A Novel Role of the Budding Yeast Separin Esp1 in Anaphase Spindle Elongation: Evidence that Proper Spindle Association of Esp1 Is Regulated by Pds1

Sanne Jensen, Marisa Segal, Duncan J. Clarke, and Steven I. Reed
Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037

Abstract. In Saccharomyces cerevisiae, the metaphase–anaphase transition is initiated by the anaphase-promoting complex–dependent degradation of Pds1, whereby Esp1 is activated to promote sister chromatid separation. Although this is a fundamental step in the cell cycle, little is known about the regulation of Esp1 and how loss of cohesion is coordinated with movement of the anaphase spindle. Here, we show that Esp1 has a novel role in promoting anaphase spindle elongation. The localization of Esp1 to the spindle apparatus, analyzed by live cell imaging, is regulated in a manner consistent with a function during anaphase B. The protein accumulates in the nucleus in G2 and is mobilized onto the spindle pole bodies and spindle midzone at anaphase onset, where it persists into midanaphase. Association with Pds1 occurs during S phase and is required for efficient nuclear targeting of Esp1. Spindle association is not fully restored in pds1 mutant expressing an Esp1-nuclear localization sequence fusion protein, suggesting that Pds1 is also required to promote Esp1 spindle binding. In agreement, Pds1 interacts with the spindle at the metaphase–anaphase transition and a fraction remains at the spindle pole bodies and the spindle midzone in anaphase cells. Finally, mutational analysis reveals that the conserved COOH-terminal region of Esp1 is important for spindle interaction.

Key words: cell cycle • Esp1/Pds1 complex • metaphase–anaphase transition • spindle elongation • budding yeast

Introduction

Faithful chromosome segregation during cell division is of fundamental importance to the continued viability of a cell. A key event is the separation of sister chromatids at the transition from metaphase to anaphase, which in budding yeast is triggered by the ubiquitin-dependent proteolysis of the anaphase inhibitor Pds1 (Cohen-Fix et al., 1996). This event is mediated by the anaphase-promoting complex (APC),1 which is also responsible for the destruction of other target proteins including the spindle component Ase1 and mitotic cyclins (Zachariae and Nasmyth, 1996; Juang et al., 1997). Degradation of Pds1 is necessary for the separation of sister chromatids, as mutant variants of Pds1 that cannot be degraded due to the absence of a destruction box block this process (Cohen-Fix et al., 1996). Recently, Pds1 was revealed to form a complex with Esp1, a protein essential for viability and crucial for sister chromatid separation (Ciosk et al., 1998). esp1 mutants exhibit a failure in proper spindle behavior at the time of elongation at anaphase, and although sister chromatid separation is blocked at the restrictive temperature, cell cycle progression is not arrested in these mutants, leading to a catastrophic mitosis (McGrew et al., 1992; Ciosk et al., 1998). Esp1 is thought to be inhibited by its association with Pds1, and only when the latter is degraded is Esp1 activated to trigger proteolysis and dissociation of cohesin proteins such as Scc1 from chromosomes. This event presumably allows sister chromatids to separate when pulled by microtubules connecting their kinetochores to opposite poles of the mitotic spindle. A failure to separate sisters in the presence of a noncleavable version of Scc1 in cells with functional Esp1 protein is accompanied by a block in spindle elongation, consistent with the idea that loss of cohesion triggers anaphase (Uhlmann et al., 1999). It is not yet clear how the separation of duplicated sister chromatids (anaphase A) is coordinated with spindle elongation (anaphase B).

Recently, Pds1 was revealed to form a complex with Esp1, a protein essential for viability and crucial for sister chromatid separation (Ciosk et al., 1998). esp1 mutants exhibit a failure in proper spindle behavior at the time of elongation at anaphase, and although sister chromatid separation is blocked at the restrictive temperature, cell cycle progression is not arrested in these mutants, leading to a catastrophic mitosis (McGrew et al., 1992; Ciosk et al., 1998). Esp1 is thought to be inhibited by its association with Pds1, and only when the latter is degraded is Esp1 activated to trigger proteolysis and dissociation of cohesin proteins such as Scc1 from chromosomes. This event presumably allows sister chromatids to separate when pulled by microtubules connecting their kinetochores to opposite poles of the mitotic spindle. A failure to separate sisters in the presence of a noncleavable version of Scc1 in cells with functional Esp1 protein is accompanied by a block in spindle elongation, consistent with the idea that loss of cohesion triggers anaphase (Uhlmann et al., 1999). It is not yet clear how the separation of duplicated sister chromatids (anaphase A) is coordinated with spindle elongation (anaphase B).

Within the context of this framework, there are two key challenges that must be addressed before the mechanism of anaphase spindle elongation can be understood. First, we must determine how the separation of duplicated sister chromatids (anaphase A) is coordinated with spindle elongation (anaphase B). Second, we must understand how Esp1 is regulated during anaphase to allow it to function as a novel protease cleaving cohesin proteins, if indeed it functions in this manner. In this paper, we show that Esp1 has a novel role in promoting anaphase spindle elongation and that proper spindle association of Esp1 is regulated by Pds1.

1Abbreviations used in this paper: APC, anaphase-promoting complex; CFP, cyan fluorescent protein; DIC, differential interference contrast; GFP, green fluorescent protein; HA, hemagglutinin; NLS, nuclear localization sequence; SPB, spindle pole body.

Address correspondence to Steven I. Reed, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037. Tel.: (858) 784-9836. Fax: (858) 784-2781. E-mail: sreed@scripps.edu

Dr. Jensen’s present address is Division of Yeast Genetics, National Institute for Medical Research, London NW7 1AA, UK.

© The Rockefeller University Press, 0021-9525/2001/01/27/14 $5.00
The Journal of Cell Biology, Volume 152, Number 1, January 8, 2001 27–40
http://www.jcb.org/cgi/content/full/152/1/27
but the exact mechanism of action and the regulation of this essential protein has not been elucidated. Functional homologues of the Esp1/Pds1 complex have been identified in \textit{Schizosaccharomyces pombe} (Cut1/Cut2) (Uzawa et al., 1990; Funabiki et al., 1996) and Esp1-related proteins exist in \textit{Aspergillus nidulans} (BimB) (May et al., 1992), \textit{Xenopus}, and human (Zou et al., 1999), suggesting that control of sister chromatid separation and anaphase onset is conserved in evolutionarily diverse organisms.

In this study, we show that Esp1 activity is required past loss of cohesion at the metaphase–anaphase transition, revealing a direct role for Esp1 in spindle elongation. This is consistent with the localization of Esp1 to the spindle pole bodies (SPBs) and mitotic spindle observed in live cells and the kinetics of Esp1 spindle association. We show that Pds1 interaction is required to obtain efficient transport of Esp1 to the nucleus and for subsequent binding of the protein to the mitotic spindle, which appears to be crucial for proper anaphase progression. Furthermore, evidence is provided that an intact putative calcium-binding site in the conserved COOH-terminal region of Esp1 is important to form and maintain spindle association during anaphase.

