# The budding yeast polo-like kinase Cdc5 regulates the Ndt80 branch of the meiotic recombination checkpoint pathway

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**ABSTRACT** **Defects in chromosome synapsis and/or meiotic recombination activate a surveillance mechanism that blocks meiotic cell cycle progression to prevent anomalous chromosome segregation and formation of aberrant gametes. In the budding yeast *zip1* mutant, which lacks a synaptonemal complex component, the meiotic recombination checkpoint is triggered, resulting in extremely delayed meiotic progression. We report that overproduction of the polo-like kinase Cdc5 partially alleviates the meiotic prophase arrest of *zip1*, leading to the formation of inviable meiotic products. Unlike vegetative cells, we demonstrate that Cdc5 overproduction does not stimulate meiotic checkpoint adaptation because the Mek1 kinase remains activated in *zip1 2μ-CDC5* cells. Inappropriate meiotic divisions in *zip1* promoted by high levels of active Cdc5 do not result from altered function of the cyclin-dependent kinase (CDK) inhibitor Swe1. In contrast, *CDC5* overexpression leads to premature induction of the Ndt80 transcription factor, which drives the expression of genes required for meiotic divisions, including *CLB1*. We also show that depletion of Cdc5 during meiotic prophase prevents the production of Ndt80 and that CDK activity contributes to the induction of Ndt80 in *zip1* cells overexpressing *CDC5*. Our results reveal a role for Cdc5 in meiotic checkpoint control by regulating Ndt80 function.**

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## INTRODUCTION

In sexually reproducing organisms, meiosis generates haploid gametes from diploid parental cells because a single phase of DNA duplication is followed by two consecutive rounds of nuclear division. During the first (reductional) meiotic division, homologous chromosomes (homologues) segregate, whereas during the second (equational) meiotic division, sister chromatids separate as in mitosis. To accurately accomplish this complex behavior, meiotic chromosomes undergo a series of interactions during the lengthy meiotic prophase, including pairing, synapsis, and recombination between homologues (Roeder, 1997; Petronczki *et al.*, 2003). In response to defects in chromosome synapsis and meiotic recombination, the so-called pachytene checkpoint or meiotic recombination is triggered and blocks or delays entry into meiosis I until those crucial processes have been completed (Roeder and Bailis, 2000; Borner, 2006; Hochwagen and Amon, 2006; Longhese *et al.*, 2009). Thus the pachytene checkpoint ensures the accuracy of meiotic chromosome segregation and the genomic integrity of the meiotic progeny contributing to the formation of healthy gametes. In humans, defects in meiosis are a prominent cause of genetic syndromes, spontaneous abortions, and infertility disorders (Hassold and Hunt, 2001).

In *Saccharomyces cerevisiae*, the structurally conserved coiled-coil Zip1 protein is a major component of the central region of the synaptonemal complex (SC). In the *zip1* mutant, homologues pair but fail to synapse; in addition, crossover formation is reduced (Sym *et al.*, 1993; Storlazzi *et al.*, 1996; Tung and Roeder, 1998; Borner *et al.*, 2004). As a consequence of the *zip1* meiotic defects, the pachytene checkpoint is triggered in the mutant, resulting in a robust meiotic block or delay. The Dmc1 protein is a meiosis-specific homologue of Rad51 involved in strand invasion during meiotic recombination (Bishop *et al.*, 1992). In the *dmc1* mutant, hyperresected unrepaired DNA double-strand breaks accumulate, leading also to activation of the meiotic recombination checkpoint (Lydall

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Abbreviations used: CDK, cyclin-dependent kinase; MSE, middle sporulation element; PGK, phosphoglycerate kinase; SC, synaptonemal complex.

*et al.*, 1996; Xu *et al.*, 1997). Thus the budding yeast *zip1* and *dmc1* mutants are useful genetic tools to elicit the meiotic recombination checkpoint response, and they are widely used to investigate this meiotic surveillance mechanism. Mutations in components of this checkpoint alleviate the meiotic arrest or delay of *zip1* and *dmc1* but lead to the formation of largely inviable spores.

Meiotic defects, such as incomplete synapsis or accumulation of meiotic recombination intermediates, are initially detected by the checkpoint sensors, including the Mec1/Ddc2 and the "9-1-1" complexes (Lydall *et al.*, 1996; Hong and Roeder, 2002; Refolio *et al.*, 2011). Although these sensors are shared with the DNA damage checkpoint, the Rad9 adaptor and the Rad53 effector kinase do not function in the meiotic recombination checkpoint. In turn, the meiosis-specific Red1 and Hop1 proteins, which localize to the lateral elements of the SC, contribute to the activation of the meiotic Mek1 effector kinase (Bailis *et al.*, 2000; Woltering *et al.*, 2000; Wan *et al.*, 2004; Niu *et al.*, 2007; Carballo *et al.*, 2008; Eichinger and Jentsch, 2010). Like Rad53, Mek1 belongs to the family of checkpoint kinases containing forkhead-associated domains (Durocher *et al.*, 1999; Li *et al.*, 2002; Perez-Hidalgo *et al.*, 2003). Meiotic checkpoint activity is also regulated by the FK506-binding protein Fpr3, which counteracts the Glc7 phosphatase to modulate checkpoint adaptation (Bailis and Roeder, 2000; Hochwagen *et al.*, 2005). In addition, chromatin-silencing factors, such as Dot1 and Sir2, as well as the meiosis-specific nucleolar-enriched Pch2 protein, participate in meiotic checkpoint control (San-Segundo and Roeder, 1999, 2000; Borner *et al.*, 2008).

Downstream targets of the meiotic recombination checkpoint responsible for establishing the cell-cycle delay include the Swe1 kinase and the meiosis-specific Ndt80 transcription factor. Swe1 catalyzes the inhibitory phosphorylation of Cdc28 at tyrosine 19 (Booher *et al.*, 1993). It is striking that, although this phosphorylation contributes to the pachytene checkpoint-induced meiotic arrest (Leu and Roeder, 1999), it is dispensable for the function of the DNA damage and replication checkpoints in *S. cerevisiae* vegetative cells (Amon *et al.*, 1992; Sorger and Murray, 1992). However, Swe1 does play an important role in the budding yeast morphogenesis checkpoint (Lew and Reed, 1995; McMillan *et al.*, 1998, 1999).

The Ndt80 transcription factor promotes the expression of a large set of genes required for exit from pachytene (i.e., *CDC5*) (Sourirajan and Lichten, 2008), nuclear divisions (i.e., *CLB1*) (Chu and Herskowitz, 1998), and spore morphogenesis (i.e., *SMK1*) (Pierce *et al.*, 1998). Ndt80 binds to the middle sporulation elements (MSEs) present in the promoter of its target genes, including *NDT80* itself. The Sum1 repressor, which also binds to a subset of the same sites in vegetative cells, decreases during midmeiosis but is stabilized in checkpoint-arrested cells. It has been proposed that competition between Ndt80 and Sum1 for binding to MSEs controls middle gene expression (Chu and Herskowitz, 1998; Lindgren *et al.*, 2000; Pierce *et al.*, 2003). Phosphorylation of Ndt80 and Sum1 by the meiosis-specific cyclin-dependent kinase (CDK)–like Ime2 kinase modulates this regulatory network (Sopko *et al.*, 2002; Sopko and Stuart, 2004; Benjamin *et al.*, 2003; Ahmed *et al.*, 2009). In addition, Sum1 is also phosphorylated by the CDK Cdc28 (Shin *et al.*, 2010). Abundant hyperphosphorylated Ndt80 is present in midmeiotic wild-type cells but not in mutants that trigger the meiotic recombination checkpoint, which suggests that phosphorylation of Ndt80 may modulate its activity (Hepworth *et al.*, 1998; Tung *et al.*, 2000). On the other hand, a recent study showed an additional posttranslational control on Ndt80, revealing that the meiotic recombination checkpoint regulates its nuclear localization (Wang *et al.*, 2011).

The *CDC5* gene, encoding the budding yeast polo-like kinase (PLK), is a crucial member of the set of genes under Ndt80 control (Sourirajan and Lichten, 2008). PLKs carry out a vast variety of cellular functions in a range of organisms from yeast to mammals during both mitotic and meiotic cell cycles (Barr *et al.*, 2004; Archambault and Glover, 2009), being important targets of DNA damage checkpoints (Sanchez *et al.*, 1999; Smits *et al.*, 2000). During meiosis in *S. cerevisiae*, the functions of Cdc5 include mono-orientation of sister kinetochores at meiosis I, dissolution of sister-chromatid cohesion from chromosome arms, Holliday junction resolution, and SC disassembly (Clyne *et al.*, 2003; Lee and Amon, 2003; Hollingsworth, 2008; Sourirajan and Lichten, 2008), but its possible role in meiotic checkpoint control is not well defined (Iacovella *et al.*, 2010).

Here, we report that Cdc5 overproduction partially alleviates the pachytene checkpoint-induced meiotic block of the *zip1* mutant but does not suppress the spore viability defects. We demonstrate that high doses of Cdc5 do not alter the Swe1-dependent checkpoint response but do lead to premature induction of Ndt80 production, which depends on Cdc5. We also provide molecular evidence indicating that, unlike DNA-damaged vegetative cells, bypass of *zip1* meiotic delay by *CDC5* overexpression does not result from enhanced checkpoint adaptation. We propose that regulation of Ndt80 by Cdc5 is important for the meiotic recombination checkpoint response.

