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Destruction of the securin Pds1p occurs at the onset of anaphase during both meiotic divisions in yeast

Suhal-Maria Salah[1], **Kim Nasmyth**[2]

[1] Vienna Biocenter, Institute of Biochemistry and Molecular Biology, Dr. Bohrgasse 9, 1030 Vienna, Austria
[2] Institute of Molecular Pathology, Dr. Bohrgasse 7, 1030 Vienna, Austria

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Abstract. Sister chromatid cohesion is established during DNA replication and depends on a multiprotein complex called cohesin. At the onset of anaphase the cohesive structures that hold sisters together must be destroyed to allow segregation of sisters. In the budding yeast *Saccharomyces cerevisiae* loss of sister chromatid cohesion depends on a separating protein (separin) called Esp1. At the metaphase to anaphase transition, separin is activated by proteolysis of its inhibitory subunit (securin) called Pds1. This process is mediated by the anaphase promoting complex and an accessory protein Cdc20. In meiosis a single round of DNA replication is followed by two successive rounds of segregation. Thus loss of cohesion is spun out over two divisions. By studying the mechanisms that initiate anaphase in meiotic division we show that the yeast securin Pds1p is present in meiotic nuclei and is destroyed at the onset of each meiotic division. We also show that securin destruction depends on Cdc20p which accumulates within nuclei around the time of Pds1p's disappearance.

Introduction

Cohesion between sister chromatids is essential for their attachment to microtubules that extend to opposite poles of the cell when cells enter mitosis (Miyazaki and Orr-Weaver 1994; Rieder and Salmon 1998). It is also essential for opposing the tendency of mitotic spindles to separate sisters until all chromosomes have congressed to the metaphase plate. On the other hand, the sudden destruction of sister chromatid cohesion is thought to be responsible for the segregation of sister chromatids to opposite poles of the cell during anaphase (Miyazaki and Orr-Weaver 1994; Nicklas 1988). In budding yeast a multisubunit complex called cohesin has an essential role in holding sister chromatids together during G_2 and M phase (Guacci et al. 1997; Losada et al. 1998; Michaelis et al. 1997; Skibbens et al. 1999; Toth et al. 1999). Sister chromatid cohesion is established during DNA replication (Uhlmann and Nasmyth 1998). At least in budding yeast sister chromatid separation at the metaphase to anaphase transition appears to be triggered by the sudden disappearance from chromosomes of Scc1p, one of cohesin's subunits (Michaelis et al. 1997). Furthermore, the proteolytic cleavage of Scc1p is essential for sister chromatid separation (Uhlmann et al. 1999). Cleavage of Scc1p is induced by a separin protein (Esp1p), whose activity is inhibited by the binding of an inhibitory „securin" protein (Pds1p; Ciosk et al. 1998; Uhlmann et al. 1999). Separin is activated at the metaphase to anaphase transition by proteolysis of its inhibitory securin subunit; a process that is mediated by a ubiquitin protein ligase called the anaphase promoting complex/cyclosome and an accessory protein called Cdc20p (APC/C^{Cdc20}; Ciosk et al. 1998; Cohen-Fix et al. 1996). In the presence of lagging chromosomes, APC/C^{Cdc20} is inhibited by Mad2p, which prevents separin activation and ensures that sister chromatids all separate simultaneously and only when all chromosomes have correctly aligned on the mitotic spindle (Alexandru et al. 1999; Fang et al. 1998).

Sister chromatid cohesion and its destruction also have key roles during meiosis. Premeiotic DNA replication produces sister chromatids that are tightly bound. Cohesion between sister chromatid arms distal to chiasmata are possibly crucial for holding homologous chromosomes together during metaphase of meiosis I. Indeed, destruction of arm cohesion is presumably essential for homologue segregation. Meanwhile, however, cohesion between sister chromatids in the neighborhood of centromeres is maintained until the second meiotic division. Centromeric cohesion is used to align sister chromatids on the meiosis II spindle, whereas its destruction permits the separation of sister chromatids and the formation of haploid gametes. In yeast the Scc1p cohesin subunit has little or no role in chromosome segregation during meiosis (Klein et al. 1999). It is replaced by a meiotic variant called Rec8p (DeVeaux and Smith 1994; Michaelis et al.

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Correspondence to: K. Nasmyth
e-mail: nasmyth@nt.imp.univie. ac.at

Table 1. List of strains used in this study and their relevant genotypes

Strain	Relevant genotype
K6151	MATa, ho::LYS2, lys2, leu2::hisG, his4X, ura3, trp1::hisG
K7210	MATa, ho::LYS2, CenV-GFP
K7238	MATα, ho::LYS2, lys2, leu2R, ura3, trp1
K7412	MATa, ho::LYS2, PDS1-myc18::TRP1,
K7413	MATa/α, ho::LYS2, PDS1-myc18::TRP1
K7833	MATa/α, ho::LYS2, CenV-GFP
K7852	MATα, N-myc18-TRP1-CDC20
K7853	MATa, N-myc18-TRP1-CDC20
K7854	MATa/α, N-myc18-TRP1-CDC20
K7872	MATα, PDS1-myc18::TRP1, *cdc20–3*
K7873	MATa, PDS1-myc18::TRP1, *cdc20–3*
K8025	MATa/α, PDS1-myc18::TRP1, *cdc20–3*

1997; Molnar et al. 1995; Parisi et al. 1999). Because Rec8p contains two motifs that resemble the separin cleavage sites within Scc1p, it is possible that separin activation has important roles during the first and/or the second meiotic division. If so, one would expect securins to be present prior to each meiotic division and to be destroyed shortly before chromosome segregation. In this contribution we report that the yeast securin Pds1p is indeed present within nuclei for much of meiosis, and that it disappears shortly before each meiotic division. We also show that securin destruction depends on Cdc20p, which is absent for much of meiosis but accumulates within cells around the time of Pds1p's disappearance. These observations suggest that the principle of the mechanism by which chromosome segregation is triggered during meiosis is similar to that employed during mitosis.