**Materials and Methods**

### Yeast Strains and Methods

The relevant genotypes of the yeast strains used in this study are listed in Table 1. All strains are isogenic derivatives of BF264-15 SDU: a ura3Δ ade1 his2 leu2-3,112 trp1-1 (Richardson et al., 1989). Yeast media and genetic procedures were performed according to Guthrie and Fink (1991). Gene disruptions were performed by PCR-targeting technique (Wach et al., 1994).

Induction of integrated ESP1GFP from the \textit{GAL1} promoter was performed as follows. Approximately 10\(^6\) cells of a YEPRAffinose culture was filtered onto 47-mm 0.45 \(\mu\)m GN-6 Metrical membrane (Gelman Sciences). Filters were placed on YEPGalactose plates for 15–30 min at room temperature. Cells were eluted into 1 M sorbitol and Esp1GFP fluorescence visualized by microscopy. For induction of Esp1GFP in time-course experiments, cells were diluted in YEPRaﬃnose to an OD\(_{600}\) of 0.15, a-factor (200 ng/ml) was added and cells were incubated at 30°C for 1 h. 2% galactose was added, and incubation was continued for 1 h. Cells were released in YEPDextrose at 30°C and samples were collected for analysis of cell-cycle progression by DAPI and protein fluorescence by microscopy.

For loss of cohesion assays, the region adjacent to the centromere of chromosome IV was visualized by using the binding of tetR-GFP fusion protein to the COOH terminus of Esp1. This plasmid is a derivative of pRS406 (Sikorski and Hieter, 1989), where the isolated by selecting for growth on dex-ura media. The parental pRSG plasmid was digested with StuI and integrants were isolated by selecting for G418 resistance.

### Plasmids

Esp1-pRSG is an integrative plasmid carrying the \textit{ESP1} open reading frame in addition to 200 bases of the 5' flanking sequence under the control of the \textit{GAL1} promoter. ESP1GFP-pRSG is derived from this plasmid by inserting a PCR-generated sequence encoding the GFP epitope (F64L, S65T, Q80R mutant) into a Smal site introduced just before the stop codon. All pRSG-derived plasmids are linearized with StuI and integrants isolated by selecting for growth on dex-ura media. The parental pRSG plasmid was digested with StuI and integrants were isolated by selecting for growth on G418 plates.

Esp1myc18-pRSG was used to tag endogenous Esp1 protein with 18 myc epitopes at the COOH terminus. The integrative pTRP1 plasmid carries the sequence encoding six myc epitopes, which can be fused to a protein of interest at the NotI site (Mondesert et al., 1997). A NotI-Smal fragment spanning the last 480 bases of the \textit{ESP1} gene was cloned into this plasmid. An additional fragment encoding 12 myc epitopes was subsequently introduced into the NotI site generating the final pTRP1myc18-pTRP1 construct. The plasmid was linearized with XbaI and integrants isolated by selecting for growth on dex-trp.

The ESP1GFP fusion was cloned into the SV40 NLS sequence, which was used to fuse the endogenous Esp1 protein to GFP. pKGFP was designed with the GFP-encoding sequence inserted after NotI, allowing in frame fusion to any protein of interest. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted in pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.

Plasmid ESP1(1-1568)-pRSG, ESP1(D1568A)-pRSG, and ESP1(S175G)-pRSG were used to produce strains expressing the COOH-terminal truncation mutant and mutants in the putative calcium-binding site of Esp1 from the \textit{GAL1} promoter, respectively. To generate ESP1(1-1568)-pRSG, a Sall-Mal plasmid carried the sequence encoding residue 1568 was in-frame fused to Esp1. To fuse the Esp1(1-1568) fragment into the \textit{ESP1}(1-1568)-pRSG plasmid described previously (Kaiser et al., 1999) was used to construct the strain expressing the nondegradable version of Pds1.

The vector pOC78 (Cohen-Fix et al., 1996) was employed for tagging endogenous Pds1 internally with three hemagglutinin (HA) epitopes. To tag the Pds1-128 protein in a similar fashion, the XbaI-Avrl fragment of pds1-128 YEp24 constituting the 3' end of the gene with the mutation was cloned into the XbaI/AviII sites of a pBsiI-derived plasmid containing the SacI-ApaI Pds1(1-128) fragment from pOC78. The AvrII-ApaI pds1-128(1-128) fragment was introduced into the plasmid ESP1GFPNLS-pRSG and the resulting plasmid was used to tag the endogenous Esp1 protein to GFP. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted into pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.

Plasmid ESP1(1-1568)-pRSG was used to tag endogenous Esp1 protein to GFP. pKGFP was designed with the GFP-encoding sequence inserted after NotI, allowing in frame fusion to any protein of interest. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted in pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.

Plasmid ESP1(1-1568)-pRSG was used to tag endogenous Esp1 protein to GFP. pKGFP was designed with the GFP-encoding sequence inserted after NotI, allowing in frame fusion to any protein of interest. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted in pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.

Plasmid ESP1(1-1568)-pRSG was used to tag endogenous Esp1 protein to GFP. pKGFP was designed with the GFP-encoding sequence inserted after NotI, allowing in frame fusion to any protein of interest. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted in pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.

Plasmid ESP1(1-1568)-pRSG was used to tag endogenous Esp1 protein to GFP. pKGFP was designed with the GFP-encoding sequence inserted after NotI, allowing in frame fusion to any protein of interest. The ESP1 Sall-NotI fragment used to construct ESP1myc18-pTRP1 was inserted in pKGFP cut with SalI and NotI. The plasmid was linearized with XbaI and integrants were isolated by selecting for G418 resistance.
and SacI (Sikorski and Hieter, 1989). To generate ESP1(1-1568)-pRS415, the SpeI fragment from ESP1(1-1568)-pRS415 containing the 3’ end of the ESP1 gene was introduced into the ESP1-pRS415 plasmid cut with SpeI. The ESP1 calcium-binding site mutants were cloned into pRS415 by substituting the NdeI fragment derived from ESP1(D1568A)- and ESP1(D1568A/D1570A)-pRS415 with the corresponding fragment in ESP1-pRS415. All plasmids were subjected to sequencing to verify their integrity.

Cell Biology Protocols

Fluorescence and differential interference contrast (DIC) microscopy was performed using an Eclipse E800 microscope (Nikon) with a 100× objective. Cell images were captured with a Quantix CCD (Photometrics) camera using IPLab Spectrum software (Signal Analytics Co.). Spindle measurements were performed on captured images using the NIH Image measuring tool calibrated with a stage micrometer. For microscopy of live cells expressing either wild-type/mutant Esp1GFP or Pds1GFP, cells were grown in YEPRaffinose/YEPGalactose or YEPDextrose, respectively, cells expressing either wild-type/mutant Esp1GFP or Pds1GFP, cells were grown in YEPRaffinose/YEPGalactose or YEPDextrose, respectively.

