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Ime2p and Cdc28p: Co-Pilots Driving Meiotic Development

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Abstract Meiosis can be considered an elaboration of the cell division cycle in the sense that meiosis combines cell-cycle processes with programs specific to meiosis. Each phase of the cell division cycle is driven forward by cell-cycle kinases (Cdk) and coordinated with other phases of the cycle through checkpoint functions (Hartwell and Weinert [1989]: Science. 246:629–634). Meiotic differentiation is also controlled by these two types of regulation (Murakami and Nurse [2000]: Biochem J. 349:1–12; Roeder and Bailis [2000]: Trends Genet. 16:395–403); however, recent study in the budding yeast *S. cerevisiae* indicates that progression of meiosis is also controlled by a master regulator specific to meiosis, namely the Ime2p kinase (Benjamin et al. [2003]: Genes Dev. 17:1–16; Schindler et al. [2003]: Mol Cell Biol 23:8718–8728). Below, I describe the overlapping roles of Ime2p and Cdk during meiosis in yeast and speculate on how these two kinases cooperate to drive the progression of meiosis. J. Cell. Biochem. 92: 1025–1033, 2004.

Key words: sporulation; Cln; Ime1

TWO PROGRAMS: TWO CENTRAL KINASES

Comparing Meiosis and the Cell Division Cycle

Meiosis is the process by which haploid germ cells are generated from diploid cells. Meiotic differentiation in yeast consists of six sequential stages, some of which correspond to stages in the cell division cycle and some of which are specific to meiosis (Fig. 1). In the first stage, meiotic initiation, early meiotic genes are induced, including *IME1*, which encodes a transcription factor and *IME2*, which encodes a protein kinase. The second stage, meiotic DNA replication (also termed premeiotic DNA replication), is equivalent to S-phase in the cell division cycle. In the third stage, homologous chromosomes pair and recombine, and a long proteinaceous structure, termed the synaptonemal complex (SC), forms along the paired chromosomes. This state, termed pachytene, does not occur during the cell division cycle. The fourth stage is the first meiotic nuclear division; during this stage, chromosomes segregate from their homologous partner to opposite poles of the nucleus. The fifth stage is the second meiotic nuclear division; during this stage, each pair of chromatids segregate to opposite poles, similar to chromosome segregation in mitosis. To allow these two sequential nuclear divisions without an intervening round of DNA replication, kinetochores (the protein structures at the centromeres that attach to the spindle) are modified differently in meiosis than in mitosis (reviewed in [Petronczki et al., 2003]). After the second meiotic division, the resulting nucleus has four separate chromosome masses, each containing a haploid genome. The final stage of meiotic differentiation is gametogenesis, the development of these haploid nuclei into gametes. In yeast, spore walls form around each haploid genome. The resulting four spores, held together in the remnant of the cell wall, form a tetrad ascus. For this reason, meiotic differentiation in yeast is also termed sporulation.

Comparing Ime2p and Cdc28p

In metazoans, multiple Cdks regulate different stages of the cell-cycle, but in yeast a single Cdk, termed Cdk1 or Cdc28p, is the only Cdk driving the cell-cycle. Many other kinases besides Cdc28p regulate the cell-cycle, but Cdc28p

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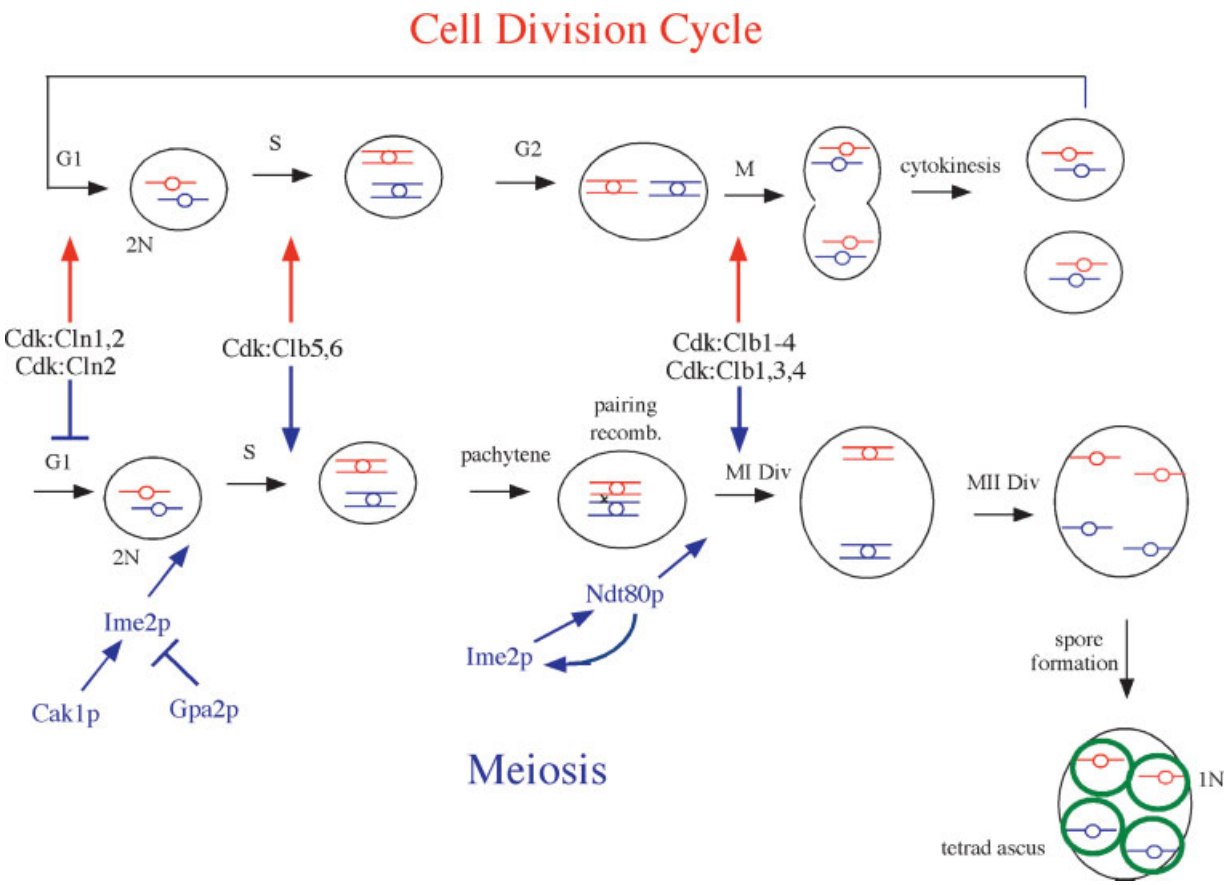