Materials and methods

Strains and media

All yeast strains were derivatives or were back-crossed at least six times to SK1 (if originally W303). Strains used in this study are listed in Table 1. Unless otherwise stated, strains were sporulated as described previously (Nairz and Klein 1997). Briefly, cells were grown on a YPD plate for about 24 h, then transferred to liquid YPA, and incubated until they reached a density of approximately 2×10^7 cells/ml upon which the medium was replaced by half the volume of sporulation medium 2% potassium acetate (t=0).

Introducing myc-tagged PDS1 into SK1

For introducing this tag into SK1 genomic DNA was prepared from W303 strain K7218 from containing C-terminally myc18-TRP1 tagged PDS1. This cassette plus 500 bp each upstream and downstream of the PDS1 stop codon (total size 2.4 kb) were amplified by PCR. The 500-bp-long flanks were needed to ensure integration of the cassette into the SK1 genome when transformed since a high region of homology is needed to overcome the possible differences in the sequence between W303 and SK1 strains. The PCR-amplified 2.4-kb cassette was then transformed into the SK1 strain K7238. To check whether the positive $TRP1^+$ transformants had really integrated the cassette in the C-terminus of PDS1, genomic DNA was prepared from colony purified transformants and then checked by PCR and by immunostaining for myc-tagged PDS1. The strain K7411 (MATα, PDS1-myc18::TRP1) was obtained, then crossed to K6151; the heterozygous diploid was in turn sporulated to yield the haploid K7412 (MATa, PDS1-myc18::TRP1). By crossing K7411 to K7412 the diploid and PDS1-myc18::TRP1 homozygous strain K7413 was obtained.

Introducing Myc-tagged CDC20 into SK1

For introducing N-terminally Myc18-tagged CDC20 (18 Myc epitopes), the plasmid GY3 (a gift from Masaki Shirayama; Shirayama et al. 1998) was used. The plasmid contains a fragment of 500 bp of the N-terminus of CDC20 fused to a Myc18-TRP1 construct. The linearized plasmid was transformed into the SK1 strains K7238 (MATα) and K6151 (MATa), resulting in the transformant strains K7852 (MATα, N-Myc18-TRP1 tagged CDC20) and K7853 (MATa, N-Myc18-TRP1 tagged CDC20), respectively. The transformant strains were checked by in situ immunolocalization. The diploid homozygous N-Myc18-TRP1 tagged CDC20 SK1 strain K7854 was produced by mating K7852 and K7853.

Introducing the Ts cdc20-3 *allele into SK1*

The Ts allele of *cdc20-3* was back-crossed 5x to SK1 strain K7412 (MATa, PDS1-myc18::TRP1), starting with crossing the W303 strain K7107 (MATα, *cdc20-3*) to the SK1 strain K7412. From the last back-cross, the diploid was sporulated and the chosen haploids K7873 (MATa, *cdc20-3*, PDS1-myc18::TRP1) and K7872 (MATα, *cdc20-3*, PDS1-myc18::TRP1) were then mated creating the diploid K8025 homozygous for *cdc20-3* and PDS1-myc18::TRP1.

In situ immunolocalization

In situ immunofluorescence was performed according to Piatti et al. (1996). Cells were fixed in the 0.1 M K-phosphate buffer pH 6.4, with 0.5 mM $MgCl_2$ and 3.4% formaldehyde at 4°C overnight. They were washed with the same buffer without formaldehyde and once with K-phosphate buffer pH 7.4, 0.5 mM $MgCl_2$ containing 1 M sorbitol. Cells were then spheroplasted in the same buffer to which 1/500 volume mercaptoethanol was added, and 100-T Zymolase (ICN Biochemicals) to final concentration 1 mg/ml at 37°C for 10–15 min.

For detection of Myc-tagged proteins, spheroplasts attached to the polylysine-covered microscope well-slide and dehydrated in methanol and acetone, were first incubated with monoclonal anti-Myc antibody 9E10 hybridoma supernatant (diluted 1:5) at 25°C for 1 h and after washing with an Cy3-coupled anti-mouse antibody from sheep (Sigma).

For detection of tubulin spheroplasts were first incubated with anti-tubulin antibody A447 (rat) at 25°C for 1 h and after washing with an rhodamin-coupled anti-rat antibody. For double staining of Myc-tagged proteins and tubulin the same procedure of staining was followed: first, spheroplasts were incubated with anti-myc antibody and with Cy3-coupled anti-mouse antibody for 1 h each. Then in a second round of staining the spheroplasts were incubated with anti-tubulin antibody A447 for 1 h and then with a FITC-coupled anti-rat antibody from goat. DNA was stained with DAPI.

Assay for spore viability

Spore viability was assayed either by tetrad dissection or, in the case of very low spore viability by plating spores to rich medium, followed by killing unsporulated cells using ether (Prinz et al. 1997). Ascus frequencies were determined under phase contrast.

