Global Gene Expression in *Coprinopsis cinerea* Meiotic Mutants Reflects Checkpoint Arrest

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ABSTRACT The basidiomycete *Coprinopsis cinerea* is well-suited to studies of meiosis because meiosis progresses synchronously in 10 million cells within each mushroom cap. Approximately 20% of *C. cinerea* genes exhibit changing expression during meiosis, but meiosis and mushroom development happen concurrently and therefore differentially expressed genes might not be directly involved in meiotic processes. By using microarrays, we examined global gene expression across a meiotic time course in two mutants in which meiosis arrests but mushrooms develop normally. Genes differentially expressed in the mutants compared with the wild type are likely to be involved in meiosis and sporulation as opposed to mushroom development. In *rad50-1*, which arrests in late prophase, RNA abundance for a group of early meiotic genes remains high, whereas the expression of a group of late meiotic genes is never induced. In contrast, in *msh5-22* (which fails to undergo premeiotic DNA replication), both early and late meiotic genes are underexpressed relative to wild type at late meiotic time points as the cells die. Genes that are differentially expressed relative to wild type in both mutants are particularly strong candidates for playing roles in meiosis and sporulation.

KEYWORDS
checkpoint arrest
MRN complex
microarray analysis
mushroom development
meiotic S phase

Meiosis is a specialized process of cell division in which one round of DNA replication is followed by two divisions to produce four haploid daughter nuclei. Thousands of genes are differentially expressed in meiotic cells compared with nonmeiotic cells, but genes that are specifically induced in meiosis are not necessarily essential for meiosis (Schlecht and Primig 2003). In addition, a large proportion of genes exhibit changing expression during the course of meiosis (Chu *et al.* 1998; Primig *et al.* 2000; Mata *et al.* 2002; Burns *et al.* 2010). In *Saccharomyces cerevisiae* meiosis, the timing of gene induction has been shown to correspond to the time of protein function in many cases, for example, in genes involved in recombination, the pachytene checkpoint, and spore wall formation (Schlecht and Primig 2003).

The basidiomycete mushroom *Coprinopsis cinerea*, also known as *Coprinus cinereus* (Redhead *et al.* 2001), is particularly well suited to studies of gene expression in meiosis because meiosis occurs synchronously in basidial cells, facilitating tissue collection at defined meiotic stages (Figure 1). Burns *et al.* (2010) examined meiotic expression of genes that have orthologs in *C. cinerea*, *S. cerevisiae*, and *Schizosaccharomyces pombe* and found that genes with meiotic function are more likely to be coinduced on entry to meiosis and more likely to have correlated expression patterns during meiosis than genes not known to be meiotic. This is true even though transcription factors that are major regulators of meiosis differ among the three fungi. For example, in *S. cerevisiae* Ndt80 induces the expression of hundreds of middle meiotic genes (Chu *et al.* 1998), but *C. cinerea* and *S. pombe* lack identifiable orthologs of this transcription factor. Similarly, in *S. pombe* the forkhead transcription factor Mei4 is required for the expression of middle meiotic genes (Mata *et al.* 2002), but its ortholog in *S. cerevisiae* is not meiosis-specific (Pramila *et al.* 2006). The *C. cinerea* genome contains predicted orthologs of forkhead transcription factors but the roles of the encoded proteins are not known.

Nearly 3000 of the approximately 13,400 *C. cinerea* genes (Stajich *et al.* 2010) change in transcript level during meiosis. These genes are grouped into nine clusters based on their expression patterns over a time course spanning meiosis from before premeiotic DNA replication

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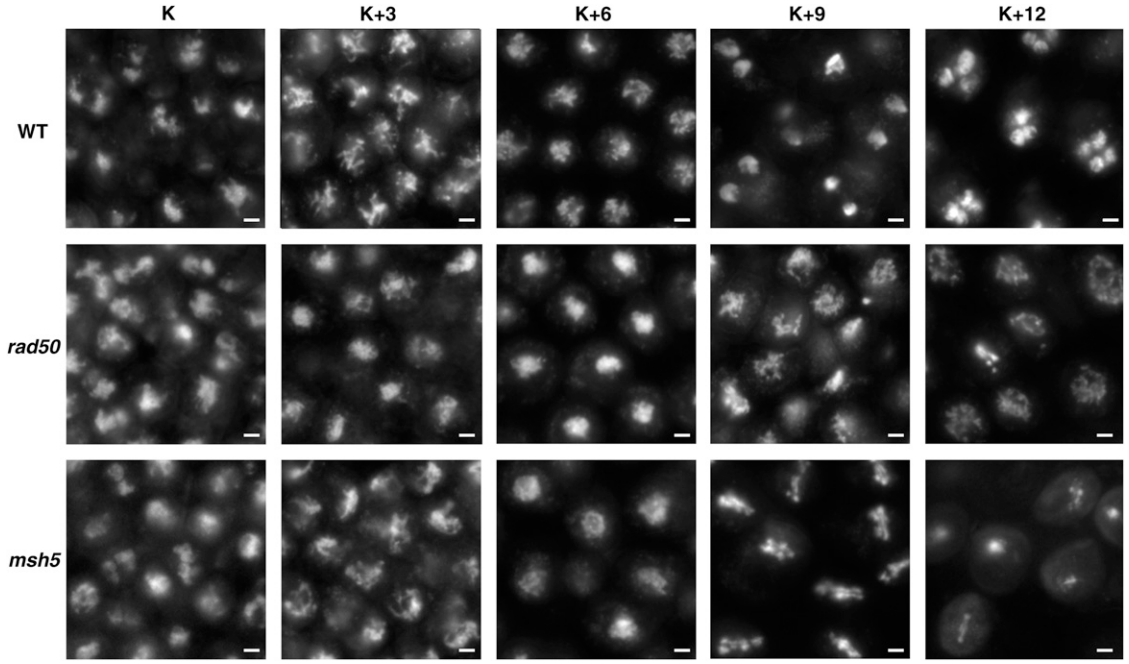