Protein isolation was essentially as previously described (Kaiser et al., 1987). Protein was examined in synchronized cells expressing endogenous Esp1 fused to 18 myc epitopes at its COOH-terminus after release from an α-factor–induced G1 block (Fig. 1 A). The level of this fully functional fusion protein is approximately threefold lower in G1 than in the rest of the cell cycle, where it appears to be constant. The synchrony of approximately threefold lower in G1 than in the rest of the cell cycle, where it appears to be constant. The synchrony of

Results

Cell Cycle–dependent Distribution of Esp1 in Live Yeast Cells

To gain insight into the regulation and function of Esp1 in S. cerevisiae, we initiated a study of the localization of this protein in live cells. Unlike Pds1, the level of Esp1 is not highly regulated in the cell cycle. The abundance of Esp1 protein was examined in synchronized cells expressing endogenous Esp1 fused to 18 myc epitopes at its COOH-terminus after release from an α-factor–induced G1 block (Fig. 1 A). The level of this fully functional fusion protein is approximately threefold lower in G1 than in the rest of the cell cycle, where it appears to be constant. The synchrony of the cells was verified by flow cytometry (data not shown).
To monitor if the localization profile of Esp1 in living cells changes through the cell cycle, a gene encoding Esp1 tagged with the green fluorescent protein (GFP) was integrated into a wild-type strain. This fusion protein complements an esp1Δ mutation, and cells overexpressing Esp1GFP under the control of the GAL1 promoter grow normally (not shown). The strain was arrested in G1 using α-factor and Esp1GFP protein induced transiently by addition of galactose 30 min before release from the arrest into glucose-containing medium. At given intervals, the localization of Esp1GFP was monitored in live cells by microscopy and the cell-cycle distribution followed by DAPI staining (Fig. 1 B). The fluorescent signal of Esp1GFP was delocalized throughout the entire cell in unbudded cells (G1). In G2, the protein was concentrated in the nucleus, and shortly thereafter was mobilized to the SPBs and the metaphase spindle (but see below). As cells progressed into anaphase, Esp1 signal was observed almost exclusively at the 2-μm region of the spindle midzone in addition to the SPBs. The localization of Esp1GFP at different stages of the cell cycle is illustrated in Fig. 2 A, where the protein was induced to ~10-fold higher levels compared with endogenous level (not shown). The distribution of Esp1 observed under these circumstances is not a result of overexpression, as the same localization pattern is observed in a strain expressing Esp1GFP from the endogenous promoter (Fig. 2 A, i). The association of Esp1 with SPBs and spindle was confirmed in colocalization experiments with strains expressing galactose-inducible Esp1GFP and endogenous levels of the SPB marker Spc29 fused to CFP (Spc29CFP) or a CFP-tagged version of tubulin (CFPTub1), respectively (Fig. 2 B). The length of the spindles labeled by Esp1GFP exhibited a broad distribution (Fig. 2 C). Relatively few Esp1-decorated spindles were measured as 2 μm and shorter, and in all cases the spindles were already oriented along the mother-bud axis. This is in contrast with tubulin staining of an analogous population, where 2-μm metaphase spindles are the most frequently observed class, and anaphase spindles of intermediate length are quite rare (data not shown). These data indicate that Esp1 labels the spindle at the metaphase–anaphase transition and not in G2 or during the bulk of metaphase. The majority of Esp1GFP anaphase spindles fell into the 3–6-μm intermediate range, but even spindles up to 9 μm were observed, although fully extended anaphase spindles of 10–12 μm did not contain Esp1. The appearance of Esp1 on spindles at the metaphase–anaphase transition, its persistence on anaphase spindles, and the specific enrichment of Esp1 at the spindle midzone, a structure important for anaphase spindle elongation in yeast (Pellman et al., 1995) is suggestive of a role of Esp1 in spindle elongation at anaphase.

Esp1 Is Required for Anaphase Spindle Movement

We next asked whether Esp1 indeed plays a direct role in spindle elongation. For this purpose, mutants deleted for the cohesin SCC1 were employed to circumvent the obstacle of Esp1 being required for the earlier event in loss of cohesion. In scc1 mutants, cohesion is defective and sister chromatid separation occurs prematurely (Michaelis et al., 1997). scc1Δ, esp1Δ scc1Δ (kept alive with galactose-induced Scc1), and esp1Δ mutant cells were synchronized in G1 with α-factor and released into YEPDextrose at the restrictive temperature of the esp1Δ mutation. Wild-type cells were included for reference. The timing of budding, loss of cohesion (visualized using a GFP marker on chromosome IV; Clarke et al., 1999), and spindle morphology (visualized by CFP-labeled tubulin) were analyzed as the cells progressed through the cell cycle (Fig. 3). All strains budded and assembled bipolar spindles with similar kinetics. Whereas scc1Δ cells separated sister chromatids prematurely and were capable of elongating their spindles (Fig. 3 B), esp1Δ cells, as originally reported, failed to separate sister chromatids and maintained short spindles until the cells exited the cell cycle.

Figure 1. Cell cycle–dependent regulation of Esp1. (A) Esp1 protein level during the cell cycle. Strain carrying epitope-tagged Esp1 integrated at the chromosomal locus (SY108) was arrested in G1 with α-factor. Cells were released into YEPDextrose at 25°C and aliquots removed at indicated times for analysis of cell morphology, Esp1 and Cdc28 protein levels, and FACS® analysis. (B) Cell cycle changes in Esp1 localization in live cells. Strain carrying GAL1-inducible ESP1GFP (SY101) was arrested in G1 with α-factor. 30 min before release, 2% galactose was added to induce Esp1GFP. Cells were released into YEPDextrose at 30°C, and aliquots were removed at intervals for analysis by real time microscopy, determination of budding index and cell-cycle progression by DAPI stain. (□) Cells with preanaphase nuclear morphology; (■) cells with anaphase and telophase nuclear morphology; (▲) budding index; (●) nuclear localization of Esp1; (○) spindle localization of Esp1.
If Esp1 activity is solely required to promote loss of cohesion, one would predict that an esp1^tscc1Δ double mutant would elongate spindles similarly to the scc1Δ mutant. However, there were virtually no elongated spindles observed in the esp1^tscc1Δ mutant in the absence of cohesion and, as in the case of the esp1^ts cells, the short spindles were maintained for the time it took the scc1Δ mutant to proceed through anaphase (Fig. 3 D). These data unequivocally show that Esp1 is required for spindle elongation during anaphase, and not merely to promote loss of cohesion at the metaphase-to-anaphase transition. Consistent with the role of Esp1 in activation of spindle elongation, ndc10-1 cells, which elongate spindles in the absence of chromosome segregation due to defective kinetochores (Goh and Kilmartin, 1993), fail to elongate spindles in the absence of Esp1 activity (data not shown).

