1 remains to be established, but it is worth noting that overproduction of *fin1⁺* in HU arrested cells does not affect bulk Plo1 activity, or alleviate the spindle formation defects of *plo1.ts* mutants (data not shown). Furthermore, Plo1 associates with the SPB in *fin1.ts* monopolar spindles (data not shown).

Table III. Strains used in this study

Strain		Source/reference
IH365	*leu1.32 ura4.D18 h⁻*	Laboratory stock
IH571	*cut12.NEGFP leu1.32 ura4.D18 h⁻*	Bridge *et al*. (1998)
IH646	*cdc2.1w h⁻*	Thuriaux *et al*. (1976)
IH666	*cut12.s11 leu1.32 h⁺*	Hudson *et al*. (1990)
IH740	*cdc25.22 cut12.s11 leu1.32 ura4.D18 h⁺*	Laboratory stock
IH754	*cut12.1 ade6.704 ura4.D18 h⁻*	Bridge *et al*. (1998)
IH776	*wee1.50 ura4.D18 h⁻*	Thuriaux *et al*. (1976)
IH1307	*mad2::ura4⁺ leu1.32 ura4.D18 h⁻*	He *et al*. (1997)
IH1308	*ura4.D18 h⁻*	Laboratory stock
IH1361	*cut12.NEGFP mam2 leu1.32 ura4.D18 his1.102 h⁹⁰*	This study
IH1363	*mph1::ura4⁺ leu1.32 ura4.D18 h⁻*	He *et al*. (1998)
IH1545	*cdc2.3w leu1.32 ura4.D18 h⁻*	Thuriaux *et al*. (1976)
IH1688	*fin1.ts1 ura4.D18 leu1.32 h⁻*	This study
IH1705	*fin1.ts1 ura4.D18 leu1.32 his2 h⁺*	This study
IH1754	*wee1.6 h⁺*	Thuriaux *et al*. (1976)
IH1824	*fin1.Δ ura4.D18 leu1.32 h⁺*	Krien *et al*. (1998)
IH1865	*fin1.ts1 cdc25.22 leu1.32 ura4.D18 h⁻*	This study
IH1911	*fin1.Δ cdc25.22 leu1.32 ura4.D18 h⁻*	This study
IH1912	*fin1.ts1 cut12.s11 leu1.32 ura4.D18 h⁻*	This study
IH1915	*fin1.ts1 cut12.NEGFP leu1.32 ura4.D18 h⁻*	This study
IH1917	*fin1.ts1 wee1.50 leu1.32 h⁻*	This study
IH1951	*fin1.ts1 dma1::LEU2 leu1.32 ura4.D18 h⁻*	This study
IH1953	*fin1.Δ mad2::ura4+ leu1.32 ura4.D18*	This study
IH1960	*cdc25.22 ade6.M216 leu1.32 ura4.D18 h⁻*	Fantes (1981)
IH1964	*Δbub1::LEU2 leu1.32 ura4.D18 ade6.M216 h⁺*	Bernard *et al*. (1998)
IH1978	*fin1.Δ ura4.D18 leu1.32 h⁻*	This study
IH1980	*fin1.Δ cut12.NEGFP leu1.32 ura4.D18*	This study
IH1981	*fin1.ts1 cdc2.1w ura4.D18 h⁻*	This study
IH1982	*fin1.ts1 cdc2.3w leu1.32 ura4.D18 h⁻*	This study
IH1987	*fin1.ts1 mad2::ura4⁺ leu1.32 ura4.D18 h⁻*	This study
IH2031	*fin1.Δ cut12.s11 leu1.32 ura4.D18 h⁻*	This study
IH2049	*fin1.ts1 wee1.6 leu1.32*	This study
IH2051	*fin1.Δ dma1::LEU2 leu1.32 ura4.D18*	This study
IH2061	*fin1.ts1 cdc25.22 cut12.s11 leu1.32 ura4.D18 h⁻*	This study
IH2062	*fin1.Δ cdc25.22 cut12.s11 leu1.32 ura4.D18 h⁻*	This study
IH2249	*fin1.Δ ura4.D18 h⁺*	This study
IH2273	*leu1::fin1.A5:ura4⁺ ura4.d18 h⁻*	This study
IH2274	*leu1::fin1.A5:ura4⁺ fin1.Δ ura4.d18 h⁻*	This study
IH2285	*leu1.32 ura4.D18 h⁻ + pREP1fin1⁺*	This study
IH2286	*leu1.32 ura4.D18 h⁻ + pREP41fin1⁺*	This study
IH2287	*fin1.ts1 ura4.D18 leu1.32 his2 h⁺ pREP3Xcdc25.d1*	This study
IH2288	*fin1.Δ ura4.D18 leu1.32 h⁻ pREP3Xcdc25.d1*	This study
IH2330	*leu1.32 ura4.D18 h⁻ pREP81fin1⁺*	This study
IH2331	*cut12.1 leu1.32 ura4.D18 his2 h⁺ pREP81fin1⁺*	This study
IH2332	*cut12.1 ade6.704 ura4.D18 h⁻ pREP41fin1⁺*	This study
IH2333	*cdc25.22 ade6.M216 leu1.32 ura4.D18 h⁻ pREP81fin1⁺*	This study
IH2334	*cdc25.22 ade6.M216 leu1.32 ura4.D18 h⁻ pREP41fin1⁺*	This study
IH2335	*mad2Δ::ura4⁺ leu1.32 ura4.D18 ade6 h⁻ pREP41fin1⁺*	This study
IH2336	*Δbub1:: ura4⁺ leu1.32 ura4-DS/E his1.102 ade6.M216 pREP41fin1⁺*	This study
IH2337	*leu1::fin1⁺:ura4⁺ fin1.ts1 ura4.d18 h⁻*	This study
IH2338	*fin1.ts1/fin1⁺ leu1.32/leu1⁺ ura1.d18/ura4⁺ ade6.M210/ade6/M216 h⁻/h⁻*	This study
IH2339	*fin1.Δ/fin1⁺ leu1.32/leu1⁺ ura4.d18/ura4⁺ ade6.M210/ade6/M216 h⁻/h⁻*	This study
IH2340	*leu1::fin1.A5:ura4⁺/leu1⁺ fin1.Δ/fin1⁺ ura4.d18/ura4⁺ ade6.M216/ade6.M210 h⁻/h⁻*	This study
IH2350	*leu1.32/leu1⁺ ura4.d18/ura4⁺ ade6.M216/ade6.M210 h⁻/h⁻*	This study