Figure 1 Wild-type, *rad50-1*, and *msh5-22* nuclei during meiosis. Wild-type, *rad50-1*, and *msh5-22* nuclei at the five time points were stained with DAPI, and images were taken at 1000× magnification. Nuclei fuse at karyogamy, condense at K+3, and are fully synapsed at K+6. At K+9, wild-type nuclei are undergoing the first meiotic division, *rad50-1* nuclei have arrested in either a diffuse or metaphase-like state, and *msh5-22* nuclei are arrested at metaphase I. At K+12, wild-type nuclei have undergone the second meiotic division, *rad50-1* nuclei are still arrested, and many *msh5-22* cells have died. Scale bars are 2 μm.

to after tetrad formation [Figure 2 (Burns *et al.* 2010)]. Overall, genes in clusters 1 through 5 (early clusters) have relatively high transcript levels at the beginning of meiosis, and these clusters are enriched for genes involved in early meiotic processes with functions such as nucleic acid binding, cytoskeleton organization, chromosome cohesion, and damaged-DNA binding. Genes in clusters 6 through 9 (late clusters) have their greatest transcript abundance at the end of meiosis, and these clusters are enriched for genes involved in gill maturation and sporulation. Mushroom development occurs concurrently with meiosis and spore formation, so only a subset of the genes with changing expression is likely involved in meiosis and sporulation. Therefore, by comparing wild-type gene expression with expression in mutants that do not complete meiosis but have normal mushroom development, we can identify the genes most likely to be responsible for meiosis and sporulation. In this study, we used a mutant that arrests in diplotene (*rad50-1*) and one that arrests in metaphase I (*msh5-22*).

Mre11, Rad50, and Nbs1 form the highly conserved MRN complex, which is involved in DNA double-strand break (DSB) repair. At the beginning of meiosis, DSBs are induced by the endonuclease Spo11. The MRN complex, along with the nucleases Ctp1 and Exo1, resects the 5′ ends of the DSBs (Nicolette *et al.* 2010) and the resulting single-strand end is coated with Rad51. This single-strand end then invades the homologous chromosome and subsequent intermediates can be resolved as crossovers. Rad50 is a structural maintenance of chromosomes protein that can dimerize to create a complex of the proper size to bridge sister chromatids or DSB ends (Hopfner *et al.* 2002). The *C. cinerea* mutant *rad50-1* produces a putative Rad50 protein that is truncated after 360 of the 1309 amino acids of the wild-type protein and is likely unstable (Acharya *et al.* 2008). *S. cerevisiae rad50S*-like mutations (certain mutations of *RAD50* and null mutations of *SAE2*) inhibit the resection of DSBs, causing *rad50S* mutants to delay for several hours in prophase before eventually entering meiosis (reviewed by Hochwagen and Amon 2006). In *C. cinerea rad50-1*, the number of Rad51 foci is not significantly different from the number of Rad51 foci in a *spo11* mutant in which no DSBs are made (Acharya *et al.* 2008), implying that DSBs are not resected properly in *rad50-1*. Presumably due to a pathway similar to that causing the arrest of *rad50S* mutants, 82% of *C. cinerea rad50-1* cells arrest in diffuse diplotene and an additional 16% arrest in a metaphase-like state (Figure 1). This mutant has only 2% spore viability, compared with more than 90% in wild type (Acharya *et al.* 2008).

The *C. cinerea msh5-22* mutant fails to replicate DNA before entering meiosis (Kanda *et al.* 1990). Msh5, a homolog of bacterial MutS, forms a heterodimer with Msh4 that is involved in crossover formation, possibly promoting or stabilizing single-strand invasion intermediates (reviewed by Lynn *et al.* 2007). Mouse Msh5-deficient spermatocytes and oocytes arrest in zygotene with aberrant chromosome synapsis and then undergo apoptosis (De Vries *et al.* 1999). Though the absence of premeiotic S phase in *msh5* mutants is apparently unique to *C. cinerea*, cell death is similar; *msh5-22* cells die after arresting at metaphase I [Figure 1 (Lu *et al.* 2003)].

We used microarrays to compare gene expression in *rad50-1* and *msh5-22* to that in wild type during a 12-hour time course spanning meiosis and identified genes that are differentially expressed at each time point. Our work identified genes whose encoded proteins are strong candidates for playing roles in meiosis and sporulation.