## RESULTS

### Overexpression of *CDC5* partially suppresses *zip1* meiotic delay

Previous studies described a role for the nucleolar-enriched Pch2 and Sir2 proteins in the meiotic recombination checkpoint (San-Segundo and Roeder, 1999, 2000). The nucleolus also plays an important functional role in the regulation of other cell cycle events—for example, the Cdc14-dependent exit from mitosis regulated by the Cdc14 early anaphase release (FEAR) and mitosis exit network (MEN) pathways (Jaspersen *et al.*, 1998; Stegmeier *et al.*, 2002; Yoshida *et al.*, 2002; Marston *et al.*, 2003; D'Amours and Amon, 2004; Rahal and Amon, 2008; Mohl *et al.*, 2009; Rock and Amon, 2009). Therefore, we examined whether components of these pathways may also participate in meiotic checkpoint control. We tested whether overexpression of *CDC14*, *CDC5*, *TEM1*, *DBF2*, and *CDC15* from *2μ* high-copy plasmids (Jaspersen *et al.*, 1998) could alleviate the pachytene checkpoint-dependent meiotic arrest of the *zip1* mutant. As shown in Figure 1A, only overexpression of the *CDC5* polo-like kinase gene reproducibly conferred a significant bypass of the *zip1* arrest, leading to increased dityrosine fluorescence and higher sporulation efficiency compared with the *zip1* mutant containing empty vector.

To confirm this initial observation, we followed the kinetics of meiotic progression. As expected, the *zip1* mutant displayed a quite robust meiotic arrest, undergoing meiotic divisions very inefficiently and only after prolonged incubation under sporulation conditions (Figure 1B) (Sym *et al.*, 1993; Refolio *et al.*, 2011). Overexpression of *CDC5* in *zip1* resulted in a faster and more efficient meiotic progression but below wild-type levels (Figure 1B). Of significance, overproduction of a kinase-dead version of Cdc5 from the *cdc5-N209A* allele (Bartholomew *et al.*, 2001) did not have any effect on *zip1* bypass (Figure 1B), implying that the Cdc5 kinase activity is required for its function in the meiotic checkpoint. On the other hand, *CDC5* overexpression did not have any significant effect in an otherwise unperturbed wild-type meiosis (Figure 1, B and D).

Cdc5 overproduction also accelerated the slow meiotic progression of the *dmc1* mutant (Figure 1C), indicating that the partial suppression of the checkpoint-imposed meiotic delay by high doses of Cdc5 is not exclusive to the *zip1* mutant. In any case, throughout

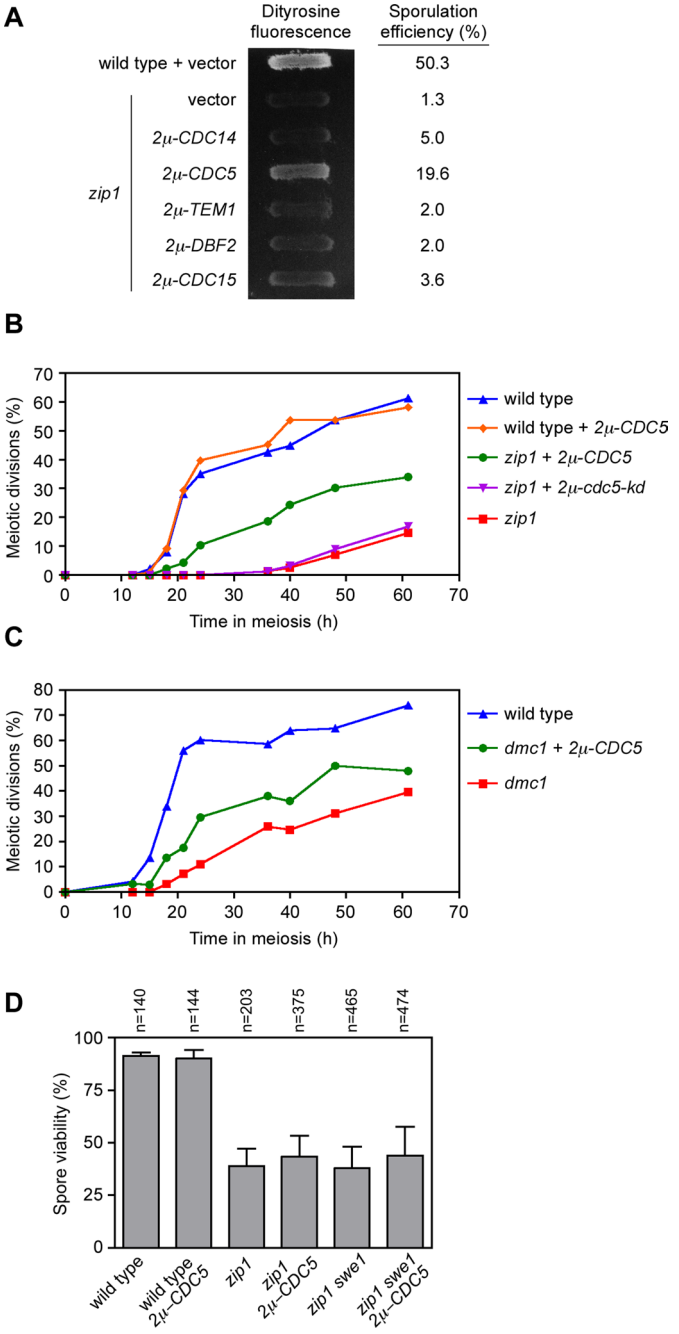


FIGURE 1: *CDC5* overexpression partially suppresses the checkpoint-dependent meiotic delay of *zip1* but does not improve spore viability. (A) Overexpression of *CDC5*, but not that of other FEAR/MEN genes, partially alleviates the sporulation defect of the *zip1* mutant. Dityrosine fluorescence after 3 d on a sporulation plate is shown as an indicator for the formation of mature asci. The sporulation efficiency, assessed by microscopic counting of asci, is also presented. Strains are BR2495 (wild type) and MY63 (*zip1*) transformed with vector alone (YEp352) or with the indicated plasmids: pPD2 (*2μ-CDC14*), pJC29 (*2μ-CDC5*), pSJ56 (*2μ-TEM1*), pSJ57 (*2μ-DBF2*), and pSJ103 (*2μ-CDC15*). (B) Suppression of *zip1* meiotic arrest by *CDC5* overexpression. Time course of meiotic nuclear divisions; the percentage of cells containing more than two nuclei is represented. Strains and plasmids used are wild type (DP396/pRS426), wild type + *2μ-CDC5* (DP396/pJC29), *zip1* + *2μ-CDC5* (DP386/pJC29), *zip1* + *2μ-cdc5-kd* (DP386/pSS127), and *zip1* (DP386/pRS426). (C) *CDC5* overexpression partially alleviates the meiotic delay of the *dmc1* mutant. Strains are wild type (BR1919-2N/pRS426), *dmc1* (DP456/pRS426), and *dmc1 2μ-CDC5* (DP456/pJC29). (D) *CDC5* overexpression does not suppress the spore viability defect of *zip1* and *zip1 swe1*. Spore viability as determined by asci dissection is plotted. Averages and standard deviations from two to six experiments, in which independent colonies were dissected, are represented. Strains are wild type (DP396/pRS426), wild type + *2μ-CDC5* (DP396/pJC29), *zip1* (DP386/pRS426), *zip1 2μ-CDC5* (DP386/pJC29), *zip1 swe1* (DP393/pRS426), and *zip1 swe1 2μ-CDC5* (DP393/pJC29). The total number of spores scored for each strain is indicated (n).

this work, we generally used the *zip1* mutant as a genetic tool to activate the meiotic recombination checkpoint.

### Cdc5 overproduction alters the meiotic recombination checkpoint response

Bypass of the prophase arrest of a meiotic mutant can be achieved by interfering with the checkpoint mechanism triggering the block or by repairing or eliminating the defects that initially activated the checkpoint. The *zip1* mutant is defective in SC development but also in crossover formation, resulting in chromosome nondisjunction and formation of aneuploid spores (Sym *et al.*, 1993; Sym and Roeder, 1994; Storlazzi *et al.*, 1996; Borner *et al.*, 2004). Because Cdc5 promotes resolution of joint molecules (JMs) as crossovers (Sourirajan and Lichten, 2008); in principle, the suppression of *zip1* delay by *2μ-CDC5* could result from a more efficient resolution of recombination intermediates, leading to a weaker checkpoint-inducing signal. If this were the case, an enhanced viability of the meiotic products could be expected; therefore, we examined spore viability of *zip1* and *zip1 2μ-CDC5* and found similar levels of viable spores (Figure 1D). Nevertheless, sporulation efficiency in *zip1* is very poor; few tetrads with four spores are formed and only after prolonged incubation in sporulation conditions. Thus, for a better comparison, we also analyzed the effect of *CDC5* overexpression on spore viability in *zip1 swe1* strains, where more mature asci are generated as compared with *zip1* (see below). As shown in Figure 1D, high levels of Cdc5 did not improve spore viability in either *zip1* or *zip1 swe1* strains, suggesting that alleviation of the *zip1* meiotic arrest does not result from suppression of the recombination defect but rather from compromised checkpoint function.