Results

Securin disappears shortly before the first and second meiotic divisions

To detect whether the yeast securin Pds1p is expressed during meiosis the endogenous *PDS1* gene was replaced

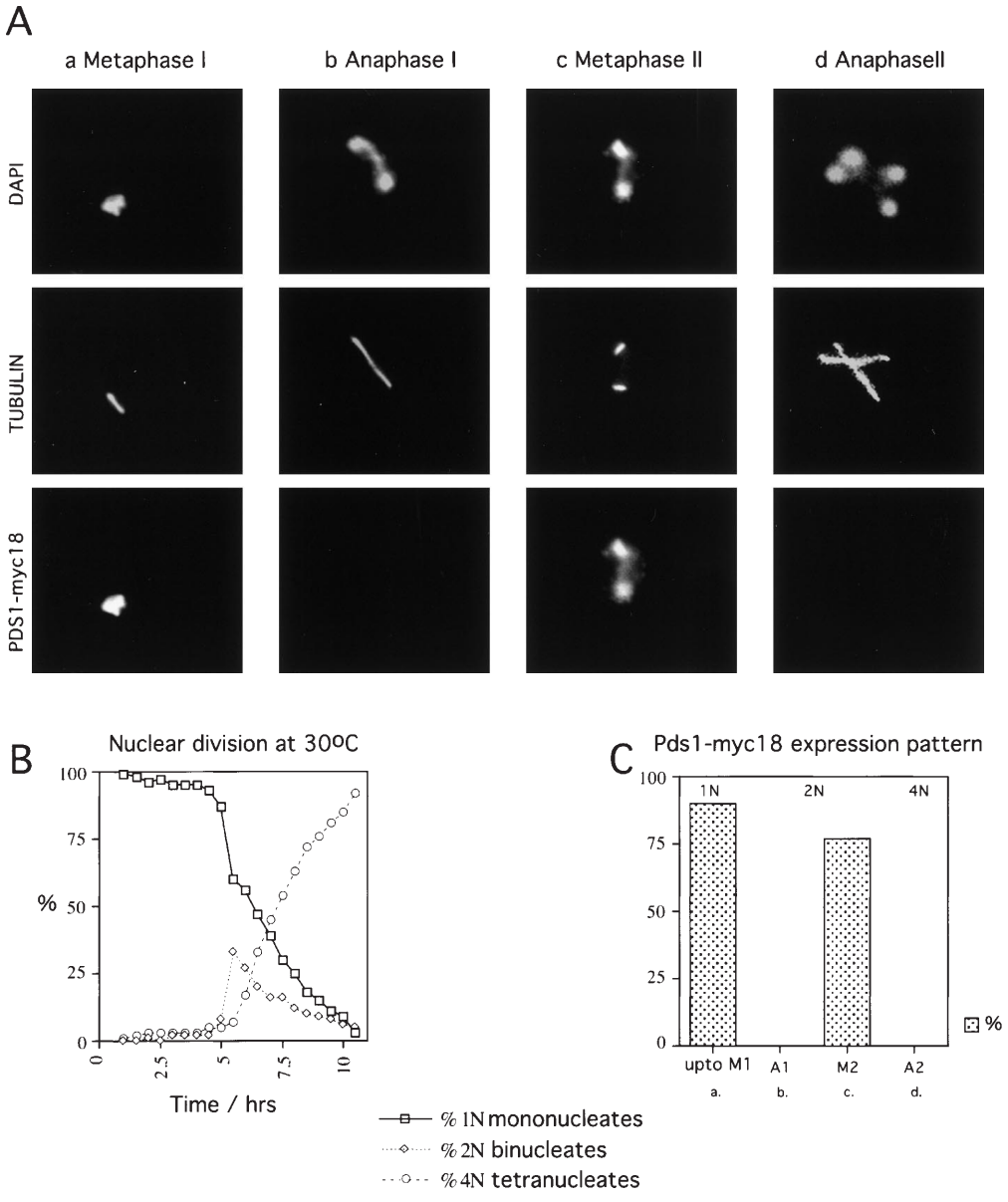

Fig. 1A–C. Fluctuation of Pds1p expression during meiosis. Cells of the diploid strain K7413 (homozygous for Pds1-Myc18::TRP1) were transferred to sporulation medium at 30°C. Progression of nuclear division and Pds1p expression was monitored by DAPI staining and in situ immunofluorescence, based on double labeling of tubulin and Pds1p. Several classes of cells were identified whose frequency peaked in a defined order consistent with their being successive meiotic stages. In this way Pds1p was shown to be expressed twice during meiosis: **A** Pds1p is expressed from premeiotic S phase through prophase and persists until metaphase I (*a*). At the onset of anaphase I (*b*), Pds1p disappears from nuclei, here showing a binucleate with elongated spindle at anaphase I. At metaphase II Pds1p is again expressed in these binucleates (*c*), only to disappear again at the onset of anaphase II (*d*) and is not expressed in tetranucleates. To check that Pds1-Myc18 was functional in cells even after 10 h of incubation in sporulation medium at 30°C, tetrads were taken from sporulation medium and dissected on rich medium plates. Spores showed normal viability (*growth*). **B** Monitoring nuclear division and sporulation. Samples taken at hourly intervals were DAPI stained and examined for mononucleates, binucleates and tetranucleates; starting with 100% mononucleate cells at *t*=0, 92% of cells were tetranucleates (completed both meiotic divisions) 10 h after shifting to sporulation medium. Although asynchronous, the amount of cells undergoing first division peaked at about 5–6 h after shift, showing approx. 30% binucleates. **C** Quantitation of the Pds1p expression pattern. Pds1p is expressed in cells from premeiotic S phase through prophase until metaphase I. Pds1p is expressed at metaphase I in 91% of spindle-positive mononucleates and in about 80% metaphase II spindle-positive binucleates. In each stage 200 cells were counted. *Lowercase letters (a, b, c, d) below columns* show the corresponding picture of each of the stages in **A**