MATERIALS AND METHODS

Wild-type monokaryon strains J6;5-5 and J6;5-4 (Valentine *et al.* 1995) and mutant strains *rad50-1* (Ramesh and Zolan 1995) and *msh5*-22 (F. Kennedy, O. Savytskyy, M. Zolan, unpublished data) were used. Mushrooms were grown, RNA was isolated from gill tissue,

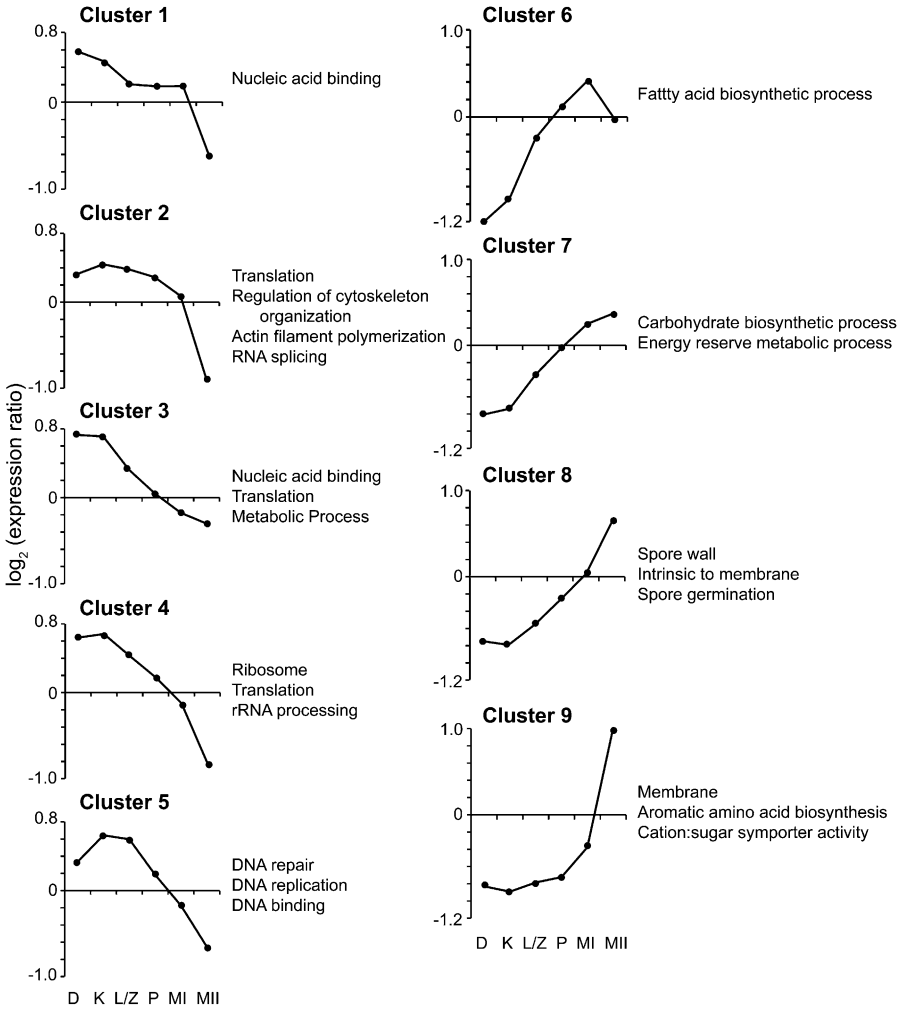

Figure 2 Clusters of genes differentially expressed during wild-type meiosis. Line graphs give the average expression profile of each cluster. The Y-axis shows $\log_2$-transformed expression ratios. Representative Gene Ontology categories enriched in each cluster are listed. Adopted from Burns *et al.* (2010).

arrays were hybridized and scanned, spots were flagged, data were normalized, and *S. cerevisiae* orthologs were identified as previously described (Burns *et al.* 2010), with the exception that spots on the arrays were not flagged for omission if the percentage of pixels above background plus 1 standard deviation in both channels <60. For each mutant at each time point, RNA was isolated from four biological replicates (each containing seven to ten mushrooms). Samples were collected 3 hr before karyogamy (*msh5-22* only), at karyogamy, and 3, 6, 9, and 12 hr after karyogamy. Single layers of gill tissue were stained with DAPI as in Acharya *et al.* (2008) to confirm that samples were at the correct stage. Each biological replicate was hybridized to a separate microarray with a reference pool of 10 wild-type biological replicates from the same time point. Significance Analysis of Microarrays software was used to identify differentially expressed genes at each time point as well as genes that have patterns of expression across the time course that are significantly different from wild type. We used a false-discovery rate (FDR) cutoff of 5%.

RESULTS

Two meiotic mutants have different overall patterns of differential expression in comparison with wild-type expression

We collected meiotic tissue from *rad50-1* and *msh5-22* mushrooms at five time points spanning meiosis. In *C. cinerea*, two haploid nuclei fuse (karyogamy) at the beginning of meiosis, and time points were measured in hours from karyogamy (K). Gill tissue was collected at K, K+3 (leptotene/zygotene), K+6 (pachytene), K+9 (first meiotic division), and K+12 (after tetrads have formed) as well as at K-3 for *msh5-22* only (Figure 1). For both *rad50-1* and *msh5-22*, microarrays were used to compare gene expression in four separate biological replicates at each time point to that of a wild-type reference pool of the same time point. We identified genes that are significantly differentially expressed compared to wild type in each mutant with an FDR cutoff of 5%. This means that 5% of the genes included in a list of differentially expressed genes are expected to be false positives.