### Overexpression of *CDC5* does not promote adaptation of the meiotic recombination checkpoint

In DNA-damaged vegetative yeast cells, high doses of Cdc5 inhibit Rad53 hyperphosphorylation, leading to checkpoint adaptation and release of the checkpoint-imposed cell cycle block (Donnianni *et al.*, 2010; Schleker *et al.*, 2010; Vidanes *et al.*, 2010). In addition, it has been recently reported that Cdc5 is required for checkpoint adaptation during meiosis (Iacovella *et al.*, 2010). Therefore, to determine whether the suppression of the pachytene checkpoint-dependent meiotic delay upon Cdc5 overproduction was due to faster checkpoint adaptation, we monitored the phosphorylation of Mek1, which is the meiosis-specific Rad53 paralogue effector kinase (Perez-Hidalgo *et al.*, 2003). Activation of the meiotic checkpoint by the lack of Zip1 leads to Mek1 hyperphosphorylation (Figure 2A). Mek1 activation depends on meiotic recombination, since it is abolished in a *spo11* mutant (Figure 2A), and also depends on the Mec1/Ddc2 meiotic checkpoint sensor complex (Refolio *et al.*, 2011), since it is abolished in a *mec1* mutant (Figure 2A). We monitored Mek1 activation throughout meiotic time courses of wild-type, *zip1*, and *zip1 2μ-CDC5* cells. In the wild type, only a weak and transient activation of Mek1 was detected during prophase coincident with ongoing meiotic recombination (Figure 2B). In contrast, in the *zip1* mutant,

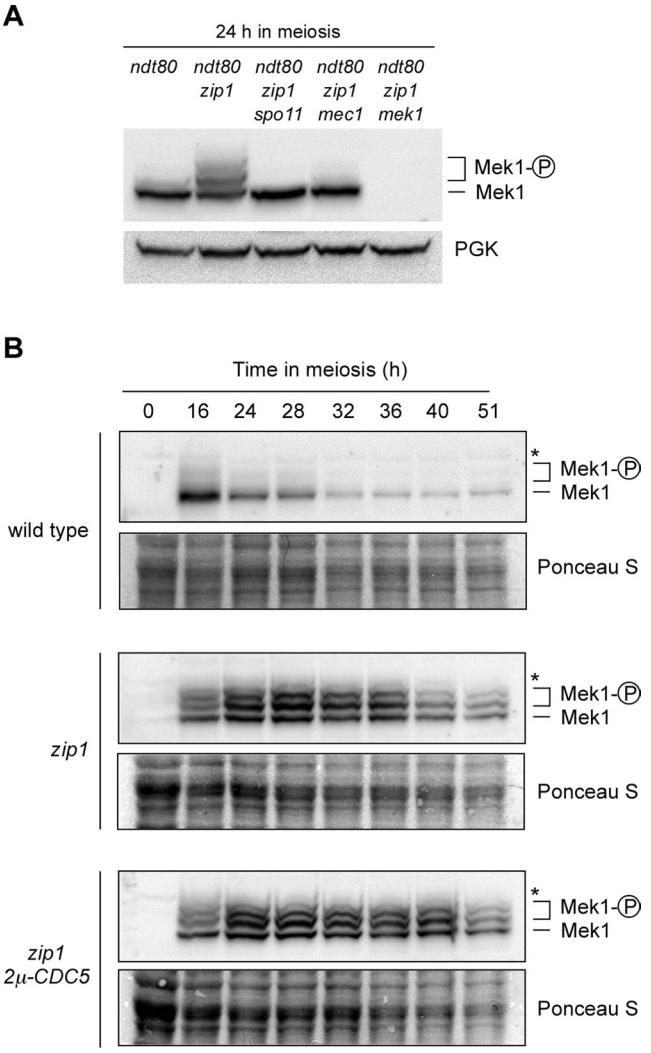


FIGURE 2: Suppression of the *zip1* meiotic delay by *CDC5* overexpression does not result from checkpoint adaptation. (A) Analysis of Mek1 phosphorylation as an indicator for activation of the meiotic recombination checkpoint effector kinase. Note that, for a better comparison, the experiment was performed in *ndt80*-arrested cells to avoid possible differences that could be due to the different meiotic progression of the various mutants. PGK was used as a loading control. Strains are *ndt80* (DP424), *ndt80 zip1* (DP428), *ndt80 zip1 spo11* (DP728), *ndt80 zip1 mec1* (DP680), and *ndt80 zip1 mek1* (DP674). (B) Western blot analysis of Mek1 activation throughout meiosis in wild type (DP396/pRS426), *zip1* (DP386/pRS426), and *zip1 2μ-CDC5* (DP386/pJC29). Ponceau S staining of the membranes is shown as a loading control.

robust Mek1 hyperphosphorylation was present until late time points (Figure 2B), consistent with the pronounced meiotic delay of the mutant (Figure 1B). Of interest, in the *zip1* mutant overexpressing *CDC5*, high levels of hyperphosphorylated Mek1 persisted throughout the whole meiotic time course (Figure 2B), despite the fact that the meiotic delay has been partially suppressed (Figure 1B).

Thus these findings demonstrate that the effect of Cdc5 overproduction on pachytene checkpoint bypass does not result from premature checkpoint adaptation to the persistent meiotic defects, because Mek1 is not down-regulated. Moreover, this analysis also indicates that Cdc5 acts downstream of Mek1 in the meiotic recombination checkpoint pathway.

### Overexpression of *CDC5* does not alter the Swe1-dependent branch of the meiotic recombination checkpoint

Activation of the pachytene checkpoint blocks meiotic progression by a dual mechanism restraining CDK activity to prevent entry into meiosis I: 1) hyperactivation and stabilization of Swe1 to maintain inhibitory phosphorylation of Cdc28 at tyrosine 19 and 2) inhibition of the Ndt80 transcription factor, which is required for expression of the *CLB1* meiotic cyclin (Figure 3A; Chu and Herskowitz, 1998; Hepworth *et al.*, 1998; Leu and Roeder, 1999). The fact that *2μ-CDC5* only partially alleviates *zip1* arrest suggested that just one of the two branches could be influenced by high levels of Cdc5; therefore, we first tested the effect of Cdc5 overproduction in the absence of *SWE1*. As described (Leu and Roeder, 1999), the *zip1 swe1* double mutant showed a partial suppression of the *zip1* meiotic arrest (Figure 3B). Notably, the additional overexpression of *CDC5*, but not that of *cdc5-N209A*, led to nearly wild-type meiotic kinetics (Figure 3B). Therefore, the additive effect of *SWE1* deletion and Cdc5 overproduction suggests that Cdc5 acts on the Swe1-independent branch of the checkpoint pathway.

To confirm this possibility, we monitored the levels of $Cdc28^{Tyr19}$ phosphorylation upon Cdc5 overproduction (Figure 3C). In the wild type, Swe1-dependent phosphorylation of $Cdc28^{Tyr19}$ peaked during prophase and then declined as meiosis and sporulation progressed. However, in the *zip1* mutant, phosphorylation of $Cdc28^{Tyr19}$ persisted. Likewise, the *zip1* mutant overexpressing *CDC5* displayed high levels of $Cdc28^{Tyr19}$ phosphorylation at late time points. Thus the suppression of the meiotic arrest of *zip1* by *2μ-CDC5* does not result from impaired Swe1 function.

### Cdc5 affects the *NDT80-CLB1* branch of the meiotic checkpoint

We next tested the effect of combining *CDC5* overexpression with that of the Ndt80 target *CLB1*. It was reported that Clb1 overproduction has only a slight effect on promoting sporulation in the checkpoint-arrested *hop2* mutant but significantly restores meiotic divisions in a *hop2 swe1* double mutant (Leu and Roeder, 1999). Likewise, we also observed a partial bypass of *zip1* by *2μ-CLB1* (Figure 4A), and, of interest, we found almost identical meiotic kinetics in *zip1* cells overproducing either Cdc5 alone or both Cdc5 and Clb1 together (Figure 4A). Moreover, overexpression of either *CDC5* or *CDC5-CLB1* also had a similar additive effect with *SWE1* deletion on suppressing the *zip1* meiotic delay (Figure 4B). These observations argue that Cdc5 overproduction does not affect the Swe1 branch of the meiotic checkpoint pathway and are consistent with Cdc5 playing a role in regulating Ndt80.