Fig. 1. Cdc28p and/or Ime2p regulation at different stages of meiosis and phases of the cell division cycle. The top diagram represents the phases of the cell cycle whereas, the bottom diagram represents the stages of meiosis. The effects of regulators at each stage are represented by an arrow (activation) or a T-bar (repression).

kinase is unique in the cell division cycle in that it plays a critical role at each sequential phase of the cycle (Fig. 2). The fundamental mechanism by which the same kinase drives each sequential stage of the cell-cycle is that this kinase associates with a different set of regulatory cyclins at each phase. In G_1, Cdc28p associates with Cln3p cyclin, whereas during the transition from G_1 to S-phase it associates primarily with Cln1p and Cln2p. During S-phase, Cdc28p associates with Clb5p and Clb6p, and in the transition from G_2 to M, Cdc28p associates first with Clb3 and Clb4 and then with Clb1 and Clb2.

Ime2p, like Cdc28p, is a serine/threonine protein kinase of the CMGC family, but Ime2p is not in the same branch of this family as Cdc28p [Hunter and Plowman, 1997; Krylov et al., 2003]. In contrast to Cdc28p, Ime2p kinase does not require cyclins for activity in vitro [Hui et al., 2002], and based on sequence comparisons, Ime2p may have evolved earlier than the Cdk kinases [Krylov et al., 2003]. Both Cdk and Ime2p are highly conserved kinases. For example, Ime2p paralogs are found in the mouse, rat, and human genomes. It is not known whether Ime2p is required for meiosis and gametogenesis in higher organisms.

Cdc28p substrates are phosphorylated at consensus sites—in its least stringent form these sites are simply a serine or a threonine followed by proline. Currently, there are three experimental tests for candidate Cdc28p substrates. First, *cdc28* mutants fail to phosphorylate the substrate. Second, purified Cdc28p:cyclin complexes phosphorylate the substrate in vitro. Third, mutations in consensus Cdk phosphorylation sites deregulate the substrate. Because phosphorylation sites have not been identified in candidate Ime2p substrates, only the first two tests are possible for these substrates.

Cdc28p regulates several cell-cycle processes that are not involved in meiosis. First, Cdc28p:Cln1,2p phosphorylates Cdc3p, causing