In *rad50-1*, dozens of genes are differentially expressed at the first three time points, increasing to roughly 1000 by K+12 (FDR 5%; Table 1). To determine whether changes in gene expression reflect the *rad50-1* prophase arrest phenotype, we categorized the genes that are differentially expressed in *rad50-1* according to their cluster in the wild type. As expected, genes that are overexpressed in *rad50-1* compared with the wild type at the end of meiosis tended to be in early clusters whereas those that are underexpressed compared with the wild type at K+12 tended to be in late clusters (Figure 3). Of the genes that are differentially expressed in *rad50-1* at K+12 and are in clusters, 91% of those that had greater transcript abundance than wild type were in clusters 1 through 6, whereas 94% of those that are lower than wild type were in clusters 7 through 9. This finding suggests that the expression of a group of genes whose wild-type expression

Table 1 Number of differentially expressed genes in *rad50-1*

	K	K+3	K+6	K+9	K+12
Greater in *rad50-1*	18	9	45	236	726
Lower in *rad50-1*	38	30	94	621	209

The numbers of genes whose transcripts are more and less abundant in *rad50-1* at FDR 5% than in wild type at each of five time points are shown. Expression ratios and FDRs for all genes are given in Table S3. FDR, false-discovery rate.

decreases remains high in *rad50-1*. Conversely, a group of genes that are induced at the end of wild-type meiosis fails to be transcribed in *rad50-1*. This pattern is consistent with our previous observation that *rad50-1* cells arrest in late prophase I (Ramesh and Zolan 1995; Acharya *et al.* 2008).

We hypothesized that in arrested *rad50-1* cells, genes involved in cell-cycle progression would be differentially expressed compared with the wild type. Indeed, of the 12 *C. cinerea* genes whose *S. cerevisiae* orthologs are involved in regulation of cyclin-dependent kinase activity, six are differentially expressed at one or both of the last two time points. The most striking example is the single *C. cinerea* homolog of the *S. cerevisiae* cyclin genes *PCL1*, *2*, and *9* [CC1G_09963]. In the wild type *C. cinerea*, transcript abundance of this gene increases more than 10-fold from the beginning to the end of meiosis. The gene has significantly lower transcript abundance in *rad50-1* than in wild type at K+9 and K+12. Interestingly, it also is underexpressed in *msh5-22* at the same time points. Taken together, this evidence suggests that transcription of this gene increases dramatically throughout meiosis but that this increase is halted at K+9 when the mutants arrest.

Other cell-cycle-related genes have elevated expression in *rad50-1* compared with the wild type at one or both of the last two time points (supporting information, Table S1). These include genes that encode the orthologs of *S. cerevisiae* B-type cyclins (Clb1-4) [CC1G_06397, CC1G_05320] and the catalytic subunit of the main cyclin-dependent kinase (Cdc28) [CC1G_02703], as well as Cdc20 [CC1G_12115], the activator of the anaphase-promoting complex (APC), and an APC component (Apc2) [CC1G_02840]. Thus, control of the checkpoint activated by aberrant repair of meiotic DSBs is likely mediated, at least in part, by transcriptional regulation of cyclins, Cdks, and APC components.

Gene expression in *msh5-22* follows a generally different pattern from that seen in *rad50-1*, particularly at the beginning of the time course. At K-3, 390 genes are expressed below wild-type levels, and 479 genes, 88 of which are in cluster 3, have above wild-type expression levels (Figure 4). Among the genes with higher-than-wild-type transcript levels are several that encode orthologs of budding yeast proteins involved in DNA replication and repair (Table S2): Cdc7 [CC1G_08833], which is required for firing of origins of DNA replication and replication fork progression (Bousset and Diffley 1998); Mcm7 [CC1G_01238], which is part of a complex that promotes DNA melting and elongation during S phase (Tye 1999); Pri2 [CC1G_11942], a component of DNA primase (Foiani *et al.* 1989); and Rfa2 [CC1G_13904], which is a subunit of the single-stranded DNA binding protein RPA that is involved in DNA replication, repair, and recombination (Longhese *et al.* 1994). *rad50* transcript levels also are greater in *msh5-22* than in the wild type at K-3. Genes encoding the ribonucleotide reductase complex (*RNR1-4*) are transcriptionally induced in response to DNA damage and replication blocks in *S. cerevisiae* (Huang *et al.* 1998). This complex catalyzes the rate-limiting step of deoxyribonucleotide biosynthesis and is required for DNA replication and repair (Elledge *et al.* 1993). In *C. cinerea*, the ortholog of *RNR1* and *RNR3* [CC1G_03383] and the ortholog of

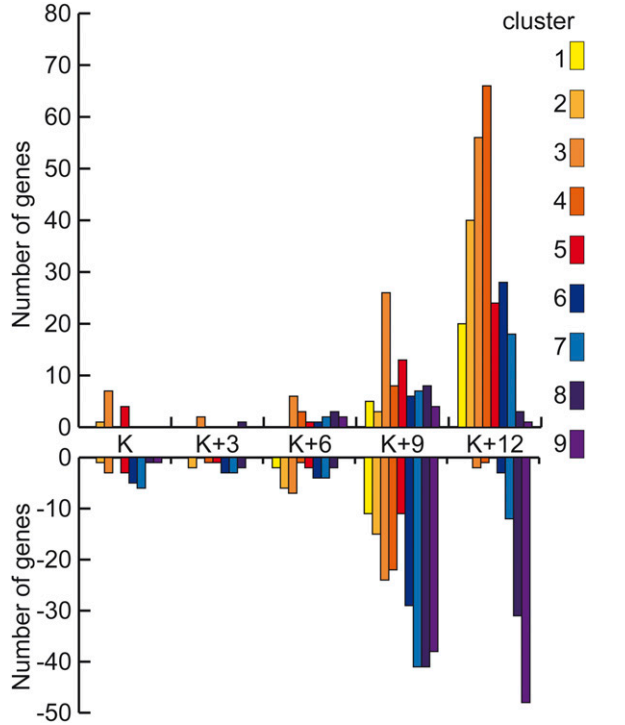

Figure 3 Number of differentially expressed genes in *rad50-1* by expression cluster. At each time point, the number of genes in each cluster whose transcripts are significantly more and less abundant in *rad50-1* than in the wild type is shown. Clusters 1−5, which contain genes that are expressed at greater levels at the beginning of meiosis, are represented with warm colors, and clusters 6−9, which contain genes that are expressed at greater levels at the end of meiosis, are represented with cool colors.