by a version that encodes multiple (18) myc epitopes at its C-terminus (Pds1-myc). Diploid SK-1 cells homozygous for Pds1-myc were transferred to sporulation medium and their progression through meiosis monitored by recording the proportion of cells with one, two, and four nuclei (Fig. 1A). Due to the poor synchrony of meiotic cultures we analyzed Pds1-myc's abundance not using protein blotting, which gives average values, but instead using in situ immunofluorescence, which permits the detection of Pds1p within individual cells. Meiotic cell

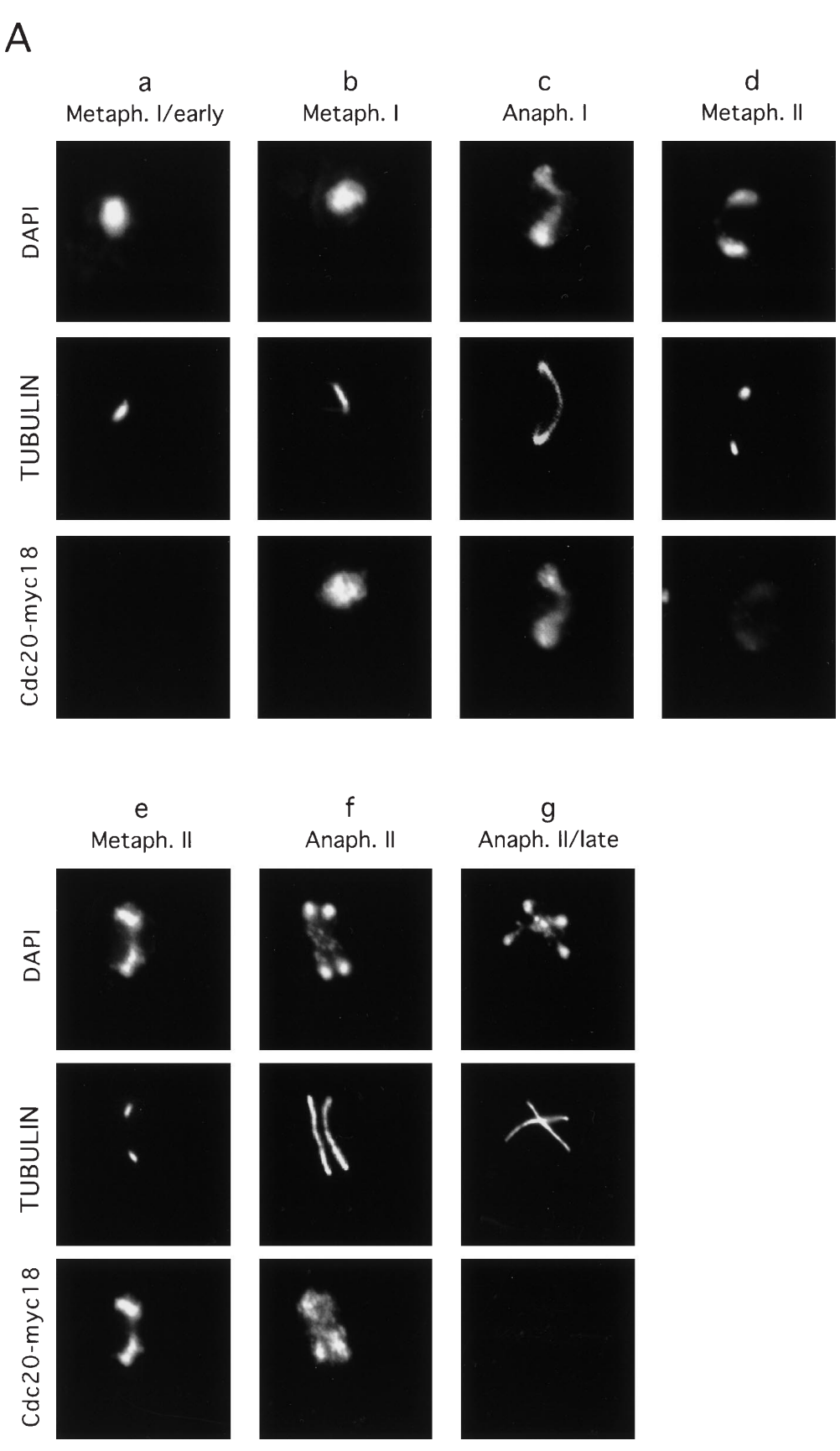
A
a
Metaph. I/early
b
Metaph. I
c
Anaph. I
d
Metaph. II
DAPI
TUBULIN
Cdc20-myc18
e
Metaph. II
f
Anaph. II
g
Anaph. II/late
DAPI
TUBULIN
Cdc20-myc18