NIMA and commitment to mitosis

The relationship between NIMA and MPF is consistent with a function in the feedback loop. Mitotic activation of NIMA is dependent upon prior activation of MPF (Ye *et al*., 1995). MPF directly phosphorylates NIMA *in vitro* in a manner that mimics the modification of NIMA during mitotic commitment *in vivo* (Ye *et al*., 1995). NIMA kinase activity peaks before MPF activity peaks at metaphase (Ye *et al*., 1995). Finally, although NIMA acts after MPF, the cells remain in interphase when NimA function is compromised (Oakley and Morris, 1983). This last fact could argue against a feedback loop function. If NIMA acts in the feedback loop it might be expected that, like Plx (Abrieu *et al*., 1998), it would alter the rate rather than the extent of mitotic commitment. However, the need to divide multiple nuclei in a single cell compartment could be a driving force to make a control that is peripheral to mitotic commitment in many systems, and central to mitotic commitment in a syncitial filamentous fungus. If, as Cut12 data suggest is true in *S.pombe*, events at the SPB play an important part in mitotic commitment in *A.nidulans*, it would be critical to co-ordinate the waves

of MPF amplifying loop emerging from each SPB of the multi-nucleated cell compartments. The activity of the amplification loop that would propagate the trigger signals is a logical point at which to interpose such co-ordination. Thus, NIMA *A.nidulans* may have been co-opted to become a rate limiting step in mitotic commitment while Fin1 remains a fine tuning control in *S.pombe*.

Common functions for all Nrks?