**Association with Pds1 Is Essential for Proper Esp1 Localization**

As an initial attempt to understand how this novel function of Esp1 might be regulated, we set out to investigate how Esp1 spindle localization is controlled. Given the accumulation of Esp1 protein at the midzone, we examined the possible connection between this association and a known component of the spindle midzone in yeast, Ase1. Although this protein is important for efficient anaphase spindle elongation, it is not essential, and ase1 mutants are able to faithfully segregate chromosomes despite their unstable spindles (Pellman et al., 1995; Juang et al., 1997). The ESP1GFP gene was integrated into an ase1Δ mutant and the protein visualized after transient induction, as described above. The localization of Esp1 to the nucleus and the SPBs was not affected in this mutant; however, no sig-
The Journal of Cell Biology, Volume 152, 2001

The spindle midzone was observed in >500 cells analyzed (Fig. 4 A). There are two possible explanations for this result. Either Ase1 interaction is required to mediate midzone binding of Esp1 or the structure of the midzone in *ase1* mutants is perturbed, hampering Esp1 association or detection. Coimmunoprecipitation experiments on a strain with epitope-tagged versions of Esp1 and Ase1 expressed from their chromosomal loci revealed no interaction between the two components under a variety of test conditions (not shown). It is therefore more likely that the absence of Esp1 at the midzone in *ase1* mutants is due to structural alterations of the midzone. Deletion of *ASE1* in an *esp1* mutant exacerbates the temperature sensitivity of the strain, consistent with the idea that the spindle midzone is important for Esp1 function (data not shown).

The only protein identified to date that interacts with Esp1 is the anaphase inhibitor Pds1. To examine what effect Esp1/Pds1 complex formation has on the subcellular distribution of Esp1, the Esp1GFP protein was expressed and followed in a *pds1* mutant at the permissive temperature. Surprisingly, the fluorescent signal was delocalized within the whole cell throughout the entire cell cycle. There was no detectable accumulation of Esp1GFP in the nucleus or spindle association (Fig. 4 B). Although the *pds1* strain is viable, the mutant grows poorly and the cells die at 28°C in the genetic background used for these studies. We therefore examined the localization of Esp1 in the less severe *pds1-128* mutant, which has a restrictive temperature of 37°C. Esp1GFP exhibited a similar localization profile in this mutant at the permissive temperature to that observed in the *pds1* mutant (Fig. 4 C). Indirect immunofluorescence on cells expressing endogenous myc18-tagged Esp1 further confirmed the requirement of a functional Pds1 for correct localization of Esp1, as *pds1-128* cells failed to show nuclear or spindle staining of Esp1 (data not shown).

By two-hybrid analysis we found that the NH2-terminal domain of Esp1 spanning residues 1–535 interacts with the COOH-terminal region (residues 300–373) of Pds1 (not shown). Since the *pds1-128* mutation affects the extreme COOH terminus of Pds1 (D.J. Clarke and S.I. Reed, unpublished data), it is possible that the mutant Pds1 protein is incapable of interacting with Esp1. To test this directly, immunoprecipitation was performed on extract from strains encoding endogenous myc18-tagged Esp1 and wild-type Pds1 or Pds1-128 protein tagged with three copies of the HA epitope. The level of coprecipitated Pds1-128 protein was significantly lower than the amount of Pds1 present in the immunoprecipitated wild-type sample (Fig. 4 D). The reduction in Esp1/Pds1 complex formation in the *pds1-128* strain, however, is not due to an inability of the mutant Pds1 protein to interact with Esp1, as revealed by two-hybrid analysis (not shown), but rather reflects the high instability and low steady state level of the Pds1-128 protein. Thus, we conclude that complex formation between Esp1 and Pds1 is required to ensure proper movement of Esp1 to the nucleus and spindle, where it can exert its anaphase functions.

**Pds1 Transports Esp1 into the Nucleus and Promotes Spindle Association**

To address whether the lack of Esp1 spindle association in strains compromised for Pds1 function could simply reflect the absence of nuclear accumulation of Esp1, we investigated the effect of fusing a nuclear localization sequence (NLS) to Esp1 on its localization. For this purpose, a peptide encoding the potent SV40 NLS (KKKRRKV) was
fused to the COOH terminus of the Esp1GFP protein. This chimeric protein retains the ability to complement an esp1I mutation when overexpressed from the GAL1 promoter (not shown). In wild-type cells, the Esp1GFPNLS protein exhibited a localization profile similar to that of Esp1 fused to GFP alone (Fig. 5 A, top). In contrast, expression of Esp1GFPNLS in the pds1-128 mutant now resulted in accumulation of the protein in the nucleus and subsequent labeling of the SPBs and weak spindle association. However, increasing the nuclear import of Esp1 in pds1-128 cells was not sufficient to achieve a strong spindle fluorescence, as seen in wild-type cells (Fig. 5 A, middle), suggesting that Pds1 association may also be required to obtain efficient Esp1 spindle interaction. This is consistent with the observation that there is no detectable spindle fluorescence in pds1D cells expressing Esp1GFPNLS (data not shown). Esp1 did label the SPBs in this mutant, demonstrating that Esp1 has the ability to bind the SPBs in the absence of Pds1 (Fig. 5 A, bottom). This is perhaps not surprising as mutants deleted for PDS1 are viable at low temperatures and unless the essential function of Esp1 is independent of its spindle localization, Esp1 must have intrinsic spindle binding activity sufficient for viability. This association, however, is clearly more efficient when Pds1 is present. Expressing the Esp1GFPNLS version in a pds1D mutant leads to partial suppression of the temperature-sensitive phenotype at 30°C, whereas the pds1D mutant expressing Esp1GFP is dead at this temperature (not shown). This further supports the notion that an important function of Pds1 is to target Esp1 to the nucleus and subsequently to the spindle.

Surprisingly, the timing of nuclear concentration of Esp1GFPNLS in wild-type cells is unaltered compared with that of Esp1 fused to GFP alone. Both proteins accumulate in the nucleus in G2. This suggests that additional mechanisms regulate nuclear translocation of Esp1 (see Discussion).

To further confirm that association with Pds1 directs Esp1 localization, we constructed a strain expressing Esp1GFP and a nondegradable version of Pds1 (Pds1DDB) from the GAL1 promoter. Induction of Pds1DDB protein led to a strong nuclear accumulation of Esp1GFP in unbudded cells, which was never observed in unbudded control cells, where the level of endogenous Pds1 is low due to active Pds1 proteolysis early in the cell cycle (Fig. 5 B). Of >300 unbudded cells counted, ~65% showed a strong Esp1 signal always colocalizing with the DAPI signal. This number is probably a low estimate as fixation often reduced the intensity of the Esp1GFP signal. Thus, expressing Pds1 prematurely in the cell cycle promotes early translocation of Esp1 to the nucleus.