RNR2 [CC1G_00201] have increased expression in *msh5-22* at K-3. These examples suggest that by K-3, *msh5-22* cells have already encountered problems in DNA replication and in response have up-regulated transcription (or down-regulated mRNA degradation) of certain DNA replication and repair genes.

From K through K+9, the number of genes differentially expressed in *msh5-22* is fairly constant, with fewer than 40 higher in *msh5-22* compared with the wild type and roughly 150 lower (FDR 5%; Table 2). At K+12, the number of differentially expressed genes increases to 75 with greater and 773 with lower expression than wild type. At this point, genes from all nine clusters are underexpressed, as opposed to in *rad50-1* in which nearly all the genes with low transcript abundance compared with the wild type are in late clusters (Figure 4). Genes that are expressed at lower-than-wild-type levels in *msh5-22* at K+12 include some presumably involved in cell-cycle progression (*e.g.*, homologs of *S. cerevisiae CDC53* [CC1G_10644] and *SWE1* [CC1G_01961]), DNA replication (*e.g.*, homolog of *S. cerevisiae DBF4* [CC1G_07408]), and DNA repair (*e.g.*, *rad51* [CC1G_10538]).

rad50-1 and *msh5-22* do not prepare for postmeiotic DNA replication

After wild-type meiosis, the daughter nuclei migrate into four separate spores and then undergo mitosis to produce binucleate spores (Lu and Jeng 1975; Kamada *et al.* 1976). The postmeiotic DNA replication that occurs shortly before or after nuclei migrate into the spores cannot take place in arrested *rad50-1* and *msh5-22* cells, and, as expected, this is reflected in gene expression differences (Table S1 and Table S2).

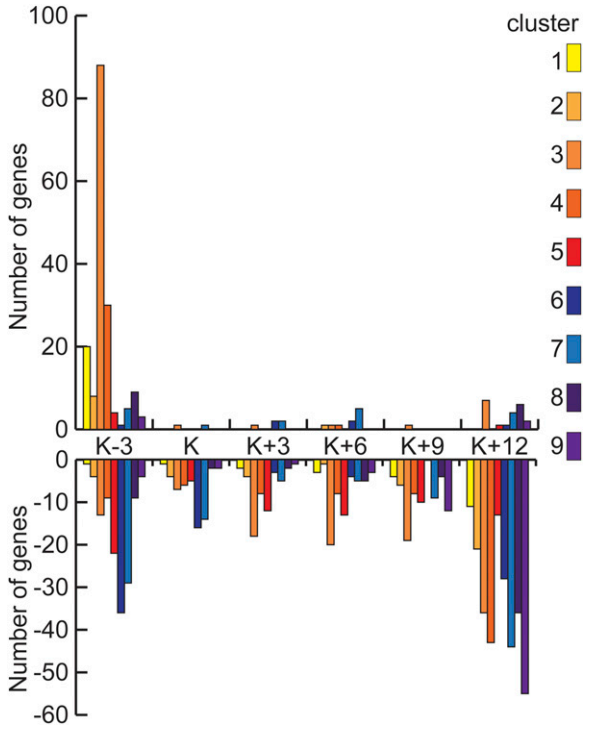

Figure 4 Number of differentially expressed genes in *msh5-22* by cluster. At each time point, the number of genes in each cluster whose transcripts in *msh5-22* are significantly more and less abundant than in the wild type is shown. Warm-colored bars represent clusters 1–5, which contain genes whose expression is greatest at the beginning of meiosis, and cool-colored bars represent clusters 6–9, which contain genes whose expression is greatest at the end of meiosis.

The transcript of the prereplicative complex component protein Cdc6 [CC1G_03323] is less abundant in both mutants at K+9 than in the wild type. Also, *pri1* [CC1G_07347] and *pri2* [CC1G_11942] transcripts (genes encoding subunits of DNA primase) are less abundant at K+9 in *rad50-1*, and *pri2* is differentially expressed over the time course in *msh5-22*. Furthermore, expression of the core histones (H2A [CC1G_03522, CC1G_03575, CC1G_07640], H2B [CC1G_03523, CC1G_07639], H3 [CC1G_04396, CC1G_05766, CC1G_08799], and H4 [CC1G_05458, CC1G_05756, CC1G_05765]) as well as the linker histone (H1 [CC1G_03810, CC1G_03813, CC1G_13972]) is lower than in the wild type at K+9 in both mutants.

There seem to be selective pressures that favor both confining core histone expression to S phase and maintaining histone genes in large clusters (reviewed by Marzluff and Duronio 2002). This results in the coordinate expression of histone genes, ensuring that at the time of DNA synthesis, the correct stoichiometric amount of each core histone is produced for proper nucleosome assembly (reviewed by Marzluff and Duronio 2002). Both of these trends seen in other organisms hold true in *C. cinerea*. For example, there are two pairs of adjacent H2A and H2B genes with the pairs located within 350 kb of each other on chromosome 9 (Broad Institute of Harvard and MIT).