The *fin1⁻* phenotypes reported here identify some common themes between Nrks in different organisms. Mutation of both *nimA* and *fin1* affects the timing of mitotic commitment. The spindle formation phenotypes seen with *fin1* or overexpression of *nimA* are reminiscent of the slow centrosome maturation seen upon depletion of Nek2B from *Xenopus* extracts and the centrosome splitting induced by injection of Nek2 into human cells (Osmani *et al.*, 1988a; Fry *et al.*, 1998, 2000; Uto and Sagata, 2000). Furthermore, if the prime function of the *S.cerevisiae* Nrk Kin3p is also to regulate Wee1 and Cdc25 equivalents, it would explain why *KIN3⁺* is not an essential gene (Jones and Rosamond, 1990). Phosphorylation of Tyr19 of Cdc28p is only essential in *S.cerevisiae* when cytokinesis or morphogenesis are defective (Lew, 2000). Thus, Nrk function in diverse systems may have more in common than has been appreciated previously.

Materials and methods

Cell culture and strains

The strains used in this study are listed in Table III. Cells were cultured in yeast extract media (YES), or appropriately supplemented EMM2 with or without nitrogen minimal media as described in Moreno *et al.* (1991). Thiamine (4 μM) was added to minimal EMM2 media to repress *nmt* promoters (Maundrell, 1990; Basi *et al.*, 1993). HU was added from an aqueous 1 M stock solution to a final concentration of 10 mM.

Molecular genetics

fin1.ts1-suppressing plasmids were cloned from a genomic library (Barbet and Carr, 1992) using standard procedures (Moreno *et al.*, 1991). The QuickChange Mutagenesis (Stratagene) approach along with appropriate primers was used for all site directed mutagenesis. The use of the SMART RACE kit (Clontech) confirmed that the ATG codon that was assigned to *fin1* in Krien *et al.* (1998) corresponded to the first ATG of the *fin1⁺* message. This ATG was mutated to an *Nde*I site and plasmid pfin1.1, which extended from the *Pst1* site 185 bp 5′ to this ATG to the *Xba*I site that lies 627 bp 3′ to the stop codon. This mutagenesis generated pfin1.ATG*. The 3108 bp *Nde*I–*Xba*I fragment from pfin1.ATG* was subcloned into pREP81 pREP41 and pREP1 (Basi *et al.*, 1993) to generate pREP81fin1⁺, pREP41fin1⁺ and pREP1fin1⁺, respectively. Note that pREP1fin1 differed from *nmt::fin1* used by Krien *et al.* (1998) by not having the 83-bp UTR between the *nmt1⁺* promoter and the initiator ATG that is present in *nmt::fin1*. Five independent PCRs amplified the *fin1* gene from *fin1.ts1*. These reactions were pooled and sequenced. Repeating this procedure identified the same single mutation. Nucleotide 564 of the *fin1⁺* ORF in pfin1.1 was changed from T to A to generate *fin1.A5*. The *Pst*I–*Sac*I fragment from the resultant plasmid pfin1.A5 or pfin1.1 was inserted between the *Pst*I and *Sac*I sites of pINTA (Petersen *et al.*, 2001) to generate pfin1.A5int or pfin1⁺int, respectively. The *Not*I fragment from each plasmid was independently transformed into IH1308, and ura⁺ leu⁻ colonies were isolated.

Indirect immunofluorescence microscopy

Standard anti-α-tubulin immunofluorescence procedures were used to stain spindles (Hagan and Yanagida, 1995). For detection of Plo1, glutaraldehyde was omitted from the fixation step and fixation was with 1.4% formaldehyde for 5 min. This fixation differs from that in Mulvihill *et al.* (1999). With the fixation used here, Plo1 is found on all *cut12.s11* interphase SPBs, but not on wild-type interphase SPBs. Anti-Sad1 AP9.2, TAT1 (anti-α–tubulin) and anti-Plo1 antibodies were used as described

previously (Woods *et al.*, 1989; Hagan and Yanagida, 1995; Mulvihill *et al.*, 1999). DAPI (4,6-diamidine-2-phenyl-indole) and calcofluor staining were according to Moreno *et al.* (1991). Images and cell length measurements were obtained using a Quantix camera (Photometrics) with Metamorph software (Universal Imaging). More than 200 dividing cells were measured for each strain for the cell length measurements in Table II.

Supplementary data

Supplementary data for this paper are available at *The EMBO Journal* Online.

References

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