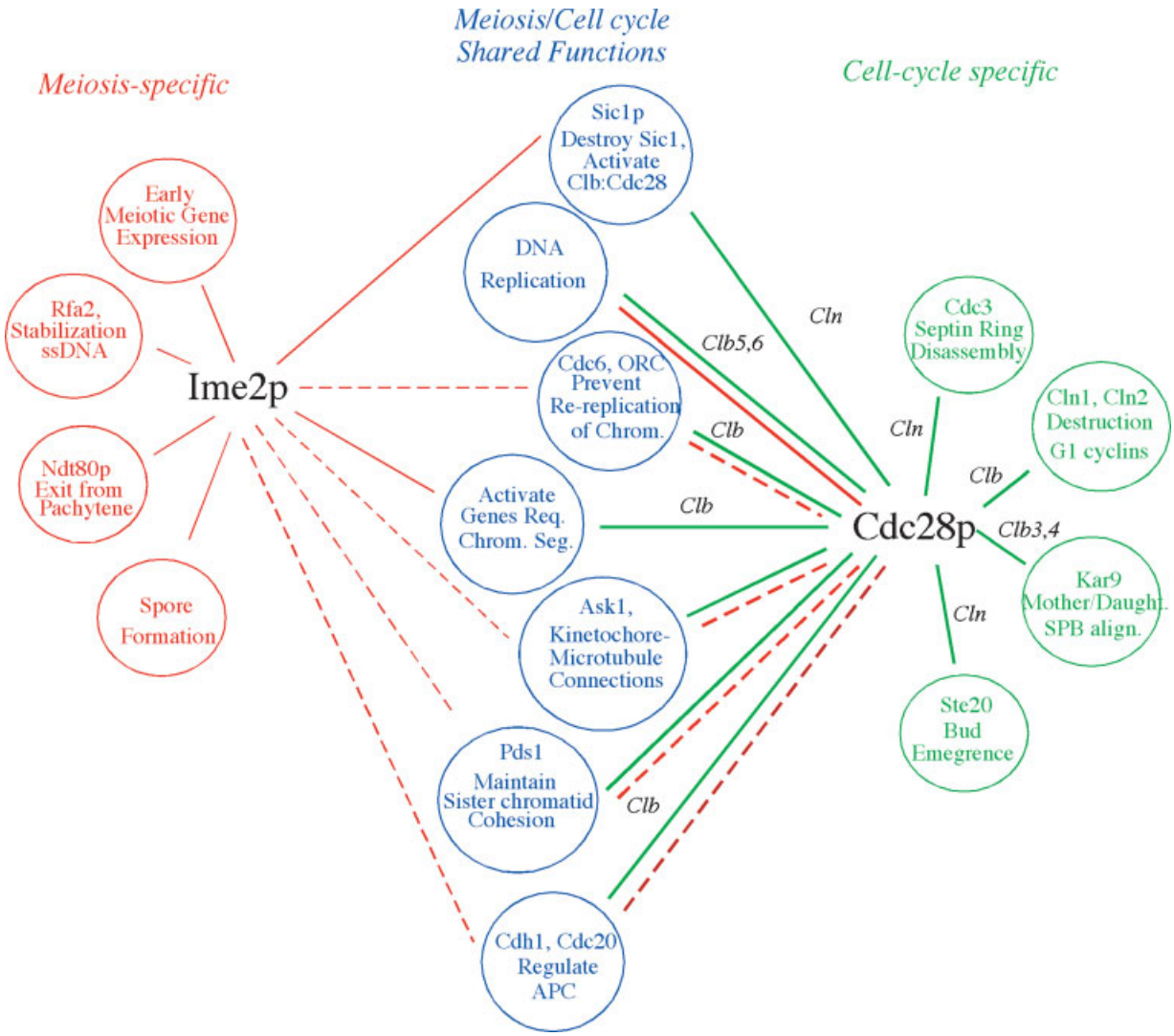

Fig. 2. Possible targets of Cdc28p and Ime2p in the cell division cycle and in meiosis. The diagram shows both Ime2p kinase and Cdc28p kinase with lines connecting each kinase to potential substrates and/or cellular processes that they regulate. Solid lines represent links between kinase and substrate supported by experimental evidence. Dotted lines indicated possible links between kinase and substrates without strong experimental evidence. Green lines indicate a link (or possible link) present during the cell division cycle, red lines indicate a link present during meiosis. Those circles colored in green are expected to function only in the cell-cycle, those in red only in meiosis, and those in blue in both programs. For Cdc28p-substrate links, where it is known which cyclin is required for the link, the cyclin is shown over the line.

disassembly of the septin ring [Tang and Reed, 2002]. Second, Cdc28p:Clb3,4p phosphorylates Kar9p, orienting spindle pole bodies with respect to mother and daughter cells [Liakopoulos et al., 2003; Maekawa et al., 2003]. Third, Cdc28p:Cln1,2p phosphorylates Cdc20p, allowing polarized cell growth [Oehlen and Cross, 1998; Wu et al., 1998]). Conversely, Ime2p regulates several meiotic processes, such as exit from pachytene, that do not occur in the cell division cycle.

OVERLAPPING ROLES OF Ime2p AND Cdc28p IN MEIOSIS

Both Ime2p and Cdc28p Are Required for Both Early and Late Stages of Meiotic Differentiation

IME2 was identified because it is required for efficient initiation of meiosis [Smith and Mitchell, 1989]. Subsequent studies revealed that separately from its role in meiotic initiation, Ime2p is also required at later stages of meiosis.

Thus, the phenotype of an ime2Δ mutant at late stages of sporulation is more severe than the phenotype at early stages [Mitchell et al., 1990; Yoshida et al., 1990; Foiani et al., 1996]. Furthermore, identification of several candidate Ime2p substrates (see below) support the idea that Ime2p is required at multiple stages of meiosis. Like Ime2p, Cdc28p is required at several stages of meiosis. Cdc28p was originally determined to be required for middle stages of meiosis [Shuster and Byers, 1989], and more recent studies reveal that it is independently required at early stages [Benjamin et al., 2003].

Meiotic Initiation

During G_1, when Cdc28p associates with the Cln family of cyclins, Ime2p and Cdc28p act in opposing fashion in regulating the choice between the cell division cycle and meiosis. Whereas Ime2p induces meiosis and inhibits growth, Cdc28p:Cln2p induces growth and inhibits meiosis. In particular, Ime2p is required for Ime1p to efficiently activate early meiotic genes [Mitchell et al., 1990], and may also inhibit progression through the cell-cycle, since forced expression of *IME2* strongly inhibits growth [Bolte et al., 2002]. In contrast, Cdc28p:Cln2p is required for the G_1 to S transition in the cell-cycle, and inhibits transcription of *IME1* [Colomina et al., 1999; Purnapatre et al., 2002]. Cdc28p probably inhibits meiotic differentiation independently of activating the G_1 to S transition because either Cln2p or Cln1p can activate the G_1 to S transition but only Cln2p (not Cln1p) can repress meiotic initiation [Purnapatre et al., 2002]. Taken together, these results indicate that Cdc28p:Cln kinase and Ime2p kinase are primary determinants in specifying which of two mutually-exclusive fates the cell adopts.