**Pds1 Localizes to the Spindle Apparatus in Budding Yeast**

Given the observation that Esp1 localization is controlled by Pds1, one would expect the two components to display similarities in their distribution at times of the cell cycle when Esp1 localization is changing. To investigate this, a strain was constructed expressing endogenous Pds1 fused at its COOH terminus to GFP, which is fully functional and regulated during the cell cycle with similar kinetics as
the wild-type protein (data not shown). Cells were synchronized with α-factor and, after release from G1, the localization of Pds1GFP was monitored by fluorescence microscopy. Nuclear morphology scored by DAPI and bud development were used as markers for cell-cycle progression (Fig. 6 A). Cells in G1 contained no Pds1GFP fluorescence, as expected, since Pds1 protein first appears after bud emergence. As cells progressed through S-phase, Pds1GFP protein accumulated almost exclusively in the nucleus. The nuclear localization of Pds1 has been shown in an earlier study by indirect immunofluorescence (Alexandru et al., 1999), where Pds1myc18 protein was shown to localize in the nucleus concomitant with bud emergence. In our study, there is a temporal lag between bud emergence and nuclear signal of Pds1 (~10–15 min), which may reflect the relatively weaker signal obtained with a single GFP tag. 

At the metaphase-to-anaphase transition, the majority of Pds1GFP disappeared, but, surprisingly, a fraction remained nuclear and often associated with the spindle apparatus. There was a strong fluorescent signal of Pds1 (~2–3 μm) long when chromosome separation begins (Straight et al., 1997). The association of Pds1 with mitotic spindles is consistent with the idea that Pds1 promotes Esp1 spindle binding by actually loading Esp1 onto the spindle apparatus at the metaphase–anaphase transition. Furthermore, the bias toward shorter spindles stained with Pds1, as compared with Esp1, suggests that Esp1 can be retained on the spindle once Pds1 is removed or degraded. Finally, the localization of Pds1 to the spindle is not altered in an esp1" mutant, suggesting that Esp1 is not required for this process (data not shown).

**Complex Formation between Esp1 and Pds1 Is Established Early in the Cell Cycle**

To establish when in the cell cycle the complex between Esp1 and Pds1 is formed, cells expressing endogenous myc18-tagged Esp1 and HA3-tagged Pds1 were arrested in G1 by α-factor. The cells were released at 22°C to slow down the cell cycle, providing a broader temporal window in which to monitor the timing of Esp1/Pds1 complex formation. After release, samples were collected at intervals for immunoprecipitation, immunoblotting, and FACS® analysis to confirm the synchrony. As is evident, the Esp1/Pds1 complex is formed at the time Pds1 first appears in the cell cycle and persists until anaphase, where Pds1 degradation is initiated (Fig. 7, A and B). Therefore, Esp1 activity is likely to be controlled by Pds1 through most of the cell cycle. A reconstitution experiment, where extract containing Esp1myc18 protein was mixed with extract containing Pds1HA3 protein followed by immunoprecipitation, showed that a complex between the two components could not be formed under these in vitro conditions (Fig. 7 C). This confirms that the Esp1/Pds1 association shown in Fig. 7 B reflects the true in vivo situation and is not an artifact of extraction. The coprecipitation result also correlates with the similar localization profile of the Esp1 and Pds1 proteins.

**A Putative Calcium Binding Site in the COOH-Terminal Domain of Esp1 Is Important for Spindle Binding**

Esp1 is a large protein composed of a total of 1,630 amino acid residues (McGrew et al., 1992). Little information can
be derived from its primary amino acid sequence. The NH₂-terminal third of the protein retains the ability to interact with Pds1 (not shown). The equivalent region in Cut1, the S. pombe homologue, has been shown to interact with Cut2, the functional homologue of Pds1, despite lack of conservation at the primary amino acid level (Kumada et al., 1998). The COOH-terminal region of Esp1 exhibits considerable homology to that of Cut1, Aspergillus BimB, and human Esp1 (30–40% identity), and it contains a putative calcium binding site (Fig. 8 A).

To address the importance of the potential calcium binding motif for Esp1 function, we constructed mutants of Esp1, where aspartic acid residues in the site were altered to alanine residues. Deletion of the extreme COOH-terminal region of Esp1, including the putative calcium binding site, rendered the mutant protein incapable of complementing an esp1ts mutation even when the protein was overexpressed from the GAL1 promoter (Fig. 8 B). In fact, overexpression at the permissive temperature is lethal in an esp1ts background (data not shown). A mutant protein with a single aspartic acid-to-alanine substitution at position 1568 could rescue an esp1ts mutation when overexpressed from the GAL1 promoter, whereas a lower dose derived from a low copy plasmid and the endogenous promoter only provided weak suppression of the esp1ts phenotype (Fig. 8 B). A second point mutation introducing the D1570A substitution yielded a mutant Esp1 protein unable to complement growth of an esp1ts mutant even when expressed from the GAL1 promoter. In fact, overexpression of the D1568A/D1570A protein in the esp1ts background, as in the case of 1-1568 protein, was lethal at the permissive temperature. That the mutant Esp1 proteins exacerbated the esp1ts phenotype but did not alter the growth of wild-type cells (not shown) suggests a weak dominant interfering effect, possibly due to competition for Pds1. These data taken together indicate that the putative calcium binding site in the COOH-terminal region plays a crucial role for Esp1 function.

We set out to characterize the essential function of this site in Esp1 by analyzing the nature of the defects of the
Esp1 mutant proteins. Immunoprecipitation experiments on extracts derived from strains expressing endogenous levels of HA3-tagged Pds1 in combination with the different Esp1GFP mutants induced from the GAL1 promoter demonstrated that the Esp1 mutants are not defective in Pds1 interaction (Fig. 8 C). The localization of the Esp1 mutant proteins in live cells was subsequently examined after a brief induction resulting in a comparable 10-fold overexpression in all strains (not shown). The COOH-terminal truncation mutant 1-1568 was delocalized throughout the cell at all stages of the cell cycle, suggesting that the COOH-terminal region of Esp1 is required for nuclear translocation and interaction with the spindle (Fig. 8 D, a). The Esp1 D1568A mutant protein exhibited a distribution similar to wild-type Esp1 protein (Fig. 8 D, b, and Table II), consistent with the fact that overexpression of the mutant from the GAL1 promoter rescues an esp1ts mutation. However, the Esp1 protein carrying two aspartic acid-to-alanine substitutions (D1568A/D1570A) had a severely reduced ability to bind the spindle (Fig. 8 D, c, and Table II). Of >400 large-budded cells examined, only 1.5% displayed spindle labeling, compared with 28.8% in wild-type cells. In addition, no spindles were observed exceeding 4 μm in length. The reduction in spindle association may account for the loss of complementation ability of this double mutant protein. Thus, the putative calcium binding site in the conserved COOH-terminal domain of Esp1 appears to be required to initiate and/or maintain spindle association. When we tested for a possible effect of calcium on Esp1 function, we noticed that addition of extra CaCl2 to the growth medium rescued the temperature sensitivity of esp1ts mutants. This suppression could not be achieved by other divalent cations, but was specific to Ca2+ and reversible by the addition of the calcium chelator EGTA to the medium (Fig. 8 E). It is therefore possible that calcium may regulate the activity of Esp1, perhaps by promoting spindle association at anaphase.