Fig. 2A

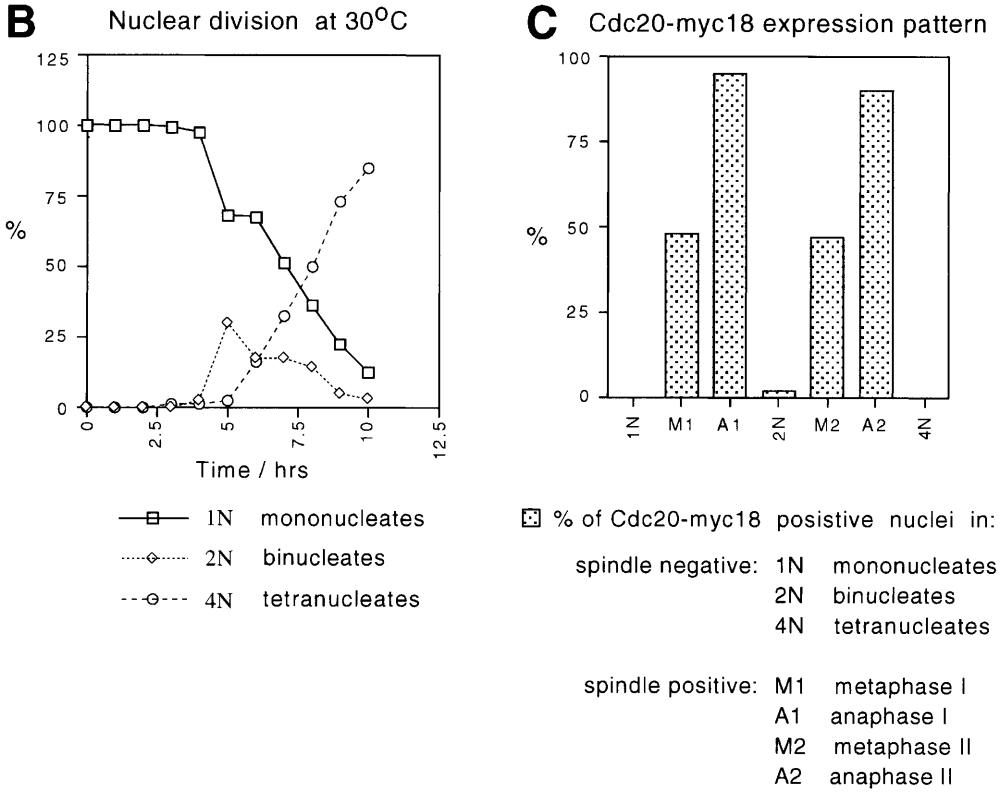

Fig. 2A–C. Fluctuation of Cdc20-Myc18 expression throughout meiosis. Cells of the strain K7854 (homozygous for Cdc20-Myc18) were transferred to sporulation medium (at 30°C). Nuclear division and Cdc20p expression were monitored by DAPI staining and in situ immunofluorescence. Based on the double labeling of tubulin and Cdc20p, seven distinct successive stages of Cdc20p expression in meiosis could be identified. **A** Cdc20p abruptly appears at anaphase onset in late metaphase I mononucleates (*a*, *b*) and persists through anaphase I in the binucleate (*c*). Cdc20p then disappears for a short time from binucleates with regressed spindles, between first and second division (*d*), but abruptly reappears at metaphase II (*e*), and persists through anaphase II (*f*) tetranucleates. Finally, no Cdc20-Myc18 signal is observed in tetranucleates after anaphase II (*g*). **B** Monitoring nuclear division and sporulation. Samples taken at hourly intervals, were DAPI stained and examined for mononucleates, binucleates and tetranucleates. As shown here, starting with 100% mononucleate cells at *t*=0, about 90% of cells were tetranucleate (completed both meiotic divisions), 10 h after shifting cells to sporulation medium. The amount of cells undergoing first division peaked at about 5 h with 28% binucleates achieved at this timepoint. **C** Quantitation of the Cdc20p expression pattern during meiosis. Based on the double labeling of tubulin and Cdc20p by in situ immunofluorescence, together with DAPI staining of chromatin, seven distinct successive stages of Cdc20p expression during meiosis could be identified (as shown in **A**). Cdc20p expression was monitored by scoring 200 cells per spindle positive stage. Cdc20p abruptly appears at anaphase onset in meiosis I. This abrupt appearance counts for the fact that only 50% of mononucleates in metaphase I (with metaphase I spindles) were scored positive for Cdc20p expression. Cdc20p expression persists through anaphase I in 95% of binucleates with elongated anaphase I spindles. Cdc20p then disappears from binucleates between first and second division (regressed spindle) but abruptly reappears at metaphase II. Again, the abrupt „re"-expression of Cdc20p was observed in only about 50% of binucleates containing metaphase II spindles in both nuclei. Cdc20p expression persists throughout anaphase II in 90% of tetranucleates with anaphase II spindles. Finally, no Cdc20-Myc18 signal is observed in tertranucleates

stage was assessed by staining DNA with DAPI and mitotic spindles by decoration with tubulin-specific antibodies (Fig. 1B). As in mitotic cells, Pds1-myc was concentrated within nuclei during meiosis. It was found in 90% or more uninucleate cells and persisted in such cells until formation of the first meiotic spindle. However, it was absent in cells that had commenced anaphase I (i.e., binucleate cells with an anaphase spindle) and in cells that had commenced anaphase II (i.e., tetranucleate cells with two anaphase spindles). In contrast, it was present in a large proportion of those binucleate cells that had just disassembled their first meiotic spindle or had just formed a pair of meiosis II spindles (Fig. 1C). These data suggest that upon transfer to sporulation medium, Pds1-myc accumulates within nuclei prior to pre-meiotic DNA replication, persists there until metaphase I, disappears (due to degradation) around the commencement of anaphase I, shortly thereafter reaccumulates within nuclei, but disappears once more around the start of anaphase II. These data are therefore consistent with the notion that securin proteolysis might be crucial for both meiotic and mitotic divisions. Diploid SK1 Cells homozygous for a Pds1 deletion failed to undergo either meiotic division (data not shown).