We asked how histone expression in *C. cinerea* meiosis compares with that in yeasts. In *S. cerevisiae*, premeiotic DNA replication occurs at roughly 2 hr after transfer to a sporulation-inducing medium. Histone expression increases sharply at this time compared with 1.5 hr earlier and then decreases over the rest of meiosis [Figure 5B (Chu *et al.* 1998)]. In *S. pombe*, histone expression also peaks at the time of replication [2 hr (Mata *et al.* 2002) Figure 5C]. Similarly, we found

Table 2 Number of differentially expressed genes in *msh5-22*

	K-3	K	K+3	K+6	K+9	K+12
Greater in *rad50-1*	479	9	19	39	2	75
Lower in *rad50-1*	390	135	146	170	152	773

The numbers of genes whose transcripts are more and less abundant in *msh5-22* at FDR 5% than in the wild type at each of six time points are shown. Expression ratios and FDRs for all genes are given in Table S4. FDR, false-discovery rate.

that core histone transcript abundance in wild-type *C. cinerea* is high around karyogamy (the approximate time of premeiotic DNA replication) and subsequently decreases. Unlike in the yeasts (in which DNA is not replicated immediately after meiosis) histone expression peaks again at K+9 (Figure 5A) in preparation for postmeiotic S phase. This does not occur in *rad50-1* and *msh5-22* (Figure 5, D and E), which is consistent with our hypothesis that the cells are not preparing for postmeiotic DNA replication.

Genes that fail to be induced in both mutants are likely involved in sporulation

For each mutant, we also asked which genes have different overall patterns of expression compared with the wild type across the time course. This type of analysis identified genes for which the expression ratio compared with the wild type changes over the time course. In contrast, the time-point analysis generated a list of the differentially expressed genes at each separate time point (Table S1 and Table S2). For example, a gene that was expressed at one half the level of wild type for the entire time course would be found only by the individual time-point analysis, whereas a gene that was initially expressed at a higher level than wild type and later at a lower level would be recognized by the time course analysis. With a cutoff of 5% FDR, there are 115 genes that have changing expression ratios over the time-course in *rad50-1* (Table S1) and 749 in *msh5-22* (Table S2; the *msh5-22* data include an additional time point (K-3), so these numbers are not directly comparable). These genes include some that were not significantly differentially expressed at any of the individual time points (perhaps because the change at each time point was very small), *e.g.*, those encoding APC components Cdc27 [CC1G_03915] in *msh5-22* and Apc1 [CC1G_09696] in *rad50-1*.

We next asked how much overlap there is between the genes that are differentially expressed over the time course in *rad50-1* and those that are differentially expressed in *msh5-22*. Four of the same genes are overexpressed compared with the wild type over the time course in the two mutants, and 53 genes are underexpressed in both mutants (FDR 5%; Figure 6). Of the 57 genes that are differentially expressed over the time course in both mutants, 60% are meiotically induced in the wild type, which is significantly more than the percentage of all genes (35%) that are meiotically induced ($P < 0.0001$, χ^2 test). This supports our prediction that genes that are differentially expressed in both mutants are likely to be involved in meiosis.

We also predicted that genes that are differentially expressed in both mutants at late time points are likely to be involved in sporulation. Twenty-four of the genes that are differentially expressed over the time course in both mutants have changing expression during wild-type meiosis. Of these, 17 are in cluster 9 (which is enriched for genes involved in spore formation) and four more are in other late clusters. For example, the ortholog of the *S. cerevisiae* gene *ARO2* [CC1G_03718] was identified by the time-course analysis as having decreasing transcript abundance with respect to wild type over the time-course in both mutants. In *S. cerevisiae*, this protein participates in aromatic amino acid synthesis and is required for sporulation