Discussion

A Novel Role of Esp1 in Anaphase Spindle Elongation

Esp1 is a key player in sister chromatid separation. Its liberation from Pds1 mediated by APC-dependent proteolysis is essential to remove cohesion between duplicated sister chromatids, allowing them to separate when microtubule-dependent forces are exerted by the spindle. To date, it has been unclear whether the Esp1/Pds1 complex also regulates aspects of anaphase spindle elongation. Here we report for the first time that a member of the separatin family is directly required for spindle elongation at anaphase. In the absence of cohesion, Esp1 activity is still required for anaphase spindle movement. The spindle function of Esp1 is consistent with its cell cycle–dependent localization, reported in this paper. Esp1 has previously been shown to bind the spindle apparatus by indirect immunofluorescence, but in this study only a fraction of the protein was reported to interact with the spindle (Ciosk et al., 1998). By monitoring the localization of Esp1 fused to GFP in real time studies, we found that the majority of Esp1 protein associates with the SPBs and the spindle cells progress from metaphase to anaphase (Figs. 1 and 2). In G2, Esp1 briefly accumulates in the nucleus before spindle binding. It is therefore tempting to speculate that Esp1 first associates transiently with chromatin to mediate sister chromatid separation, after which it serves as an anaphase signal transducer, translocating to the spindle. A significant pool of Esp1 is located at the spindle midzone during anaphase. This central region of the spindle, where
polar microtubules overlap, plays an important role in elongation of the anaphase spindle (Pellman et al., 1995; Winey et al., 1995). Although there is still controversy as to whether anaphase elongation occurs by a pushing or pulling mechanism, studies suggest that in S. cerevisiae the nuclear microtubules generate the forces for anaphase spindle elongation (Sullivan and Huffaker, 1992). A recent study of spindle dynamics reveals that elongation of the anaphase spindle in yeast is coupled to microtubule growth at the overlapping plus ends of the spindle midzone (Maddox et al., 2000). The midzone usually becomes highly organized during anaphase, with microtubules from one pole lying adjacent to those from the opposite pole. Electron microscopy studies have revealed that the midzone in esp1ts mutants lacks organization of the antiparallel microtubules (McGrew et al., 1992). Although this could be an indirect result of the inability of esp1ts mutants to separate sister chromatids, in the light of our data, it
more likely reflects a direct role for Esp1 in maintaining proper interdigitation of pole-to-pole microtubules that drive spindle elongation. The midzone association of Esp1 presumably reflects essential aspects of the Esp1 spindle role. It is likely that adequate, but undetectable, amounts of spindle-bound Esp1 remains in ase1Δ and pds1 mutants, which have impaired Esp1 spindle localization (Fig. 3), explaining why these mutants are still viable under unperturbed conditions. Deletion of ASE1 in an esp1ts mutant enhances the temperature sensitivity of the strain, supporting the idea that the midzone is important for Esp1 function. A similar genetic interaction was identified between tub3Δ and esp1ts mutations (not shown), which further links Esp1 to spindle function.

Whether the Esp1 function at the spindle involves degradation of an unidentified midzone component, regulation of an anaphase motor protein, microtubule polymerization, or a structural role in the organization of microtubules from opposite poles remains unknown. The Esp1 protein itself does not show significant homology to any known motor protein, and it does not interact directly with the known midzone protein Ase1. Identification of potential components of the midzone that interact with Esp1 will provide valuable clues to the exact mechanism of action of Esp1 and how the midzone functions during anaphase spindle elongation in general.

**Pds1 Localization Reflects a Regulatory Role of the Esp1/Pds1 Complex during Anaphase**

Pds1 has previously been shown to be a nuclear protein from the time it is produced after bud emergence until it is turned over at the onset of anaphase (Yamamoto et al., 1996). We report the novel finding that Pds1 associates with the spindle apparatus at the metaphase–anaphase transition and that a fraction of Pds1 persists at the SPBs and the spindle midzone into midanaphase. Pds1 may, therefore, like Esp1, be involved in regulating aspects of early anaphase spindle function. The surprising observation that a pool of Pds1 escapes destruction at anaphase onset raises the intriguing question whether distinct subpopulations of Pds1, with specialized tasks, exist in the cell. This would be consistent with the recent report that Pds1, besides its regulatory role at anaphase, also regulates exit of mitosis by preventing release of Cdc14 from the nucleolus, and thus inhibiting cyclin degradation (Cohen-Fix and Koshland, 1999; Shiramaya et al., 1999; Tinker-Kulberg and Morgan, 1999). To this end, it is possible that the remaining pool of Pds1 may be involved in coupling exit of mitosis with the earlier completion of anaphase, either in conjunction with or independently of Esp1. In fact, several components of the mitotic exit network that govern exit of mitosis exhibit SPB localization (Bardin et al., 2000; Song et al., 2000). The reason why a subset of Pds1 resists early degradation is not clear, but may reflect modification or interaction with specific components that delay recognition by the proteolysis machinery.

**Pds1 Targets Esp1 to the Nucleus and Mitotic Spindle**

The general view of Pds1 is that it functions as an inhibitor of Esp1 activity. However, the current model does not explain the observations that Esp1 in high dosage can suppress a pds1ts mutation or that esp1ts and pds1ts mutations exhibit synthetic lethality (D.J. Clarke and S.I. Reed, unpublished data), which argue that the two components have overlapping functions in addition to their antagonistic ones. In this study, we present evidence that Pds1 also promotes Esp1 function by ensuring efficient localization of the latter to the nucleus and the spindle, providing an explanation for this apparent paradox. Esp1 only accumulated in the nucleus and associated with the spindle in the presence of functional Pds1 (Figs. 4, b and c, and 5). It is possible that earlier association with Pds1 is needed merely to induce a conformational change in the structure of Esp1 to render it more competent to interact with the spindle. An alternative, and perhaps more plausible, explanation in the light of our data is that Pds1 is directly involved in mediating Esp1 spindle interaction. Thus, there exists a dual-step requirement for Pds1: first, to transport Esp1 into the nucleus and, second, to presumably load it onto the early mitotic spindle. A loading function of Pds1 is consistent with the observation that Pds1 itself interacts with the spindle with similar timing as Esp1, and the relatively brief kinetics of interaction manifest in the shorter spindle length distribution of Pds1-labeled spindles, as compared with Esp1-decorated spindles.

Although Pds1 is required to accumulate Esp1 in the nucleus, Esp1 is not ostensibly excluded from this compartment when Pds1 is absent, either in wild-type G1 or pds1Δ cells. This, albeit reduced, activity of Esp1 in the nucleus may indeed explain why deletion of *PDS1* is not lethal at low temperatures.