Cdc20p accumulates only immediately prior to meiosis I and II

During vegetative divisions Pds1p is destroyed shortly before anaphase through the activity of the APC/C acting

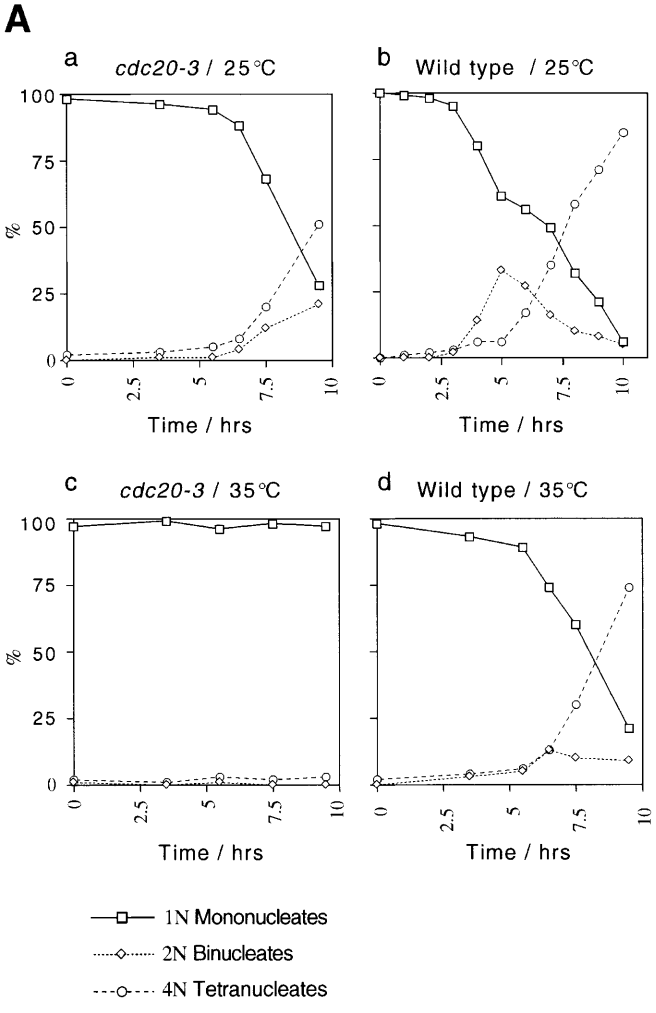

Fig. 3A–C. Cdc20p is essential for meiosis and Pds1p destruction during meiosis I. SK1 strain K8025 was constructed homozygous for both *cdc20-3* Ts allele and a Myc18-tagged version of Pds1p. Cells were grown to log phase at permissive temperature (25°C) and then shifted to sporulation medium at both permissive (25°C) and restrictive temperature (35°C). **A** Monitoring nuclear division and sporulation; samples taken at hourly intervals were DAPI stained and examined for mononucleates, binucleates, and tetranucleates (200 cells were scored per time point). Starting with 100% mononucleate cells at *t*=0 (in all cases), the graphs (*b*, *d*) show that wild-type cells successfully undergo both nuclear divisions at both temperatures producing 85% (*b*) and 74% (*d*) tetranucleate cells, 10 h after shifting cells to sporulation medium. On the other hand, *cdc20-3* mutant cells were shown to undergo both nuclear divisions at permissive temperature, producing about 60–65% tetranucleates 10 h after transfer to sporulation medium (*a*), while at 35°C 96% of *cdc20-3* mutant cells failed to undergo any nuclear division (*c*) arresting as mononucleates in metaphase I. Taking a 24-h time point, *cdc20-3* cells, at restrictive temperature, failed to undergo any meiotic division and did not sporulate. Both *cdc20-3* mutant strain K8025 (*cdc20-3*, Pds1-Myc18) and wild-type K7413 (Pds1-Myc18 samples were taken at hourly intervals, and cells were stained by in situ immunofluorescence (double labeling of tubulin and Pds1p) and DAPI staining of chromatin. **B**. In situ immunofluorescence showed that Pds1p accumulates in all *cdc20-3* mononucleate arrested cells with metaphaselike spindles. The pictures show a *cdc20-3* mononucleate cell, arrested in metaphase I, accumulating nondegraded Pds1p in the nucleus. **C** Monitoring of Pds1p accumulation in *cdc20-3* mononucleate arrested cells. Comparing Pds1p-myc18 accumulation/degradation between the *cdc20-3* mutant, and wild-type cells at 25°C and 35°C. For comparison, 200 cells were scored per time point, counting the number of metaphase I mononucleates positive for Pds1-myc18. The graphs show the proportions of Pds1-myc positive metaphase I cells from the total number of cells. *a* Pds1p expression and degradation in *cdc20-3* mutant and wild-type cells at 25°C: comparable values can be observed at permissive temperature. *b* At 35°C, comparing Pds1p accumulation in *cdc20-3* mutants and wild-type cells, shows that while wild-type cells degrade Pds1p from their nuclei (approx. 30% Pds1p positive nuclei), 90% of *cdc20-3* mutants accumulate Pds1p in their nucleus