### *CDC5* overexpression in *zip1* results in premature activation of Ndt80

To investigate the influence of Cdc5 on Ndt80 regulation, we monitored by Western blot the levels of Cdc5 and Ndt80 throughout meiosis in wild-type, *zip1*, and *zip1 2μ-CDC5* cells. In the wild type, low Cdc5 levels were detected in vegetative cells prior to entering the meiotic program; the levels increased during midmeiosis and then progressively declined. In turn, Ndt80 was absent in vegetative cells and peaked during meiotic prophase (Figure 5A). In contrast, production of both Cdc5 and Ndt80 was extremely delayed in the *zip1* mutant (Figure 5A; t = 40–48 h), consistent with the checkpoint-dependent inhibition of Ndt80 and with *CDC5* being a target of Ndt80 (Tung *et al.*, 2000; Sourirajan and Lichten, 2008). In the *zip1 2μ-CDC5* strain, high Cdc5 levels were more evenly distributed throughout the whole meiotic time course, being easily detectable even at t = 0 h, and full Ndt80 production was induced earlier than

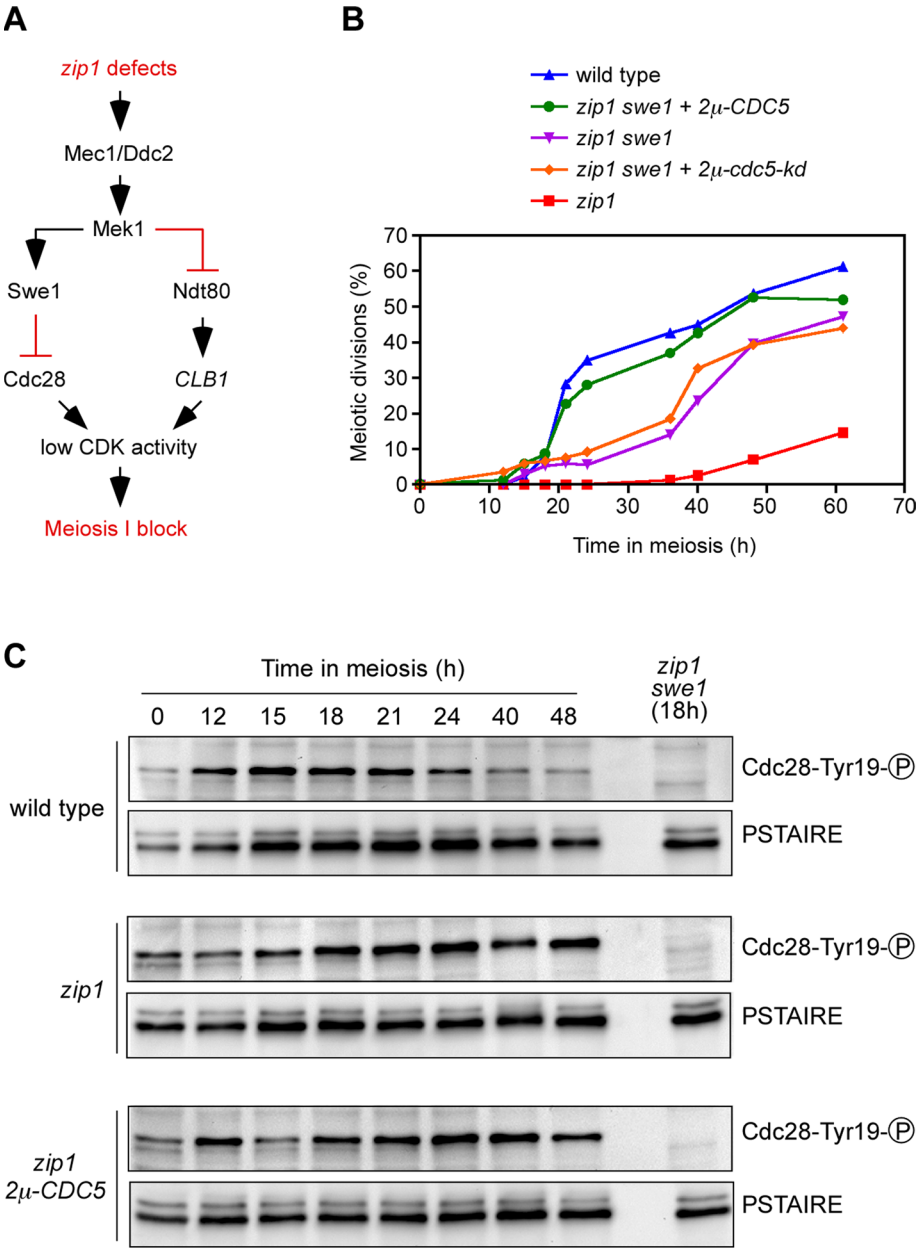


FIGURE 3: Bypass of *zip1* meiotic delay by high levels of active Cdc5 does not result from altered Swe1 function. (A) Schematic representation of the two regulatory branches targeted by the meiotic recombination checkpoint to restrain CDK activity, thus preventing meiosis I entry. Note that the positive and negative arrows connecting Mek1 to Swe1 and Ndt80, respectively, do not necessarily imply direct action. (B) Time course of meiotic nuclear divisions; the percentage of cells containing more than two nuclei is represented. Strains are wild type (DP396/pRS426), *zip1 swe1 + 2μ-CDC5* (DP393/pJC29), *zip1 swe1* (DP393/pRS426), *zip1 swe1 + 2μ-cdc5-kd* (DP393/pSS127), and *zip1* (DP386/pRS426). (C) Swe1-dependent inhibitory phosphorylation of Cdc28 is not affected by Cdc5 overproduction. Western blot analysis of phosphorylation of Cdc28 at tyrosine 19 and total Cdc28 (PSTAIRE) throughout meiosis in wild type (DP396/pRS426), *zip1* (DP386/pRS426), and *zip1 2μ-CDC5* (DP386/pJC29). Extracts from a *zip1 swe1* strain (DP393), which lacks $Cdc28^{Tyr19}$ phosphorylation, were used as control for specificity of the antibody.

in *zip1* (Figure 5A; t = 21 h), thus explaining the faster meiotic progression of *zip1* when Cdc5 is overproduced.

As in *zip1*, production of Ndt80 was delayed in *zip1 swe1* (Figure 5B; t = 40 h), but *2μ-CDC5* also led to earlier Ndt80 induction (Figure 5B; t = 18–21 h). Thus these observations suggest that the suppression of the meiotic delay of the *zip1* mutant by *CDC5* overexpression results from premature activation of Ndt80.

To corroborate that Cdc5 overproduction in *zip1* promotes Ndt80 activity, we monitored by quantitative RT-PCR the relative levels of the *CLB1* mRNA as readout of Ndt80 function. As a control for normalization, we used the *NUP85* gene, whose expression remains invariable during the meiotic program (Chu *et al.*, 1998; Primig *et al.*, 2000). We found that overexpression of *CDC5* increased (1.5-fold) the relative meiotic levels of *CLB1* mRNA in the *zip1* mutant (Figure 5C); however, they did not reach wild-type values, consistent with the partial bypass of the meiotic arrest.

## Cdc5 is required for Ndt80 production and stability

Expression of *CDC5* depends on Ndt80 (Sourirajan and Lichten, 2008), but, in turn, our results indicate that high levels of Cdc5 also stimulate Ndt80 activation. Thus, to further investigate this dependence, we generated strains carrying a heat-inducible degron allele of *CDC5* (*cdc5-dg*) under control of a doxycycline-repressible promoter to eliminate the essential Cdc5 protein specifically during meiotic prophase. At the permissive temperature (25°C) in the absence of doxycycline, the *cdc5-dg* mutant completed meiosis and sporulation, albeit with reduced efficiency compared with the wild type at the same temperature (Figure 6A and unpublished data). However, when the cultures were shifted to 33°C in the presence of doxycycline after 15 h of meiotic induction (peak of prophase in this background), the wild type displayed normal meiotic kinetics, whereas the *cdc5-dg* mutant was blocked and did not undergo meiotic divisions (Figure 6A). Western blot analysis revealed that, indeed, the Cdc5-dg protein, but not the wild-type Cdc5, was rapidly degraded upon transferring the cells to the nonpermissive conditions (Figure 6B; and unpublished data); in addition, it is striking that the levels of the fully active phosphorylated Ndt80 also dramatically dropped in the *cdc5-dg* mutant, leading to the accumulation of the fastest-mobility form of Ndt80 (Figure 6B), which is presumed to be a degradation product (Tung *et al.*, 2000; Shubassi *et al.*, 2003). Thus Cdc5 function is required for sustained Ndt80 production in its stable and active form.