Ime2p may play a second essential role in meiotic initiation, at the completion of this first stage of meiosis. Specifically, Ime2p is required to repress *IME1* expression. Ime1p is dispensable once meiosis has initiated, and in fact repression of *IME1* may be necessary for the correct timing of late meiotic events. For example, forced expression of *IME1* during late stages of meiosis causes only two of the four haploid genomes to be packaged in most cells [Lee and Honigberg, 1996]. Ime2p represses IME1 expression at two levels. First Ime2p represses IME1 transcription through an unknown mechanism [Smith and Mitchell, 1989]. Second, Ime2p phosphorylates Ime1p and is required for Ime2p degradation [Guttmann-Raviv et al., 2002].

Meiotic DNA Replication

DNA replication in meiosis initiates at the same origins in meiosis as in the cell division cycle [Collins and Newlon, 1994], and the same replication enzymes are used in both programs [Simchen, 1974; Zamb and Roth, 1977]. However, initiation of replication in the cell-cycle is much more rapid than in meiosis [Cha et al., 2000], and replication in S-phase may have a shorter duration than in meiosis [Collins and Newlon, 1994; Cha et al., 2000] indicating that DNA replication is regulated differently in meiosis and the cell-cycle.

In the cell division cycle, initiation of DNA replication requires both Cdc28p:Cln1/2p and Cdc28p:Clb5/6p. As cells enter S-phase, the Cdc28p:Cln1/Cln2p complex phosphorylates Sic1p, targeting it for ubiquitination and, ultimately, destruction by the proteosome. Since Sic1p binds and inhibits Cdc28p:Clb5p,Clb6p kinase, once Sic1p is destroyed, this cyclin/kinase complex is activated, and DNA replication ensues.

In meiosis, DNA replication requires Cdc28p:Clb5,6p [Dirick et al., 1998; Stuart and Wittenberg, 1998; Benjamin et al., 2003], but not Cdc28p:Cln1,2p. Indeed, as described above, the latter cyclin:kinase complex inhibits meiotic initiation. In meiosis, phosphorylation and degradation of Sic1p requires Ime2p rather than Cdc28p:Cln1,2p [Dirick et al., 1998]. In addition, Ime2p phosphorylates Rfa2p a component of the trimeric replication protein A (RPA), at early stages of meiosis [Clifford et al., 2004]. RPA is required for replication in both the cell division cycle and meiosis, but the effect of Rfa2p phosphorylation on RPA activity is not known. Possibly, Ime2p activates meiotic DNA replication by at least two independent mechanisms, targeting Sic1p for destruction and regulating RPA activity.

Once chromosomes replicate in the cell-cycle, they are prevented from replicating again until the next cell-cycle. This block to re-replication requires that Cdc28p:Clb5,6 phosphorylates Cdc6p and several components of the origin-replication complex (ORC) [Elsasser et al., 1999; Calzada et al., 2000; Drury et al., 2000; Nguyen et al., 2001]. It is not known whether Cdc28p:Clb5,6 inhibits re-replication during

meiosis, but some evidence suggests that Ime2p is required for this inhibition. For example, an ime2Δ mutant displays an extra round of DNA replication in meiosis [Guttmann-Raviv et al., 2001].

In addition to Cdc28p:Cln1,2p and Cdc28p:Clb5,6p, Cdc7p:Dbf4p is another cyclin: kinase complex required for DNA replication in the cell-cycle. During S-phase, the Cdc7p:Dbf4p complex activates the MCM complex bound to replication origins (reviewed in [Masai and Arai, 2002]). In contrast to the role of Cdc7p in the cell-cycle, in meiosis a cdc7-1 mutant progresses normally through meiotic DNA replication and arrests at a later stage of meiosis (pachytene) [Sclafani et al., 1988; Hollingsworth and Sclafani, 1993]. This result suggests the possibility that Ime2p or some other meiosis-specific kinase may substitute for Cdc7p during meiotic replication.

Meiotic Pairing and Recombination

It is not known whether either Ime2p or Cdc28p have a direct role in the initiation of meiotic chromosome pairing and recombination. A clb5Δ mutant is unable to initiate meiotic recombination, but this phenotype is probably an indirect effect of defects in meiotic DNA replication in this mutant [Smith et al., 2001]. In contrast, to the uncertain role of both kinases in the initiation of meiotic recombination, both kinases may be required for the completion of recombination. For example, Cdc28p is required for exit from pachytene, the final stage of recombination [Shuster and Byers, 1989]. Ime2p may also be required to exit pachytene, including resolution of Holliday structures, since both of these events depend on Ndt80p, a transcription factor activated by Ime2p [Xu et al., 1995; Allers and Lichten, 2001]. Because activation of Ndt80p by Ime2p is also required for the meiosis I nuclear division, this activation is discussed in the next section.