with its activator protein Cdc20p (Ciosk et al. 1998; Cohen-Fix et al. 1996). To investigate whether Cdc20p can play a similar role during meiosis we used in situ immunofluorescence to monitor expression of a myc-tagged Cdc20 protein (Cdc20-myc) during meiosis. Remarkably, the distribution of Cdc20-myc was largely reciprocal to that of Pds1-myc. Cdc20-myc was absent from before premeiotic DNA replication until metaphase I. It appeared within nuclei soon after the formation of the first meiotic spindle, persisted during anaphase, soon thereafter disappeared, but reaccumulated within nuclei soon after formation of meiosis II spindles, and disappeared once again during anaphase II (Fig. 2). This pattern is consistent with the notion that Cdc20p might be responsible for Pds1p's proteolysis prior to the first and second meiotic divisions.

CDC20 *is essential for meiosis and for Pds1p's disappearance during meiosis I*

To investigate Cdc20p's role during meiosis we generated a diploid homozygous for the *cdc20-3* Ts mutant (Shirayama et al. 1998) that expressed Pds1-myc18. In mitosis *cdc20-3* mutants fail to destroy Pds1p and to separate sister chromatids; thus *cdc20-3* mutants fail to initiate anaphase (Shirayama et al. 1998). Indeed, these mutant cells failed to undergo any meiotic division at 35°C and accumulated as uninucleate cells whose nuclei contained high levels of Pds1-myc and a metaphaselike mitotic spindle (Fig. 3). These data are consistent with the notion that the appearance of Cdc20p during metaphase I is responsible for the subsequent degradation of Pds1p and for the onset of anaphase I.

Discussion

We have shown that Pds1p is present within nuclei for much of meiosis but disappears transiently around the onset of anaphase I and II. Pds1p's disappearance during the first meiotic division depends on the activity of Cdc20p, which is absent for much of meiosis but appears transiently during metaphase I and II. These observations suggest that Pds1 proteolysis is required for both meiotic divisions, and that this event is triggered on each occasion by the sudden appearance of Cdc20p. During vegetative divisions Pds1p forms a stable complex with the separin Esp1p and inhibits its ability to induce the cleavage of the cohesin