Pds1 may not be the sole factor that regulates Esp1 localization. In cells expressing Esp1GFPNLS, we saw no significant difference in the timing of nuclear and spindle association of Esp1 as compared with cells expressing Esp1GFP alone. There are several possibilities that could account for this unexpected result. First, the NLS may only become exposed after a structural change in Esp1 as a result of modification induced by an unknown event in late G2. Second, a nuclear export mechanism may operate early in the cell cycle, in the absence of Pds1, to reduce the nuclear amount of Esp1, although the Esp1 protein sequence does not reveal obvious nuclear export signals. Alternatively, and perhaps more likely, a cytoplasmic component may retain most Esp1 protein in the cytoplasm in the early stages of the cell cycle. Studies on the Esp1 homologue in *S. pombe*, Cut1, have hinted at the existence of

| Table II. The Distribution of Mutant Esp1GFP Proteins in Live Cells Compared with Wild-Type Esp1GFP |
|-------------------------------------------------|---------------------------------|---------------------------------|
| %                                               | %                               | %                               |
| wt Esp1GFP                                       | 11.6                            | 28.8                            | 59.6 |
| Esp1(D1568A)GFP                                   | 10.0                            | 21.0                            | 69.0 |
| Esp1(D1568A/D1570A)                               | 5.3                             | 1.5                             | 93.2 |

Cells derived from the strains described in Fig. 8 D were analyzed for Esp1GFP localization. For each strain, >400 cells were counted and placed in the following categories: nuclear localization, spindle localization, or nondispersed fluorescent signal. The experiment was repeated at least three times with similar results.
such a cytoplasmic retention factor, although its identity remains unknown (Kumada et al., 1998). The presence of such a regulatory factor could also explain the temporal lag between Esp1 and Pds1 nuclear localization. Early work has shown that Pds1 is mostly nuclear at S phase (Yamamoto et al., 1996), whereas we have demonstrated both in live cells and by indirect immunofluorescence that Esp1 first concentrates in the nucleus in late G2. Yet we know that Esp1 and Pds1 form a complex as soon as Pds1 protein appears in the cell cycle. Therefore, the complex must be retained in the cytoplasm until late G2 unless the amount of complex formed early in the cell cycle is below the detection limit of our readout, which cannot be entirely excluded. If a cytoplasmic retention factor exists, it must be limiting as overexpression of Pds1Δdb protein from the GAL1 promoter allows nuclear accumulation of Esp1 in unbudded cells (Fig. 5 B).

Our data favor a model in which Pds1, through physical interaction, carries Esp1 into the nucleus and positions it efficiently on the spindle at the onset of anaphase. Pds1 presumably has a docking site on the spindle independent of Esp1, as Pds1 spindle localization is unperturbed in esp1Δ mutants. The Esp1/Pds1 complex binds both SPBs and the early anaphase spindle. What event actually triggers the spindle association is not clear at present, but it may require APC activity and initial degradation of Pds1 (Ciosk et al., 1998; our unpublished observations). Upon Pds1 proteolysis, Esp1 is activated to promote sister chromatid separation, and then presumably in conjunction with remaining Pds1 is translocated to the spindle to mediate anaphase elongation, perhaps through interaction with components of the spindle midzone. This model is consistent with the observation that Esp1 can promote loss of cohesion in the absence of functional kinetochores or mitotic spindles (Ciosk et al., 1998). If Pds1 is indeed directly mediating Esp1 spindle interaction, Esp1 must be loaded on the spindle when a stoichiometric amount of Pds1 still remains. Initially, there must be an excess of Pds1 in the cell, as extra Esp1 from overproduction is largely being cleared from the cytoplasm into the nucleus in a Pds1-dependent manner (compare Fig. 4, A with B). Esp1 persists on the spindle through an independent docking site after spindle-bound Pds1 is degraded.

**Function of the Conserved COOH-Terminal Domain in Esp1-related Proteins**

The ability to associate with the spindle apparatus depends on the COOH-terminal region of Esp1 as well as the NH2-terminal third of the protein, which mediates interaction with Pds1. We found, more specifically, that a putative calcium binding site in the COOH terminus of Esp1 is important for proper anaphase spindle function, as an Esp1 mutant with the two amino acid substitutions D1568A/D1579A in this motif had a strongly reduced (~20-fold) spindle binding ability and only labeled spindles shorter than 4 μm (Table II). Indeed, a single mutation at position 1568 was sufficient to yield a mutant Esp1 protein unable to complement an esp1Δ mutation. A similar behavior was recently described for Cut1 alleles mutated in the COOH-terminal region (Kumada et al., 1998). In this study, a Cut1 mutant with a single amino acid change at position 1767, which lies within the potential calcium binding motif, was shown to localize to metaphase spindles but was excluded from anaphase spindles. Thus, the COOH-terminal calcium-binding motif is likely to have equivalent essential roles in Esp1 and Cut1, ensuring proper association of the protein with the spindle during anaphase. It cannot be ruled out that these separin mutants are also defective in triggering loss of cohesion and that the observed effect on spindle binding is indirect.

Whether the conserved calcium binding motif in Esp1 and Cut1 actually mediates calcium binding remains to be shown. Interestingly, esp1Δ mutations were suppressed by the addition of calcium to the growth medium, which could be reversed by simultaneous addition of the chelating agent EGTA. This, together with our finding that mutations in two genes important for calcium homeostasis are lethal in combination with an esp1Δ mutation (our unpublished data), raises the intriguing possibility that calcium may regulate Esp1 activity. It has previously been reported that calcium oscillations occur at the metaphase-to-anaphase transition and play an important role in anaphase onset (Groïno and Whitaker, 1998), although this has yet to be established in yeast.

It is not surprising that Esp1 is regulated by several mechanisms given the importance of sister chromatid separation for the survival of the cell. Once cohesion has been established during DNA replication, it becomes pivotal that Esp1 activity is controlled to coordinate loss of cohesion with anaphase spindle elongation. The fact that pds1Δ cells do not separate sisters prematurely (Ciosk et al., 1998; D.J. Clarke, unpublished data) clearly indicates that alternative mechanisms exist to regulate Esp1 activity.

The Esp1 and Pds1 complex appears to be conserved in organisms of different origin. Their best characterized homologues in *S. pombe*, Cut1 and Cut2, perform equivalent vital roles in sister chromatid separation. Recently, both Cut1 and Cut2 were found to interact with the spindle, and this association (but not the nuclear localization of Cut1) was dependent on Cut2. Thus, given the overall similarities in the regulation and function of the Esp1/Pds1 and Cut1/Cut2 complexes, it seems reasonable to assume that similar functional relationships exist between homologues of Esp1 and Pds1 in higher eukaryotic cells.

We are grateful to D. Koshyland and O. Cohen-Fix for providing the Pds1 tagging vector, T. Davis for providing the Spc29CFP tagging construct, and N. Rhind and P. Russell for help with microscopy. We thank members of the McGowan, Reed, Russell, and Wittenberg laboratories for stimulating discussions.

S. Jensen was supported by a fellowship from the Danish Medical Research Council. M. Segal acknowledges support from the European Molecular Biology Organization (EMBO) and Human Frontier Science Program fellowships, and D.J. Clarke was funded by EMBO and the United States Army Breast Cancer Research fellowships. This research was supported by U.S. Public Health Service grant GM38328 to S.I. Reed.