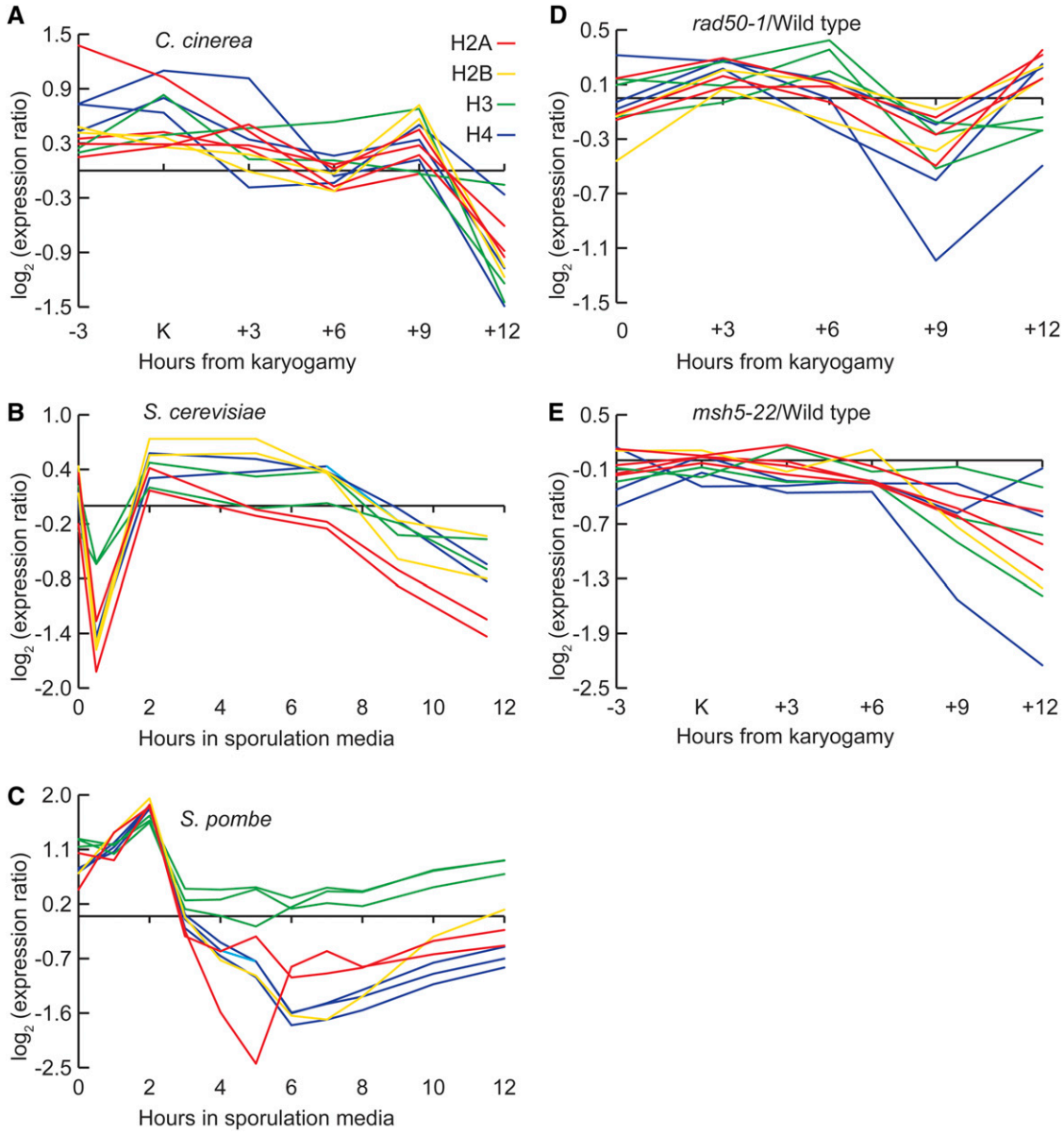

Figure 5 Histone expression during meiosis in *C. cinerea*, *S. cerevisiae*, and *S. pombe*. Histone transcript levels for each core histone gene are shown for (A) *C. cinerea*, (B) *S. cerevisiae* (data from Chu *et al.* 1998), and (C) *S. pombe* (data from Mata *et al.* 2002). Red lines represent H2A, yellow H2B, green H3, and blue H4. There are two to four copies of each histone gene in the *C. cinera* genome, and each line represents the transcript levels of one copy. The ratio of transcript abundance in *rad50-1* (D) or *msh5-22* (E) to the wild type is shown at each time point.

(Lucchini *et al.* 1978). Sporulation requires the biogenesis of the multilayered spore wall (Kues 2000), and the genes that are differentially expressed in both mutants include orthologs of *S. cerevisiae* genes involved in this process. For example, Exg1 [CC1G_06563] is involved in cell wall β-glucan assembly (Vazquez De Aldana *et al.* 1991). Other genes putatively involved in posttranslational processing of extracellular proteins are also differentially expressed: in *S. cerevisiae*, Pdi1 [CC1G_00344] is essential for formation of disulfide bonds in secretory and cell-surface proteins (Freedman 1989) and Pmt4 [CC1G_00834] is involved in O-mannosylation of secretory proteins (Strahl-Bolsinger *et al.* 1993).

At K+12, there are 120 genes whose expression is lower than that in the wild type in both mutants, and one-half of these are in late clusters in the wild type, including 31 in cluster 9. For example, the orthologs of the *S. cerevisiae* genes *ARO1* [CC1G_09809], *ARO2*, and the sporulation-specific chitinase gene *CTS2* [CC1G_04870] (Dunkler *et al.* 2008) are all in cluster 9 in the wild type. Orthologs of other *S. cerevisiae* genes encoding proteins required for cell wall synthesis and assembly, *e.g.*, *GAS1* [CC1G_13682] (Popolo *et al.* 1993) and *ROT2* [CC1G_04516] (Trombetta *et al.* 1996), which are not in any cluster in wild type, are also underexpressed in both mutants. This supports the hypothesis that genes differentially expressed at the end of the time course in both mutants are likely to play roles in sporulation. Also, some of the genes that this analysis indicates are likely to be involved in sporulation were not in wild-type clusters. This means that identifying genes not induced at the end of meiosis in the mutants and identifying genes that are up-regulated during sporulation in wild type are complementary methods of learning which genes are involved in sporulation. Genes in clusters 6 and 7 play roles in gill expansion (which happens normally in the mutants), whereas clusters

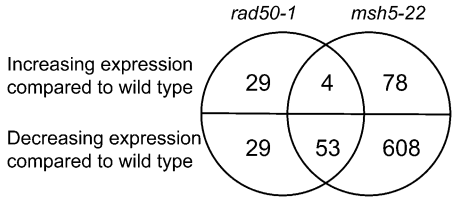

Figure 6 Overlap between genes that are differentially expressed over the time course in *rad50-1* and *msh5-22*. Based on the time course analysis, the numbers of genes whose transcripts change significantly in each mutant with respect to the wild type are shown. Four genes have increasing transcript abundance, and 53 have decreasing transcript abundance compared with the wild type in the two mutants.

8 and 9 are enriched for genes involved in spore structure and packaging and preparation for spore germination (Burns *et al.* 2010). Spores are not made in the mutants, so it is not surprising that roughly one-third of the genes that are differentially expressed in both mutants are in cluster 9.