## CDK contributes to Ndt80 activation by *CDC5* overexpression in *zip1* cells

Substrates of Polo-like kinases are often primed by CDK phosphorylation prior to the action of the polo-like kinase itself (van Vugt and Medema, 2005). Therefore we tested whether the premature activation of Ndt80 observed in *zip1* cells upon Cdc5

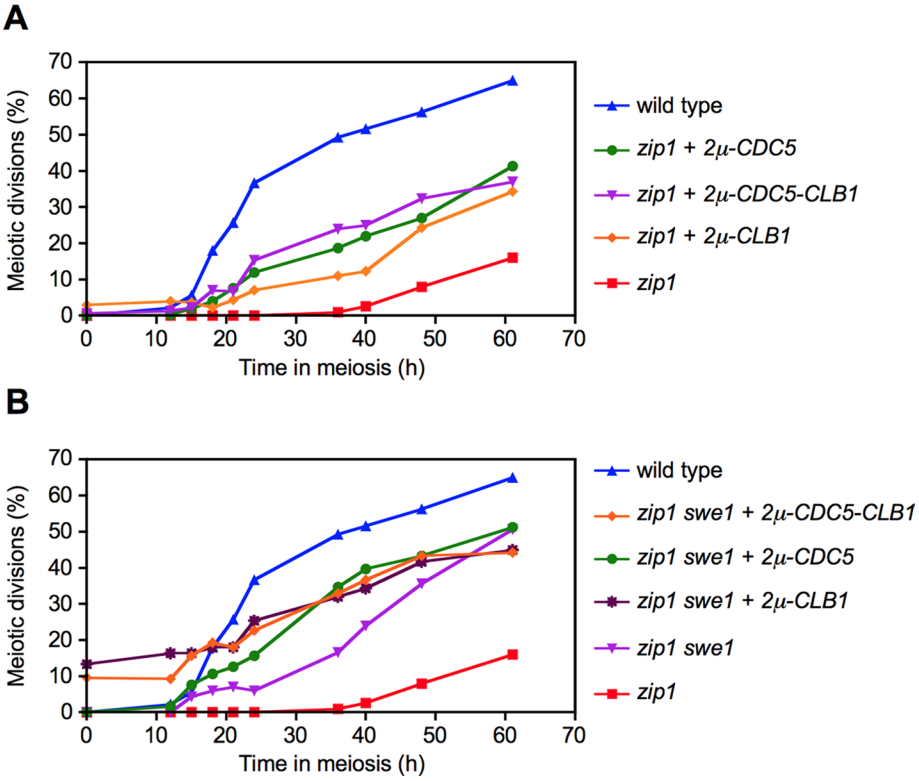


FIGURE 4: Cdc5 functions on the *NDT80-CLB1* branch of the meiotic recombination checkpoint. Time course of meiotic nuclear divisions; the percentage of cells containing more than two nuclei is represented. Strains and plasmids used in the experiments are as follows: (A) Wild type (DP396/pRS426), *zip1 + 2μ-CDC5* (DP386/pJC29), *zip1 + 2μ-CDC5-CLB1* (DP386/pSS121), *zip1 + 2μ-CLB1* (DP386/pR2045), and *zip1* (DP386/pRS426). (B) Wild type (DP396/pRS426), *zip1 swe1 + 2μ-CDC5-CLB1* (DP393/pSS121), *zip1 swe1 + 2μ-CDC5* (DP393/pJC29), *zip1 swe1 + 2μ-CLB1* (DP393/pR2045), *zip1 swe1* (DP393/pRS426), and *zip1* (DP386/pRS426).

overproduction requires CDK activity. To address this issue, we generated strains harboring an analogue-sensitive *cdc28-as1* allele. In unperturbed meiosis, Cdc28 inactivation by addition of the ATP analogue 1NM-PP1 rapidly arrested meiotic progression (Figure 7A) but had no effect or just a marginal effect on Cdc5 and Ndt80 production (Figure 7B), as previously reported (Benjamin *et al.*, 2003; Sourirajan and Lichten, 2008). Like *zip1* (Figure 5A), the *zip1 cdc28-as1* mutant in the absence of the inhibitor displayed a delayed induction of Ndt80 and Cdc5 (Figure 7B), resulting in a significantly delayed meiotic progression (Figure 7A). Notably, Cdc28 inhibition in *zip1* completely blocked meiotic divisions and largely abolished the activation of Ndt80 that occurred in the absence of the analogue at late time points (Figure 7, A and B). Likewise, overexpression of *CDC5* in *zip1 cdc28-as1* cells treated with 1NM-PP1 also resulted in reduced levels of Ndt80 compared with the untreated control (Figure 7B). Thus, although CDK activity is largely dispensable for Ndt80 activation in unperturbed meiosis when the checkpoint is not triggered, it becomes more important under conditions in which Ndt80 activation is limited by the action of the checkpoint.

## DISCUSSION

The budding yeast polo-like kinase Cdc5 plays various roles in several key aspects of meiotic chromosome metabolism (see *Introduction*); in this work, we characterized an additional function of Cdc5 in meiotic checkpoint control.

We found that overexpression of *CDC5* alleviates to some extent the prolonged meiotic delay of the *zip1* mutant but does not suppress its spore viability defect. Indeed, spore viability levels of *zip1 2μ-CDC5* (~40%) are similar to those resulting from bypassing *zip1* arrest by mutation of meiotic checkpoint genes, such as *PCH2* and *DOT1* (San-Segundo and Roeder, 1999, 2000), mutation of meiotic checkpoint cell-cycle targets, such as *SWE1* (Figure 1C; Leu and Roeder, 1999), or introduction of the dominant *NDT80-bc* allele (Wang *et al.*, 2011). These observations argue that the recombination defects of *zip1* may be a consequence, and not the cause, of the meiotic block. Thus, although Cdc5 promotes JM resolution as crossovers (Sourirajan and Lichten, 2008), our results imply that bypass of the *zip1* arrest conferred by *2μ-CDC5* results from an impaired checkpoint response rather than from resolution of the recombination intermediates accumulated in the absence of Zip1 by an alternative pathway that would lead to enhanced spore viability.

Vegetative yeast cells can adapt to persistent unrepaired DNA damage by overriding the checkpoint-imposed cell cycle arrest to eventually resume suicidal mitotic divisions. Cdc5 regulates this phenomenon, referred to as checkpoint adaptation, and the *cdc5-ad* allele is specifically defective in adaptation (Toczyski *et al.*, 1997; Pellicioli *et al.*, 2001). It was recently shown that high levels of Cdc5 counteract hyperphosphorylation of the effector DNA damage checkpoint kinase Rad53, thus resulting in a weakened checkpoint response and resumption of cell division despite the persistence of the checkpoint-inducing signal (Donnianni *et al.*, 2010; Schleker *et al.*, 2010; Vidanes *et al.*, 2010). Because we observe a suppression of the checkpoint-dependent meiotic cell cycle block on *CDC5* overexpression but the *zip1* defects are still manifest, it was tempting to hypothesize that, as in mitotic cells, high levels of Cdc5 could be promoting meiotic checkpoint adaptation. Indeed, while this work was in progress, Iacovella and coworkers showed that meiotic depletion of Cdc5 prevents meiotic divisions in *sum1 dmc1* or *fpr3 dmc1* cells and suggested that Cdc5 is involved in meiotic checkpoint adaptation (Hochwagen *et al.*, 2005; Iacovella *et al.*, 2010). To monitor meiotic checkpoint adaptation at the molecular level, we followed hyperphosphorylation of the checkpoint effector Mek1 kinase, which is the meiosis-specific counterpart of Rad53. It is striking that we found that there is no premature attenuation of Mek1 activation upon Cdc5 overproduction in *zip1* cells. These results indicate that, at least in these circumstances, alleviation of the meiotic block does not result from checkpoint adaptation to the persistent *zip1* defects. This situation contrasts with that in damaged mitotic cells, where high levels of Cdc5 reduce Rad53 hyperphosphorylation, pointing to functional differences for regulation of checkpoint adaptation in the distinct cell cycles.

To pinpoint where Cdc5 acts in the meiotic recombination checkpoint pathway, we dissected the functional interaction of *CDC5* overexpression with the two main branches targeted by the checkpoint. In vegetative cells, Cdc5-dependent phosphorylation of Swe1 at the bud neck, previously "primed" by CDK phosphorylation,