Submitted: 18 July 2000

Revised: 1 November 2000

Accepted: 14 November 2000

**References**

Alexandru, G., W. Zachariae, A. Schleiffer, and K. Nasmyth. 1999. Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage. *EMBO (Eur. Mol. Biol. Organ.)* 18:2707–2721.

Bardin, A.J., R. Visintin, and A. Amon. 2000. A mechanism for coupling exit of mitosis to partitioning of the nucleus. *Cell.* 102:21–31.

Ciosk, R., W. Zachariae, C. Michaelis, A. Shevchenko, M. Mann, and K. Nasmyth. 1998. An ESP1/PDS1 complex regulates loss of sister chromatid...
cohesion at the metaphase to anaphase transition in yeast. Cell. 93:1067–1076.
Clarke, D.J., M. Segal, G. Mondesert, and S.I. Reed. 1997. The Pds1 anaphase inhibitor and Mec1 kinase define distinct checkpoints coupling S phase with mitosis in budding yeast. Curr. Biol. 9:365–368.
Cohen-Fix, O., and D. Koshland. 1999. Pds1p of budding yeast has dual roles: inhibition of anaphase initiation and regulation of mitotic exit. Genes Dev. 13:1950–1959.
Cohen-Fix, O., J.-M. Peters, M.W. Kirschner, and D. Koshland. 1996. Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. 10: 3081–3093.
Funabiki, H., K. Kumada, and M. Yanagida. 1996. Fission yeast Cut1 and Cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes. EMBO (Eur. Mol. Biol. Organ.) J. 15:6617–6628.
Goh, P.Y., and J.V. Kilmartin. 1993. NDC10: a gene involved in chromosome segregation in Saccharomyces cerevisiae. J. Cell Biol. 121:503–512.
Groigno, L., and M. Whitaker. 1998. An anaphase calcium signal controls chromosome disjunction in early sea urchin embryos. Cell. 92:193–204.
Guthrie, C., and G.R. Fink. 1991. Guide to yeast genetics and molecular biology. Vol. 194. Academic Press, Inc., San Diego, CA.
Jiang, Y.-L., J. Huang, J.-C. Peters, M.E. McLaughlin, C.-Y. Tai, and D. Pellman. 1997. APC-mediated proteolysis of Ase1 and the morphogenesis of the mitotic spindle. Science. 275:1311–1314.
Kaiser, P., V. Moncollin, D.J. Clarke, M.H. Watson, B.L. Bertolaet, S.I. Reed, and E. Baillie. 1999. Cyclin-dependent kinase and Cks/Suc1 interact with the proteosome in yeast to control proteolysis of M-phase targets. Genes Dev. 13:1190–1202.
Kumada, K., T. Nakamura, K. Nagao, H. Funabiki, T. Nakagawa and M. Yanagida. 1998. Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis. Curr. Biol. 8:633–641.
Maddox, P.S., K.S. Bloom, and E.D. Salmon. 2000. The polarity and dynamics of microtubule assembly in the budding yeast Saccharomyces cerevisiae. Nat. Cell Biol. 2:36–41.
May, G.S., C.A. McGoldrick, C.L. Holt, and S.H. Denison. 1992. The bin3 mutation of Aspergillus nidulans uncouples DNA replication from the completion of mitosis. J. Biol. Chem. 267:15737–15743.
McGrew, J.T., L. Goetsch, B. Byers, and P. Baum. 1992. Requirement for ESPl in the nuclear division of Saccharomyces cerevisiae. Mol. Biol. Cell. 3:1443–1454.
Michaelis, C., R. Cisk, and K. Nasmyth. 1997. Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell. 91:35–45.
Mondesert, G., D.J. Clarke, and S.I. Reed. 1997. Identification of genes controlling growth polarity of the budding yeast Saccharomyces cerevisiae: a possible role of N-glycosylation and involvement of the exocyst complex.

Genetics. 147:421–434.
Pellman, D., M. Biggert, H. Tu, and G.R. Fink. 1995. Two microtubule-associated proteins required for anaphase spindle movement in Saccharomyces cerevisiae. J. Cell Biol. 130:1373–1385.
Richardson, H.E., C. Wittenberg, F.R. Cross, and S.I. Reed. 1989. An essential G1 function for cyclin-like proteins in yeast. Cell. 59:1127–1133.
Shirama, M., A. Toth, M. Galova, and K. Nasmyth. 1999. APC(Cdh1) promotes exit from mitosis by destroying the anaphase inhibitor Pds1 and cyclin Clb5. Nature. 11:203–207.
Sikorski, R.S., and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics. 122:19–27.
Song, S., T.Z. Grenfell, S. Garfield, R.L. Erikson, and K.S. Lee. 2000. Essential function of the polo box of Cdc5 in subcellular localization and induction of cytokinetic structures. Mol. Cell. Biol. 20:286–298.
Straight, A.F., W.F. Marshall, J.W. Sedat, and A.W. Murray. 1997. Mitosis in living budding yeast: anaphase A but no metaphase plate. Science. 277:574–578.
Sullivan, D.S., and T.C. Huffaker. 1992. Astral microtubules are not required for anaphase B in Saccharomyces cerevisiae. J. Cell Biol. 119:379–388.
Tang, Y., and S.I. Reed. 1993. The Cdk-associated protein Cks1 functions both in G1 and G2 in Saccharomyces cerevisiae. Genes Dev. 7:822–832.
Tinker-Kulberg, R.L., and D.O. Morgan. 1999. Pds1 and Esp1 control both anaphase and mitotic exit in normal cells and after DNA damage. Genes Dev. 13:1986–1949.
Uhlmann, F., F. Lottspeich and K. Nasmyth. 1999. Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. Nature. 400:37–42.
Uzawa, S., I. Samejima, T. Hirano, K. Tanaka, and M. Yanagida. 1990. The fission yeast cut1+ gene regulates spindle pole body duplication and has homology to the budding yeast ESPl gene. Cell. 62:913–925.
Wach, A., A. Brachut, R. Pohmann, and P. Philippsen. 1994. New heterologous modules for classical or PCR-based gene disruptions in Saccharomyces cerevisiae. Yeast. 10:1793–1808.
Winey, M., C.L. Mamay, E.T. O'Toole, D.N. Mastronarde, T.H. Giddings, K.L. McDonald, and J.R. McIntosh. 1995. Three-dimensional ultrastructural analysis of the Saccharomyces cerevisiae mitotic spindle. J. Cell Biol. 129: 1601–1615.
Yamamoto, A., V. Guacci, and D. Koshland. 1996. Pds1p is required for faithful execution of anaphase in the yeast, Saccharomyces cerevisiae. J. Cell Biol. 133:85–97.
Zachariae, W., and K. Nasmyth. 1996. TPR proteins required for anaphase progression mediate ubiquitination of mitotic B-type cyclins in yeast. Mol. Biol. Cell. 7:791–801.
Zou, H., T.J. McGarry, T. Bernai, M.W. Kirschner. 1999. Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. Science. 285:418–422.