Meiosis I Nuclear Division

Both Cdc28p and Ime2p regulate entry into the first meiotic division. The role of Cdc28p in regulating chromosome segregation in meiosis I may in some ways be analogous to its role during the G_2/M transition of the cell-cycle. However, one difference between the Cdc28p:Clb cyclins in mitosis and meiosis is the relative importance of the four M-phase cyclins in these two processes. Of these four cyclins (Clb1–4), Clb2p is the most active in mitosis, whereas this cyclin is neither expressed nor required for the meiotic divisions [Grandin and Reed, 1993; Dahlman and Futcher, 1995]. During the cell division cycle, Cdc28p has several critical roles in chromosome segregation: (1) phosphorylation of Ask1p, a component of the DASH complex, which mediates the interaction between the spindle and the kinetochore [Li and Elledge, 2003], (2) phosphorylation of Pds1/securin, a protease inhibitor that protects a protein complex (cohesin) that maintains cohesion between sister chromatids [Agarwal and Cohen-Fix, 2002], and (3) phosphorylation of Cdc20p and Cdh1p, components of two anaphase-promoting complexes (APC^{Cdc20} and APC^{Cdh1}), ubiquitin ligases that regulate both cohesin and cyclin stability [Jaspersen et al., 1999; Rudner and Murray, 2000]. It is not known whether Cdc28p phosphorylates any of these substrates in meiosis, but all three types of regulation are expected to be important in meiosis.

The ime2-as1 allele allows separation of the functions of Ime2p in early stages of meiosis from its functions in the meiosis I division [Benjamin et al., 2003]. Ime2-as1 kinase activity is blocked by addition of an inhibitor, 1-NA-PP1, but is active in the absence of this inhibitor. When 1-NA-PP1 is added to ime2-as1 mutant after completion of meiotic replication, the nuclear divisions are still inhibited, indicating a role for *IME2* in meiotic chromosome segregation that is separate from its role in DNA replication. Interestingly, forced expression of Ime2p during the cell division cycle causes Cdh1p phosphorylation, suggesting that one function of Ime2p in the meiosis I division may be to regulate APCs [Bolte et al., 2002].

In addition to the Cdc28p-substrates described above, Cdc28p activates the G_2/M transition in the cell-cycle by activating transcription of *CLB* genes and a cohort of other genes required for this transition [Amon et al., 1993]. Transcription of these genes requires the SFF transcription complex (reviewed in [Futcher, 2002]). The role of SFF in meiosis is not known, but Ime2p performs an analogous function to Cdc28p in activating transcription of genes required for chromosome segregation; Ime2p phosphorylates the Ndt80p transcription factor [Sopko et al., 2002; Benjamin et al., 2003] and stimulates binding of this transcriptional activator to middle sporulation elements (MSE) [Sopko et al., 2002]. MSEs are present in all five

CLB genes that are expressed in meiosis and in many other genes expressed in middle stages of meiosis [Chu and Herskowitz, 1998; Hepworth et al., 1998]. Ime2p further stimulating transcription of these genes by inactivating the Sum1p repressor, which competes with Ndt80p for MSEs [Lindgren et al., 2000; Pak and Segall, 2002; Pierce et al., 2003]. In addition, Ndt80p activates transcription of both its own gene [Pak and Segall, 2002] and *IME2* [Benjamin et al., 2003] further amplifying the expression of middle sporulation genes.

Meiosis II Nuclear Division and Spore Formation

Cdc28p:Clb is required for the second meiotic division but not for spore formation [Shuster and Byers, 1989; Dahlman and Futcher, 1995]. In contrast, Ime2p appears to have a direct role in both the meiosis II division and spore formation. For example, forced expression of Ndt80p in an ime2Δ mutant allows the first meiotic division but does not allow either the second meiotic division or spore formation [Benjamin et al., 2003]. However, the targets of Ime2p in these later stages of meiotic differentiation are not known.

REGULATION OF Ime2p AND Cdc28p IN MEIOSIS

Transcriptional Regulation

CDC28 is expressed at nearly constant levels throughout both the cell-cycle and meiosis. In contrast, *IME2* is under tight transcriptional control; *IME2* transcript is undetectable during the cell division cycle and is induced strongly at the initiation of meiosis. Repression of *IME2* during the cell-cycle occurs on at least two levels. On the first level, *IME2* transcription depends on the product of the *IME1* gene, which is strongly repressed during growth (reviewed in [Honigberg and Purnapatre, 2003; Kassir et al., 2003]). On a second level, assembly of Ime1p and other transcription factors at the *IME2* promoter is inhibited by both cell growth and specific nutrients such as glucose [Vidan and Mitchell, 1997; Xiao and Mitchell, 2000]. However, once meiosis initiates, IME2 transcript levels are relatively constant. Because Ime2p represses *IME1* expression as meiosis progresses (see "Meiotic initiation"), continued transcription of *IME2* during middle stages of meiosis may depend on the activation of Ndt80p by Ime2p and the resulting induction of IME2 transcription by Ndt80p (a positive feedback loop).

Post-Translational Regulation

At a post-translational level, Cdc28p activity is regulated by binding to activator proteins such as cyclins and Cks1/Suc1, by binding to inhibitor proteins, such as Sic1p, and by phosphorylation (reviewed in [Mendenhall and Hodge, 1998]). Ime2p may also be regulated both by binding to regulatory proteins and by phosphorylation. Gpa2p, the alpha subunit of a trimeric G-protein, binds Ime2p and inhibits its kinase activity in vitro [Donzeau and Bandlow, 1999]. In addition, Ime2p has at least two distinct phosphorylation states during meiosis. Ime2p is phosphorylated from its earliest appearance, and becomes hyperphosphorylated at around the time of the nuclear divisions. These two phosphorylation states correspond to two peaks of Ime2p activity as measured by in vitro kinase assays [Benjamin et al., 2003]. Thus different phospho-isoforms of Ime2p act in sequential stages of meiosis, analogous to the different Cdc28p:cyclin complexes that are active at different phases of the cell-cycle.