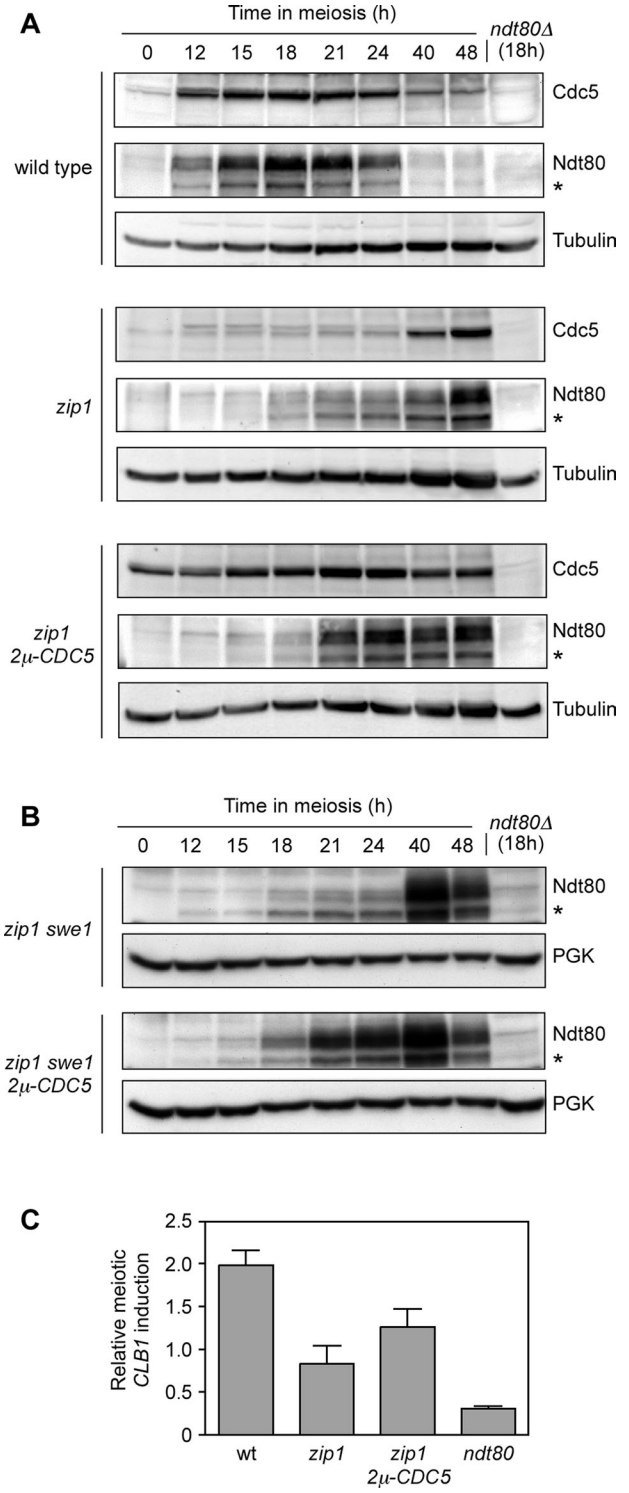


FIGURE 5: *CDC5* overexpression in *zip1* cells leads to earlier induction of Ndt80 production. (A) Western blot analysis of Cdc5 and Ndt80 production throughout meiosis in wild type (DP396/pRS426), *zip1* (DP386/pRS426), and *zip1 2μ-CDC5* (DP386/pJC29). Tubulin was used as a loading control. The asterisk marks a presumptive degradation product of Ndt80 (Tung *et al.*, 2000; Shubassi *et al.*, 2003). (B) Western blot analysis of Ndt80 production in *zip1 swe1* (DP393/pRS426) and *zip1 swe1 2μ-CDC5* (DP393/pJC29). PGK is shown as a loading control. (C) Quantification of the relative meiotic induction at the 36-h time point of the *CLB1* mRNA in wild type (DP396/pRS426), *zip1* (DP386/pRS426), *zip1 2μ-CDC5* (DP386/pJC29), and *ndt80* (DP424/pRS426).

directs its degradation to allow mitosis entry. Moreover, defective bud morphogenesis results in Swe1 stabilization and delay of the G2/M transition (Bartholomew *et al.*, 2001; Sakchaisri *et al.*, 2004; Asano *et al.*, 2005; Simpson-Lavy and Brandeis, 2010). Because Swe1 is also stabilized when the pachytene checkpoint is triggered by meiotic defects (Leu and Roeder, 1999), it was conceivable that overexpression of *CDC5* could be promoting meiotic progression in *zip1* cells by counteracting Swe1 action. However, the additive effect of *2μ-CDC5* and *SWE1* deletion on bypass of the *zip1* meiotic block and the persistence of Swe1-dependent phosphorylation of Cdc28 at Tyr19 on *CDC5* overproduction in *zip1* meiotic cells clearly argue that Swe1 function is largely unaffected by Cdc5 during the meiotic recombination checkpoint response.

On the other hand, we provided genetic and molecular evidence indicating that Cdc5 function influences the branch of the meiotic checkpoint that targets the Ndt80 transcription factor. We showed that overexpression of *CDC5* in checkpoint-arrested *zip1* cells leads to earlier induction of Ndt80, whereas depletion of Cdc5 during meiotic prophase using a *cdc5* degron allele in otherwise wild-type cells prevents production of Ndt80, suggesting that Cdc5 controls, directly or indirectly, Ndt80 activation. Regulation of production of active Ndt80 is complex, being exerted both at transcriptional and posttranslational levels. During meiosis, there is an initial wave of premiddle, Ime1-dependent *NDT80* expression at relatively low levels (Pak and Segall, 2002).The Ndt80 protein initially produced binds to the MSEs in its own promoter, displacing the competing Sum1 repressor and thus engaging an autoactivating loop that increases the levels of active Ndt80 and triggers the middle gene transcriptional program (Lindgren *et al.*, 2000; Pierce *et al.*, 2003).

We propose that, in wild-type cells, basal levels of Cdc5 could contribute to the initial activation step of Ndt80 production (Figure 8, left). Because Rad53-dependent inhibition of Cdc5 was reported in DNA-damaged vegetative cells (Cheng *et al.*, 1998; Sanchez *et al.*, 1999; Zhang *et al.*, 2009), it is reasonable to speculate that when the meiotic checkpoint is triggered by meiotic defects (i.e., *zip1* or *dmc1* mutations), it could inhibit Cdc5 function to restrain launching of the positive-feedback Ndt80 activation loop. Alternatively, it is also possible that the meiotic recombination checkpoint inhibits Ndt80 in a Cdc5-independent manner (Figure 8, right). In either case, Cdc5 overproduction in *zip1* cells would overcome the inhibitory action of the checkpoint, enabling a feedforward Ndt80 activation loop, which induces middle gene expression, promoting meiotic progression. The fact that we observe only a partial bypass of *zip1* upon *CDC5* overexpression can be explained in terms of the Swe1-dependent inhibition of Cdc28 still operating in those cells. In addition, other proteins, such as the Cdc7 kinase, also could be collaborating with Cdc5 at this step (Lo *et al.*, 2008). Nevertheless, there must be some cross-talk between both branches of the meiotic recombination checkpoint because we observe earlier induction of Ndt80 in *zip1 swe1* compared with *zip1* cells, and also we find that activation of Ndt80 is further impaired in *zip1*, and even also to some extent in *zip1 2μ-CDC5* cells, when Cdc28 activity is inhibited by an ATP analogue.

Several mechanisms by which Cdc5 could be promoting Ndt80 activation can be envisaged. Ime2- and Cdc28-dependent removal of the Sum1 repressor from the MSEs at the *NDT80* promoter is required for the initial wave of Ime1-mediated *NDT80* expression and the eventual generation of the autoactivation loop (Pak and Segall, 2002; Shin *et al.*, 2010). It is conceivable that Cdc5 could contribute to this intricate transcriptional regulation of *NDT80* by targeting some key component of the network. Indeed, a role for polo-like kinases in controlling cell-cycle

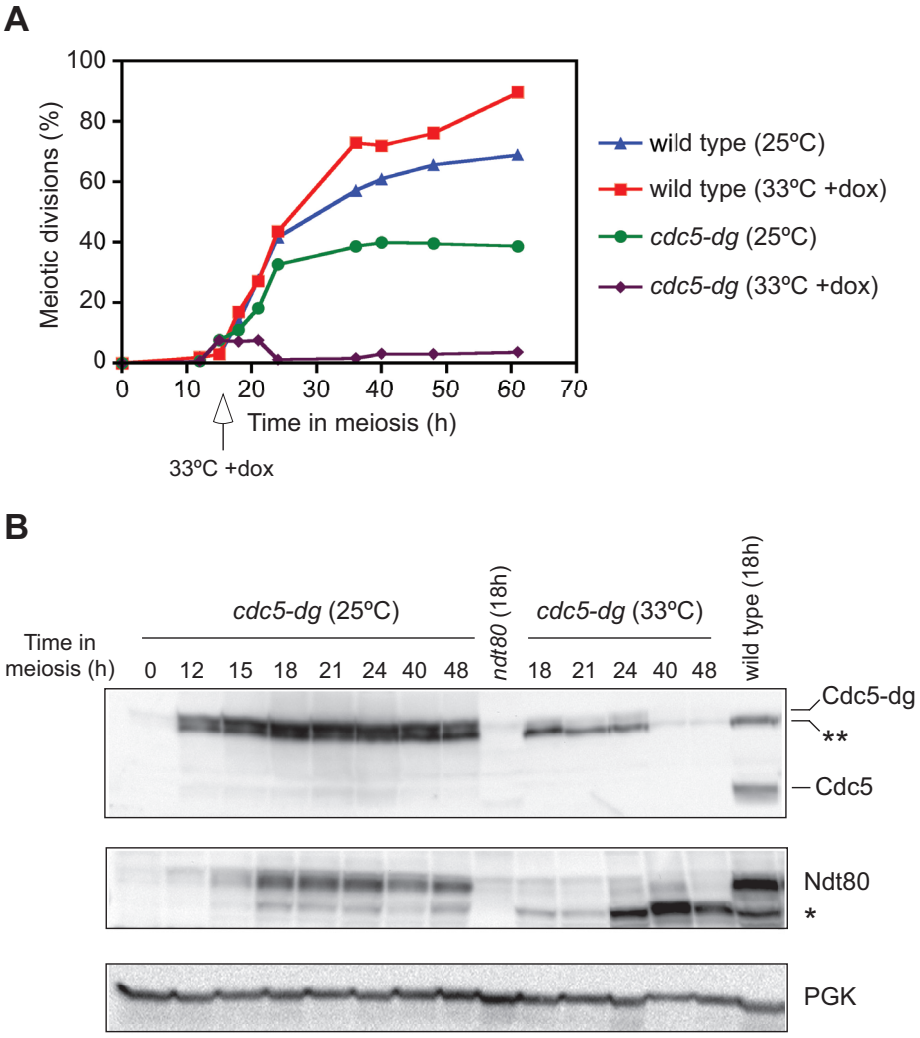


FIGURE 6: Production of active and stable Ndt80 depends on Cdc5. (A) Time-course analysis of meiotic nuclear divisions in wild-type (BR1919-2N) and *cdc5-dg* (DP402) strains. The percentage of cells containing more than two nuclei is represented. Meiotic cultures incubated at 25°C were split in two 15 h after meiotic induction (arrow), and doxycycline was added to one-half of the cultures (+dox), which were shifted to 33°C. The other half remained at 25°C without doxycycline. (B) Western blot analysis of Cdc5 and Ndt80 production in the *cdc5-dg* meiotic cultures described in A. Extracts from 18-h meiotic cultures of *ndt80* (DP424; center lane) and wild type (BR1919-2N; right lane) were also included as controls. The asterisk in the Ndt80 blot indicates the Ndt80 form reportedly resulting from degradation (Tung *et al.*, 2000; Shubassi *et al.*, 2003). The double asterisk in the Cdc5 blot marks a nonspecific band produced in midmeiosis recognized by the anti-Cdc5 antibody. PGK was used as a protein loading control.