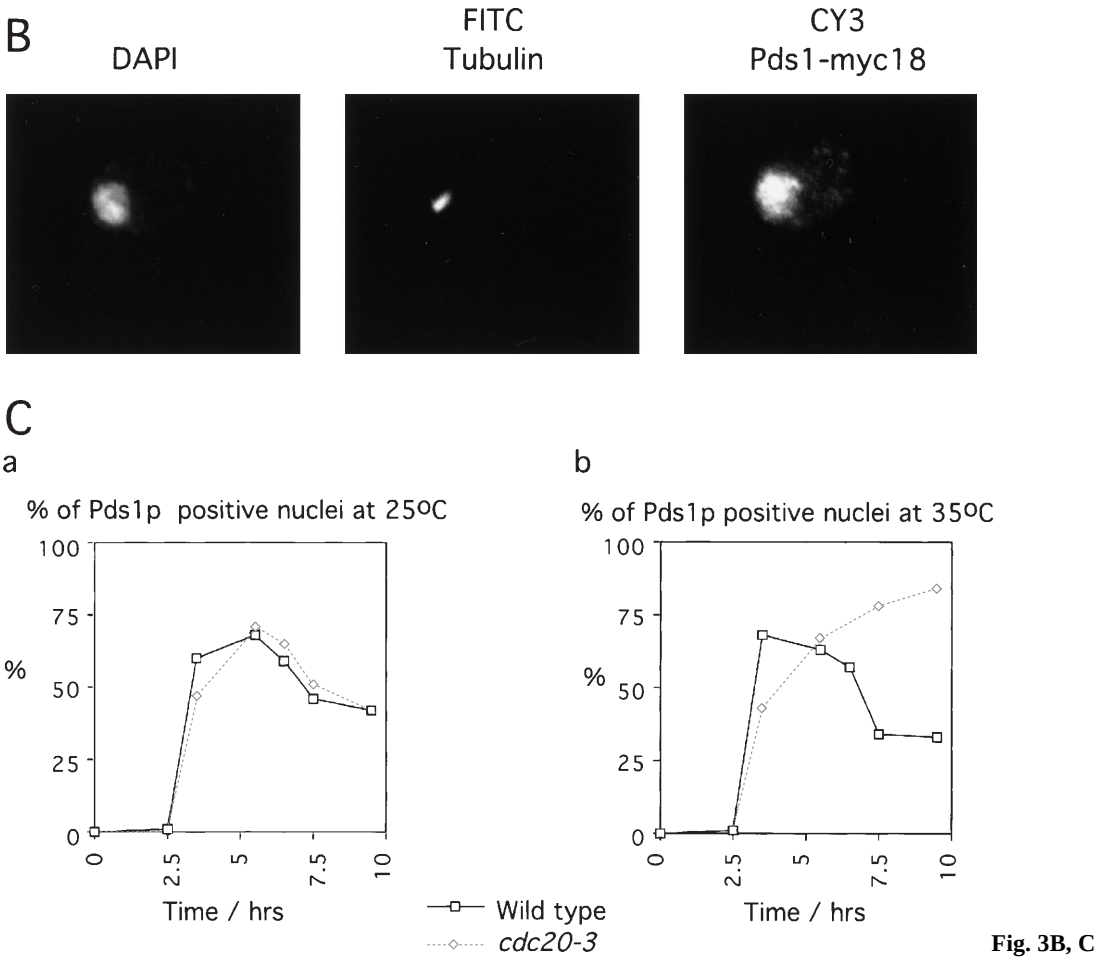

Fig. 3B, C

protein Scc1p. It is reasonable to suppose that Pds1 might fulfill a similar role during meiosis; that is, it may bind to and inhibit separin activity. Separin's target might be Rec8p, which replaces Scc1p during meiosis. This raises the possibility that the transient activation of separin due to Pds1 degradation might trigger the resolution of chiasmata and the separation of sister chromatids within chromosome arms during the first meiotic division and the separation of sister centromeres at the second division. If this hypothesis is correct, separin should be stably bound by securin until the metaphase to anaphase transition of meiosis I and its release due to Cdc20p's activation should be crucial for both reductional and equational divisions. Whether this is the case, and whether Rec8p is the target for separin during meiosis remains to be determined.

In conclusion, our data suggest that the mechanism by which sister chromatid separation is triggered during meiosis may have much in common with that employed by mitotic cells. Destruction of securins by the APC/C^{Cdc20} may well be the trigger for anaphase during meiosis I and meiosis II.

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References

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

Ciosk R, Zachariae W, Michaelis C, Shevchenko A, Mann M, Nasmyth K (1998) An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell 93:1067-1076

Cohen-Fix O, Peters J-M, Kirschner MW, Koshland D (1996) Anaphase initiation in Sacharomyces cervisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pdslp. Genes Dev 10:3081–3093

DeVeaux LC, Smith GR (1994) Region-specific activators of meiotic recombination in Schizosaccharomyces pombe. Genes Dev 8:203–210

Fang G, Yu H, Kirschner MW (1998) The checkpoint protein MAD2 and the mitotic regulator CDC20form a ternary complex with the anaphase promoting complex to control anaphase initiation. Genes Dev 12:1871–1883

Guacci V, Koshland D, Strunnikov A (1997) A direct link between sister chromatid cohesion and chromosome condensation revealed through analysis of MCD1 in *S. cerevisiae*. Cell 91:47–57

Klein F, Mahr P, Galova M, Buonomo S, Michaelis C, Nairz K, Nasmyth K (1999) A central role for cohesins in sister chromatis cohesion, formation of axial elements, and recombination during meiosis. Cell 98:91–103

Losada A, Hirano M, Hirano T (1998) Identification of Xenopus SMC protein complexes required for sister chromatid cohesion. Genes Dev 12:1986–1997

Michaelis C, Ciosk R, Nasmyth K (1997) Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. Cell 91:35–45

Miyazaki WY, Orr-Weaver TL (1994) Sister-chromatid cohesion in mitosis and meiosis. Annu Rev Genet 28:167–187

Molnar M, Bähler J, Sipiczki M, Kohli J (1995) The rec8 gene of Schizosaccharomyces pombe is involved in linear element formation, chromosome pairing and sister- chromatid cohesion during meiosis. Genetics 141:61–73

Nairz K, Klein F (1997) Mre11S – a yeast mutation that blocks double-strand-break processing and permits non-homologous synapsis in meiosis. Genes Dev 11:2272–2290

Nicklas RB (1988) The forces that move chromosomes in mitosis. Annu Rev Biophys Chem 17:431–449

Parisi S, McKay MJ, Molnar M, Thompson MA, van der Spek PJ, van Drunen-Schoenmaker E, Kanaar R, Lehmann E, Hoeijmakers JH, Kohli J (1999) Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the rad21 p family conserved from fission yeast to humans. Mol Cell Biol 19:3515–3528

Piatti S, Bohm T, Cocker JH, Diffley JF, Nasmyth K (1996) Activation of S-phase-promoting CDKs in late Gl defines a „point of no return“ after which Cdc6 synthesis cannot promote DNA replication in yeast. Genes Dev 10:1516–1531

Prinz S, Amon A, Klein F (1997) Isolation of COM 1, a now gene required to complete meiotic double-strand break-induced recombination in Saccharomyces cerevisiae. Genetics 146:781–795

Rieder CL, Salmon ED (1998) The vertebrate cell kinetochore and its roles during mitosis. Trends Cell Biol 8:310–318

Shirayama M, Zachariae W, Ciosk R, Nasmyth K (1998) The polo-like Cdc5 and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in S. cerevisiae. EMBO J 17:0:101–114

Skibbens RV, Corson LB, Koshland D, Hieter P (1999) Ctf7p is essential for sister chromatid cohesion and links mitotic chromosome structure to the DNA replication machinery. Genes Dev 13:307–319

Toth A, Ciosk R, Uhlmann F, Galova M, Schleifer A, Nasmyth K (1999) Yeast Cohesin complex requires a conserved protein, Eco1p (Ctf7), to establish cohesion between sister chromatids during DNA replication. Genes Dev 13:320–333

Uhlmann F, Nasmyth K (1998) Cohesion between sister chromatids must be established during DNA replication. Curr Biol 8:1095–1101

Uhlmann F, Lottspeich F, Nasmyth K (1999) Sister chromatid separation at the onset of anaphase triggered by cleavage of the cohesin subunit Sccl p. Nature 400:37–42