Initial phosphorylation of Ime2p requires Cak1p kinase, though this kinase does not phosphorylate Ime2p in vitro [Schindler et al., 2003]. A cak1Δ mutant, presumably because it fails to phosphorylate Ime2p, displays decreased Ime2p activity and accordingly fails to degrade Sic1p or initiate meiotic replication efficiently. Interestingly, Cak1p also phosphorylates Cdc28p during the cell-cycle, activating this Cdk. The hyperphosphorylation of Ime2p and the corresponding second peak of Ime2p kinase activity requires both Ndt80p and Cdc28p [Benjamin et al., 2003]. In addition, Ime2p hyperphosphorylation requires active Ime2p, and Ime2p displays autokinase activity in vitro, suggesting a positive feedback loop on Ime2p activity [Sopko et al., 2002; Benjamin et al., 2003].

The stability of Ime2p may also be regulated, because Ime2p is unstable when its expression is forced during the cell division cycle [Bolte et al., 2002]. Indeed an Ime2p-Gal4DB fusion protein is unstable even under sporulation conditions [Guttmann-Raviv et al., 2002]. Destabilization of Ime2p may require a PEST sequence present in the C-terminal region of this kinase, because a mutant lacking this sequence is more effective at blocking growth

than is the wild-type allele [Guttmann-Raviv et al., 2002].

FUTURE DIRECTIONS

The last several years have revealed many of the proteins that are phosphorylated by Cdc28p during the cell division cycle. Since many of these proteins regulate processes that are important in meiosis, a likely scenario is that some of the proteins will be phosphorylated by Cdc28p in meiosis as in the cell-cycle, whereas others will be phosphorylated by Ime2p or other meiosis-specific kinases. This view takes in account two sets of observations. First, Cdc28p is required for several key stages of meiosis, including meiotic DNA replication and the meiotic divisions. Second, several functions of Cdc28p in the cell division cycle are performed by Ime2p in meiosis; in particular, the targeting of Sic1p for degradation and activation of *CLB* expression.

Ime2p may substitute for Cdc28p functions in meiosis when it is necessary to impose additional controls on meiosis that are not present in the cell division cycle. For example, meiotic initiation is activated by different nutritional signals than commitment to the cell-cycle. As a result, a key regulator of commitment to the cell division cycle, Cdc28p:Cln1,2p is replaced by Ime2p in activating meiotic initiation and DNA replication. A different type of meiosis-specific control may occur at the time of meiosis I chromosome segregation. Meiotic chromosome segregation is coordinated, via checkpoint functions, with the completion of chromosome pairing and recombination, events that do not occur in the cell division cycle. Thus, regulation of meiotic chromosome segregation may require additional Ime2p-dependent controls that are unnecessary in the cell-cycle.

Regulation of Ime2p in some ways parallels Cdc28p regulation, both kinases are activated by Cak1p and both kinases exist in multiple forms with different activities. In the case of Cdc28p, the multiple forms are determined by association with different cyclins. In the case of Ime2p, the multiple forms may correspond to different phosphorylation states. How can some Cdk functions be replaced by a completely different type of kinase in meiosis? At the end of each cell division cycle the cell-cycle machinery must be reset to initiate another cycle; this continual resetting occurs because each class of cyclins regulates the expression and activity of other classes of cyclins. In contrast, the production of haploid cells in meiosis precludes a second round of meiosis, perhaps rendering resetting mechanisms and additional cyclin-dependent kinases unnecessary.

REFERENCES

Agarwal R, Cohen-Fix O. 2002. Phosphorylation of the mitotic regulator Pds1/securin by Cdc28 is required for efficient nuclear localization of Esp1/separase. Genes Dev 16:1371–1382.

Allers T, Lichten M. 2001. Differential timing and control of noncrossover and crossover recombination during meiosis. Cell 106:47–57.

Amon A, Tyers M, Futcher B, Nasmyth K. 1993. Mechanisms that help the yeast cell cycle clock tick: G_2 cyclins transcriptionally activate G_2 cyclins and repress G_1 cyclins. Cell 74:993–1007.

Benjamin KR, Zhang C, Shokat KM, Herskowitz I. 2003. Control of landmark evens in meiosis by the CDK Cdc28 and the meiosis-specific kinase, Ime2. Genes Dev 17:1–16.

Bolte M, Steigemann P, Braus GH, Irniger S. 2002. Inhibition of APC-mediated proteolysis by the meiosis-specific protein kinase Ime2. Proc Natl Acad Sci USA 99: 4385–4390.

Calzada A, Sanchez M, Sanchez E, Bueno A. 2000. The stability of the Cdc6 protein is regulated by cyclin-dependent kinase/cyclin B complexes in *Saccharomyces cerevisiae*. J Biol Chem 275:9734–9741.

Cha RS, Weiner BM, Keeney S, Dekker J, Kleckner N. 2000. Progression of meiotic DNA replication is modulated by interchromosomal interaction proteins, negatively by Spo11p and positively by Rec8p. Genes Dev 14: 493–503.

Chu S, Herskowitz I. 1998. Gametogenesis in yeast is regulated by a transcriptional cascade dependent on Ndt80. Mol Cell 1:685–696.