transitions by regulating transcription factors was described in both yeast and mammalian cells (Darieva *et al.*, 2006; Fu *et al.*, 2008). Alternatively, or in addition, Cdc5 could regulate Ndt80 activity posttranslationally. Although the relevance of Ndt80 phosphorylation remains obscure, a recent report elegantly demonstrates that regulation of Ndt80 nuclear localization by the meiotic recombination checkpoint is crucial (Wang *et al.*, 2011). Wang *et al.* (2011) proposed that Ndt80 is sequestered in the cytoplasm by an unknown checkpoint factor. It is possible that Cdc5 controls Ndt80 nuclear localization (Figure 8) and, therefore, the access to MSE-containing promoters, including its own. Future experiments will be required to pinpoint the molecular basis of Ndt80 regulation by Cdc5 in the context of the meiotic recombination checkpoint.

## MATERIALS AND METHODS

### Yeast strains and plasmids

Yeast strains used in this work are listed in Table 1. Strains are in the BR2495 or in the BR1919 background (Rockmill and Roeder, 1990), as indicated. All strains compared in every experiment were completely isogenic. The *zip1::kanMX6*, *spo11::hphMX4*, and *mek1::kanMX6* deletions were performed using a PCR-based approach (Longtine *et al.*, 1998; Goldstein and McCusker, 1999). The *zip1::LYS2*, *ndt80::LEU2*, and *swe1::LEU2* gene disruptions were previously described (Leu and Roeder, 1999; San-Segundo and Roeder, 1999; Tung *et al.*, 2000). The pRS426-derived high-copy plasmids (Christianson *et al.*, 1992) pPD.2, pJC29, pSJ56, pSJ57, and pSJ103 overexpressing *CDC14*, *CDC5*, *TEM1*, *DBF2*, and *CDC15*, respectively, were described (Jaspersen *et al.*, 1998). Plasmids pR2045 and pL165, provided by J. L. Leu and S. Roeder (Leu and Roeder, 1999), harbor a 2.3-kb *Bam*HI–*Sal*I fragment containing *CLB1* cloned into the same sites of the *2μ* vectors pRS426 and pRS424 (Christianson *et al.*, 1992), respectively. To generate plasmid pSS121, which overexpresses both *CDC5* and *CLB1*, a 2.3-kb *Bam*HI(filled-in)–*Kpn*I fragment from pL165 containing *CLB1* was cloned into the *Xho*I(filled-in)–*Kpn*I sites of pJC29. Plasmid pSS127 overproducing a kinase-dead version of Cdc5 with the N209A amino acid change was constructed as follows. A DNA fragment containing the desired mutation was generated by two-step fusion PCR with appropriate oligonucleotides (sequences and details are available upon request) and pJC29 as template. The fragment was digested with *Cla*I and used to substitute the wild-type *Cla*I fragment of *CDC5* in pJC29 spanning that region for the one containing the N209A mutation to generate pSS127. The N209A mutation was designed to create a *Ban*I site and was confirmed by digestion and by sequencing. *The cdc28-as1* allele containing a F88G mutation in *CDC28* was introduced using the *Afl*II-digested *URA3*-based pJAU1 integrative plasmid (Bishop *et al.*, 2000) using a pop-in/pop-out strategy. 5-Fluoro-orotic acid–resistant clones carrying the mutation were identified by sequencing. To inhibit Cdc28, the ATP analogue 1NM-PP1 was added to the cultures at a final concentration of 1 μM from a stock solution at 10 mM in dimethyl sulfoxide (DMSO). To generate the *cdc5-dg* allele, a cassette containing the *kanMX6* marker, followed by a doxycycline-repressible *tTA* promoter and the heat-inducible DHFR degron module (Sanchez-Diaz *et al.*, 2004), was PCR amplified from the pKL183 plasmid (kindly provided by K. Labib, Paterson Institute for Cancer Research, Manchester, United Kingdom) and fused in-frame upstream of the *CDC5* start codon, eliminating 125 nucleotides of its promoter region. To trigger Cdc5 degradation, meiotic cultures were shifted to 33°C, and,

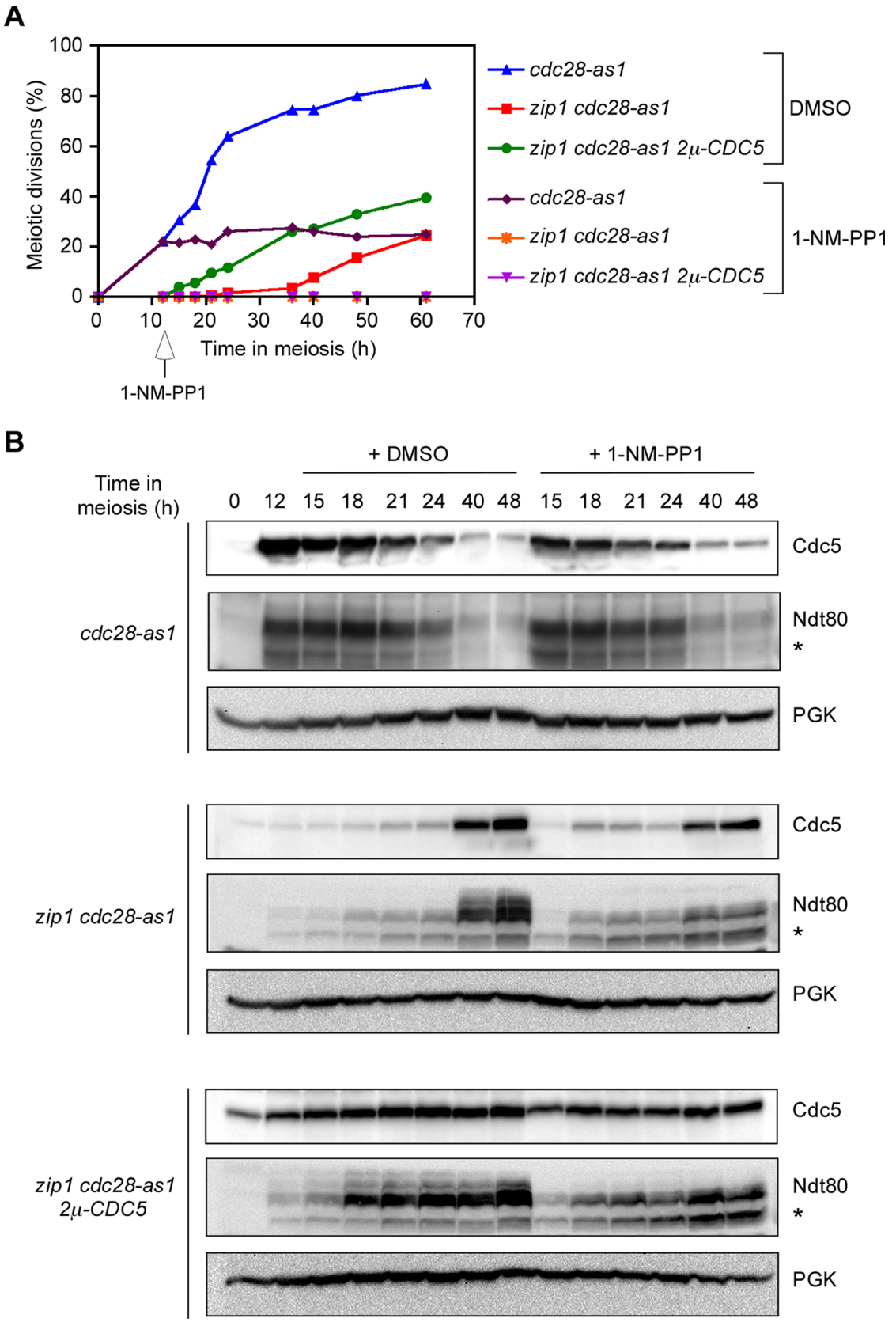


FIGURE 7: CDK inhibition further compromises Ndt80 induction in *zip1* cells. (A) Time-course analysis of meiotic nuclear divisions. The percentage of cells containing more than two nuclei is represented. Cultures were split in two 12 h after meiotic induction (arrow) to allow premeiotic S phase to occur, and 1 μM of 1NM-PP1 (dissolved in DMSO) was added to one-half of the culture. The same amount of DMSO alone was added to the other half. (B) Western blot analysis of Cdc5 and Ndt80 production in the meiotic cultures described in A. PGK was used as a loading control. Strains are *cdc28-as1* (DP430/pRS426), *zip1 cdc28-as1* (DP431/pRS426), and *zip1 cdc28-as1 2μ-CDC5* (DP431/pJC29).

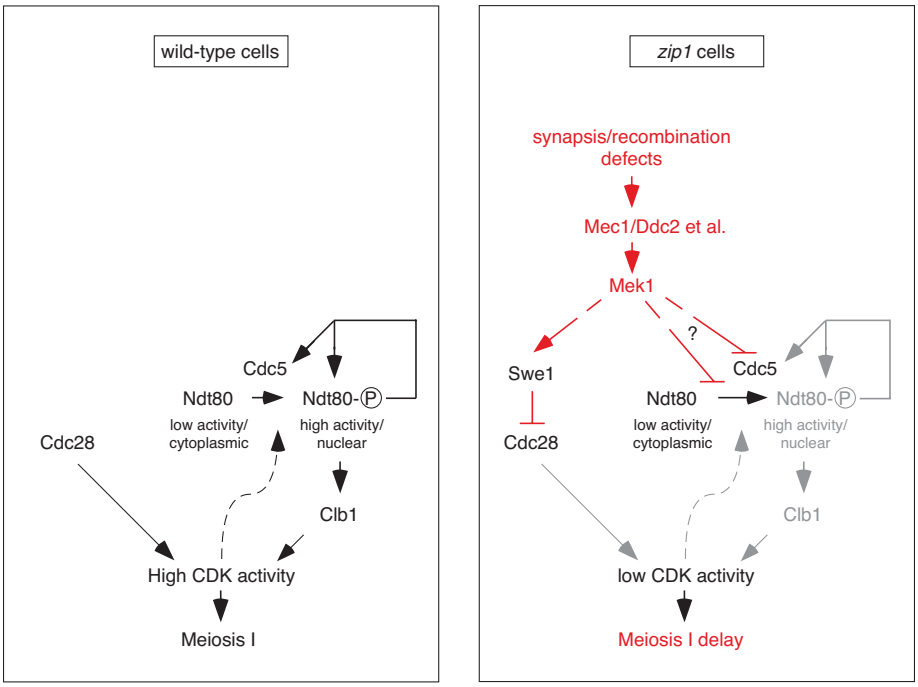


FIGURE 8: Schematic representation of a proposed model for Cdc5-dependent regulation of Ndt80. In unperturbed meiosis (left), basal levels of Cdc5 would contribute to trigger the Ndt80 autoactivation loop that, once engaged, also induces *CDC5* expression itself as a middle sporulation gene and other genes required for meiotic divisions (i.e., *CLB1*). In contrast, when synapsis or recombination is defective (i.e., *zip1* mutant; right) the checkpoint sensors relay the signal to the Mek1 effector kinase, which, in turn, directly or indirectly, affects the two main regulatory branches of the checkpoint. Our results indicate that Cdc5 acts specifically on the Ndt80 branch. It is possible that when the checkpoint is triggered, Cdc5 function is inhibited to prevent activation of Ndt80, thus contributing to the meiotic cell cycle delay. Alternatively, Cdc5-independent inhibition of Ndt80 by the checkpoint is also plausible. See *Discussion* for additional details.

simultaneously, doxycycline was added to a final concentration of 5 µg/ml to repress *cdc5-dg* gene expression.

### Meiotic time courses and sporulation

Strains were grown in 2xSC or in 2xSC-Ura (3.5 ml) for 20–24 h and then transferred to 2.5 ml of YPDA (1% yeast extract, 2% peptone, 2% glucose, 0.01% adenine) and incubated to saturation for an additional 8 h. Cells were harvested, washed with 2% potassium acetate (KAc), resuspended into 2% KAc (10 ml), and incubated at 30°C (except for the experiment shown in Figure 6) with vigorous shaking to induce meiosis and sporulation. Both YPDA and 2% KAc were supplemented with 20 mM adenine and 10 mM uracil. The culture volumes were scaled up when needed. Aliquots of cells were removed at different times for analysis. To monitor meiotic divisions, cells were fixed in 70% ethanol, washed in phosphate-buffered saline, and stained with 1 µg/µl 4′,6-diamidino-2-phenylindole for 15 min at room temperature. Nuclei were observed by fluorescence microscopy. At least 300 cells were scored for each strain at each time point in every experiment. Sporulation efficiency and dityrosine fluorescence were examined as described (Refolio *et al.*, 2011). All experiments were performed at least two times; representative time courses are shown in the figures.

| Strain | Genotype |
|---|---|
| BR2495 | *MAT***a**/MATα *leu2-27/leu2-3, 112 his4-280/his4-260 trp1-1/trp1-289 arg4-8/ARG4 thr1-1/ thr1-4 ura3-1/ura3-1 ade2-1/ade2-1 cyh10/CYH10* |
| MY63 | BR2495 *zip1::LEU2* |
| DP396 | BR2495 *lys2Δ* |
| DP386 | BR2495 *zip1::LYS2 lys2Δ* |
| DP393 | BR2495 *zip1::LYS2 swe1::LEU2 lys2Δ* |
| BR1919-2N | *MAT***a**/MATα *leu2-3112 his4-260 ura3-1 ade2-1 thr1-4 trp1-289* |
| DP402 | BR1919-2N *cdc5-dg::kanMX6* |
| DP424 | BR1919-2N *ndt80::LEU2 lys2Δ* |
| DP428 | BR1919-2N *ndt80::LEU2 zip1::LYS2 lys2Δ* |
| DP430 | BR1919-2N *cdc28-as1 lys2Δ* |
| DP431 | BR1919-2N *cdc28-as1 zip1::LYS2 lys2Δ* |
| DP456 | BR1919-2N *dmc1::kanMX6 lys2Δ* |
| DP674 | BR1919-2N *ndt80::LEU2 zip1::LYS2 mek1::kanMX6 lys2Δ* |
| DP680 | BR1919-2N *ndt80::LEU2 zip1::LYS2 sml1::kanMX6 mec1::KlURA3 lys2Δ* |
| DP728 | BR1919-2N *ndt80::LEU2 zip1::kanMX6 spo11::hphMX4* |

Unless indicated otherwise, all diploid strains are homozygous for the markers.

TABLE 1: *S. cerevisiae* strains.

### Western blotting

Cells from 5- to 10-ml aliquots of meiotic cultures were harvested, and 1 ml of 20% trichloroacetic acid (TCA) was added. The supernatant was removed after centrifugation, and the pellet was resuspended in 100 µl of 20% TCA and stored al −80°C. Samples were thawed on ice, glass beads were added, and cells were broken using a FastPrep FP120 cell disrupter (BIO 101 ThermoSavant, Obiogene, Carlsbad, CA). The lysate was recovered by punching a hole on the bottom of the tube, and the glass beads were further washed with 200 µl of 5% TCA. Lysates were centrifuged at 1000 × *g* for 3 min, and the pellet was thoroughly resuspended in 100 µl of 2× Laemmli buffer and 50 µl of 2 M Tris base. After boiling for 5 min, 10–20 µl were loaded in the gels. Ndt80 and Cdc5 production was analyzed in 10% SDS–PAGE gels with a 37.5:1 ratio of acrylamide:bisacrylamide. To resolve the phosphorylated forms of Mek1, 10% gels (acrylamide:bisacrylamide 29:1) containing 37.5 µM Phos-tag reagent and 75 µM $MnCl_2$ were used. After running, Phos-tag gels were washed with 1 mM EDTA before transfer. The anti-Ndt80 (kindly provided by K. Benjamin) and anti-Cdc5 (sc-6733; Santa Cruz Biotechnology, Santa Cruz, CA) antibodies were used at 1:5000 and 1:500 dilutions, respectively. To detect phosphorylation of Cdc28 at tyrosine 19, anti–phospho-Cdc2(Tyr15) (Cell Signaling Technology, Beverly, MA) was used at 1:1000 dilution. Total Cdc28 was detected with anti-PSTAIRE (sc-53, 1:500 dilution; Santa Cruz Biotechnology,). The anti-Mek1 antibody was previously described (Refolio *et al.*, 2011). Anti-tubulin (TAT1; 1:5000 dilution) and anti–phosphoglycerate kinase (PGK) (A-6457, 1:5000 dilution; Molecular Probes, Invitrogen, Carlsbad, CA) were used as loading controls. The ECL or ECL+ reagents (GE Healthcare, Piscataway, NJ) were used for detection. The signal was captured with a ChemiDoc XRS system (Bio-Rad, Hercules, CA), using the Quantity One software.

### mRNA quantification by quantitative PCR

RNA was isolated using the RNeasy purification kit (Qiagen, Valencia, CA) following the recommended procedures. The cDNA was generated using the PrimeScript RT reagent kit (Takara Bio, Otsu, Japan) and quantified by real-time PCR in an ABI7300 machine. Primers CLB1#2 (5′-CTTCCACAGAGCATGCACCG-3′) and CLB1#3 (5′-GCCTGTTCATCGCATCTAAG-3′) were used to analyze *CLB1* expression. *CLB1* cDNA levels were normalized to those of *NUP85*, whose expression remains constant during the meiosis and sporulation program, as described (Govin *et al.*, 2010). The values obtained from meiotic cells (t = 36 h) were relativized to those from vegetative cells (t = 0 h) to determine the relative meiotic induction. The cDNA was analyzed in triplicate in every experiment. Three independent experiments were performed.

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