Clifford DM, Marinco SM, Brush GS. 2004. The meiosis-specific protein kinase Ime2 directs phosphorylation of replication protein A. J Biol Chem 279:6163–6170.

Collins I, Newlon CS. 1994. Chromosomal DNA replication initiates at the same origins in meiosis and mitosis. Mol Cell Biol 14:3524–3534.

Colomina N, Gari E, Gallego C, Herrero E, Aldea M. 1999. G_1 cyclins block the Ime1 pathway to make mitosis and meiosis incompatible in budding yeast. Embo J 18: 320–329.

Dahlman C, Futcher B. 1995. Specialization of B-type cyclins for mitosis or meiosis in *S. cerevisiae*. Genetics 140:957–963.

Dirick L, Goetsch L, Ammerer G, Byers B. 1998. Regulation of meiotic S phase by Ime2 and a Clb5,6-associated kinase in *Saccharomyces cerevisiae*. Science 281:1854–1857.

Donzeau M, Bandlow W. 1999. The yeast trimeric guanine nucleotide-binding protein alpha subunit, Gpa2p, controls the meiosis-specific kinase Ime2p activity in response to nutrients. Mol Cell Biol 19:6110–6119.

Drury LS, Perkins G, Diffley JF. 2000. The cyclin-dependent kinase Cdc28p regulates distinct modes of

Cdc6p proteolysis during the budding yeast cell cycle. Curr Biol 10:231–240.
Elsasser S, Chi Y, Yang P, Campbell JL. 1999. Phosphorylation controls timing of Cdc6p destruction: A biochemical analysis. Mol Biol Cell 10:3263–3277.
Foiani M, Nadjar-Boger E, Capone R, Sagee S, Hashimshoni T, Kassir Y. 1996. A meiosis-specific protein kinase, Ime2, is required for the correct timing of DNA replication and for spore formation in yeast meiosis. Mol Gen Genet 253:278–288.
Futcher B. 2002. Transcriptional regulatory networks and the yeast cell cycle. Curr Opin Cell Biol 14:676–683.
Grandin N, Reed SI. 1993. Differential function and expression of *Saccharomyces cerevisiae* B-type cyclins in mitosis and meiosis. Mol Cell Biol 13:2113–2125.
Guttmann-Raviv N, Boger-Nadjar E, Edri I, Kassir Y. 2001. Cdc28 and Ime2 possess redundant functions in promoting entry into premeiotic DNA replication in *Saccharomyces cerevisiae*. Genetics 159:1547–1558.
Guttmann-Raviv N, Martin S, Kassir Y. 2002. Ime2, a meiosis-specific kinase in yeast, is required for destabilization of its transcriptional activator, Ime1. Mol Cell Biol 22:2047–2056.
Hartwell L, Weinert T. 1989. Checkpoints: Controls that ensure the order of cell cycle events. Science 246: 629–634.
Hepworth SR, Friesen H, Segall J. 1998. NDT80 and the meiotic recombination checkpoint regulate expression of middle sporulation-specific genes in *Saccharomyces cerevisiae*. Mol Cell Biol 18:5750–5761.
Hollingsworth REJ, Sclafani RA. 1993. Yeast pre-meiotic DNA replication utilizes mitotic origin ARS1 independently of CDC7 function. Chromosoma 102:415–420.
Honigberg SM, Purnapatre K. 2003. Signal pathway integration in the switch from the mitotic cell cycle to meiosis in yeast. J Cell Sci 116:2137–2147.
Hui CM, Campistrous A, Stuart DT. 2002. Purification and some properties of *Saccharomyces cerevisiae* meiosis-specific protein kinase Ime2. Protein Expr Purif 26:416–424.
Hunter T, Plowman GD. 1997. The protein kinases of budding yeast: Six score and more. Trends Biochem Sci 22:18–22.
Jaspersen SL, Charles JF, Morgan DO. 1999. Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. Curr Biol 9:227–236.
Kassir Y, Adir N, Boger-Nadjar E, Raviv NG, Rubin-Bejerano I, Sagee S, Shenhar G. 2003. Transcriptional regulation of meiosis in budding yeast. Int Rev Cytol 224:111–171.
Krylov DM, Nasmyth K, Koonin EV. 2003. Evolution of eukaryotic cell cycle regulation: Stepwise addition of regulatory kinases and late advent of the CDKs. Curr Biol 13:173–177.
Lee RH, Honigberg SM. 1996. Nutritional regulation of late meiotic events in *Saccharomycescerevisiae* through a pathway distinct from initiation. Mol Cell Biol 16:3222–3232.
Li Y, Elledge SJ. 2003. The DASH complex component Ask1 is a cell cycle-regulated Cdk substrate in *Saccharomyces cerevisiae*. Cell Cycle 2:143–148.
Liakopoulos D, Kusch J, Grava S, Vogel J, Barral Y. 2003. Asymmetric loading of Kar9 onto spindle poles and microtubules ensures proper spindle alignment. Cell 112: 561–574.
Lindgren A, Bungard D, Pierce M, Xie J, Vershon A, Winter E. 2000. The pachytene checkpoint in *Saccharomyces cerevisiae* requires the Sum1 transcriptional repressor. Embo J 19:6489–6497.
Maekawa H, Usui T, Knop M, Schiebel E. 2003. Yeast Cdk1 translocates to the plus end of cytoplasmic microtubules to regulate bud cortex interactions. Embo J 22: 438–449.
Masai H, Arai K. 2002. Cdc7 kinase complex: A key regulator in the initiation of DNA replication. J Cell Physiol 190:287–296.
Mendenhall MD, Hodge AE. 1998. Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*. Microbiol Mol Biol Rev 62:1191–1243.
Mitchell AP, Driscoll SE, Smith HE. 1990. Positive control of sporulation-specific genes by the IME1 and IME2 products in *Saccharomyces cerevisiae*. Mol Cell Biol 10:2104–2110.
Murakami H, Nurse P. 2000. DNA replication and damage checkpoints and meiotic cell cycle controls in the fission and budding yeasts. Biochem J 349:1–12.
Nguyen VQ, Co C, Li JJ. 2001. Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. Nature 411:1068–1073.
Oehlen LJ, Cross FR. 1998. Potential regulation of Ste20 function by the Cln1-Cdc28 and Cln2-Cdc28 cyclin-dependent protein kinases. J Biol Chem 273:25089–25097.
Pak J, Segall J. 2002. Regulation of the premiddle and middle phases of expression of the NDT80 gene during sporulation of *Saccharomyces cerevisiae*. Mol Cell Biol 22: 6417–6429.
Petronczki M, Siomos MF, Nasmyth K. 2003. Un menage a quatre: The molecular biology of chromosome segregation in meiosis. Cell 112:423–440.
Pierce M, Benjamin K, Mantano SP, Georgiadis MM, Winter E, Vershon AK. 2003. Sum1 and Ndt80 proteins compete for binding to middle sporulation element sequences that control meiotic gene expression. Mol Cell Biol 23:4814–4825.
Purnapatre K, Piccirillo S, Schneider BL, Honigberg SM. 2002. The CLN3/SWI6/CLN2 pathway and SNF1 act sequentially to regulate meiotic initiation in *Saccharomyces cerevisiae*. Genes Cells 7:675–691.
Roeder GS, Bailis JM. 2000. The pachytene checkpoint. Trends Genet 16:395–403.
Rudner AD, Murray AW. 2000. Phosphorylation by Cdc28 activates the Cdc20-dependent activity of the anaphase-promoting complex. J Cell Biol 149:1377–1390.
Schindler K, Benjamin KR, Martin A, Boglioli A, Herskowitz I, Winter E. 2003. The Cdk-activating kinase Cak1p promotes meiotic S phase through Ime2p. Mol Cell Biol 23:8718–8728.
Sclafani RA, Patterson M, Rosamond J, Fangman WL. 1988. Differential regulation of the yeast *CDC7* gene during mitosis and meiosis. Mol Cell Biol 8:293–300.
Shuster EO, Byers B. 1989. Pachytene arrest and other meiotic effects of the start mutations in *Saccharomyces cerevisiae*. Genetics 123:29–43.
Simchen G. 1974. Are mitotic functions required in meiosis? Genetics 76:745–753.

Smith HE, Mitchell AP. 1989. A transcriptional cascade governs entry into meiosis in *Saccharomyces cerevisiae*. Mol Cell Biol 9:2142–2152.

Smith KN, Penkner A, Ohta K, Klein F, Nicolas A. 2001. B-type cyclins CLB5 and CLB6 control the initiation of recombination and synaptonemal complex formation in yeast meiosis. Curr Biol 11:88–97.

Sopko R, Raithatha S, Stuart D. 2002. Phosphorylation and maximal activity of *Saccharomyces cerevisiae* meiosis-specific transcription factor Ndt80 is dependent on Ime2. Mol Cell Biol 22:7024–7040.

Stuart D, Wittenberg C. 1998. CLB5 and CLB6 are required for premeiotic DNA replication and activation of the meiotic S/M checkpoint. Genes Dev 12:2698–2710.

Tang CS, Reed SI. 2002. Phosphorylation of the septin cdc3 in g1 by the cdc28 kinase is essential for efficient septin ring disassembly. Cell Cycle 1:42–49.

Vidan S, Mitchell AP. 1997. Stimulation of yeast meiotic gene expression by the glucose-repressible protein kinase Rim15p. Mol Cell Biol 17:2688–2697.

Wu C, Leeuw T, Leberer E, Thomas DY, Whiteway M. 1998. Cell cycle- and Cln2p-Cdc28p-dependent phosphorylation of the yeast Ste20p protein kinase. J Biol Chem 273: 28107–28115.

Xiao Y, Mitchell AP. 2000. Shared roles of yeast glycogen synthase kinase 3 family members in nitrogen-responsive phosphorylation of meiotic regulator Ume6p. Mol Cell Biol 20:5447–5453.

Xu L, Ajimura M, Padmore R, Klein C, Kleckner N. 1995. *NDT80*, a meiosis-specific gene required for exit from pachytene in *Saccharomyces cerevisiae*. Mol Cell Biol 15:6572–6581.

Yoshida M, Kawaguchi H, Sakata Y, Kominami K, Hirano M, Shima H, Akada R, Yamashita I. 1990. Initiation of meiosis and sporulation in *Saccharomyces cerevisiae* requires a novel protein kinase homologue. Mol Gen Genet 221:176–186.

Zamb TJ, Roth R. 1977. Role of mitotic replication genes in chromosome duplication during meiosis. Proc Natl Acad Sci USA 74:3951–3955.