DISCUSSION

Identification of new meiotic and sporulation genes

Nearly 3000 genes are differentially expressed during the synchronous meiosis and mushroom development of *C. cinerea* (Burns *et al.* 2010). To determine which of these are involved in meiosis itself, we identified genes whose expression is significantly different from the wild type in two mutants that arrest in meiosis but otherwise produce morphologically normal mushrooms. In both cases, the number of differentially expressed genes increases dramatically beginning at the time point when the mutant arrests. The lists of differentially expressed genes include some whose *S. cerevisiae* orthologs are known to encode proteins with roles in cell cycle progression, S phase, and sporulation. This supports our prediction that genes differentially expressed in the mutants are indeed likely to be involved in meiotic processes. In addition to identifying genes involved in wild-type meiosis, this study contributes to an understanding of what happens when errors arise in meiosis and how changes in transcription mediate and result from checkpoint arrests. Checkpoint activation leads to transcriptional changes and these changes then effect cell cycle arrest and the observed morphological changes in the mutants, *e.g.* lack of spore formation.

rad50-1 and *msh5-22* show checkpoint arrest

Several different checkpoint pathways can be activated in response to errors in meiosis. Each checkpoint consists of a signal, which is detected by signal sensors that activate signal transduction pathways, which in turn modify targets to produce an output. Various errors in DSB processing create signals (*e.g.*, Rad51-coated single-stranded DNA), and through checkpoint pathways, these signals lead to the modification of targets with the result of controlling cell-cycle progression, DNA repair, programmed cell death, and in some cases development (reviewed by Hochwagen and Amon 2006). Mutations in *rad50* and *msh5* trigger checkpoints in other organisms (De Vries *et al.* 1999; Usui *et al.* 2001; Borner *et al.* 2004), *rad50-1* and *msh5-22* each exhibit errors in DSB processing in *C. cinerea* [(Acharya *et al.* 2008) F. Kennedy, O. Savytskyy, M. Zolan, unpublished data], and our microscopy observations indicate that meiosis stalls before the first division in both mutants (Figure 1). Moreover, our expression data show that in *rad50-1* cells, a group of early meiotic genes continue to have high transcript levels through K+12, whereas conversely the expression of dozens of genes that are expressed at the end of meiosis in wild type is never triggered. Thus, we believe that meiotic checkpoint pathways are being activated in the two mutants studied. We have shown that the arrest also prevents the induction of some genes involved in spore formation and postmeiotic DNA replication, and in *rad50-1* prevents the down-regulation of many prophase genes. Our results highlight the overlap between presumed checkpoint targets in these two mutants, which have different arrest phenotypes and presumably different checkpoint signals.

rad50-1 gene expression reflects mutant phenotypes

Few genes are differentially expressed in *rad50-1* compared with the wild type at the beginning of the time course (Table 1) meaning that the loss of Rad50 function has little effect on early expression of meiotic genes. This finding is consistent with the observation that *rad50-1* chromosomes condense, pair, and synapse with the same timing as wild type, though at much lower levels [Figure 1 (Acharya *et al.* 2008)]. Kugou *et al.* (2007) explored the effect of MRN-complex mutations on meiotic gene expression in *S. cerevisiae*. They compared gene expression at the time of transfer to sporulating media to expression 4 hr later in both wild-type and an *mre11*-null mutant. At this time point [which corresponds to K+3 in *C. cinerea* (Burns *et al.* 2010)], many key meiotic genes were similarly regulated in mutant and wild-type cells, but the expression of a group of genes enriched for spore wall biogenesis functions depended on Mre11. At the same time in *S. cerevisiae rad50Δ*, the overall induction of meiotic genes was largely unaffected (Kugou *et al.* 2007). This evidence suggests that MRN mutations may affect meiotic gene expression in *S. cerevisiae*, but because the study examined only a single early time point, it does not give a complete picture of the expression changes.

Checkpoint activation in *msh5-22*

Unlike *rad50-1*, all *msh5-22* cells progress to metaphase, arrest, and then at roughly K+10 start to die [Figure 1 (Lu *et al.* 2003)]. In *msh5-22*, DNA is not replicated before meiosis, raising the possibility that a DSB checkpoint similar to the mechanism in *S. cerevisiae* and *S. pombe* is triggered. In the yeasts, this checkpoint ensures that DSBs are not formed in a region until the replication fork has passed that location (Borde *et al.* 2000; Tonami *et al.* 2005). This mechanism is not activated unless DNA replication has been initiated (Murakami and Nurse 2001), raising the alternative possibility that a DSB checkpoint exists in *C. cinerea* but is bypassed in *msh5-22* if DNA replication is never initiated. The number of Rad51 foci (which mark resected DSBs) in *msh5-22* is the same as the number of foci in a mutant (*spo11-1*) that fails to make DSBs, implying that meiotic DSBs are not induced in *msh5-22* (F. Kennedy, O. Savytskyy, M. Zolan, unpublished data). The apparently extremely low level of DSBs is consistent with the hypothesis that a checkpoint preventing their formation is triggered in this mutant.

In *rad50-1*, there is a large group of early genes whose transcript abundance remains high at late time points; however, this pattern is not evident in *msh5-22*, likely because *msh5-22* cells do not arrest until metaphase I, which occurs just before K+9. By K+12, the end of the time course, many *msh5-22* cells have already died, which explains why genes from all clusters have low transcript abundance at this point. In wild-type cells at metaphase I, tension is established though crossovers between homologous chromosomes, cohesion between sister chromatids, and amphitelic attachment of homologs. *msh5-22* chromosomes have very few crossovers and lack sister chromatids (F. Kennedy, O. Savytskyy, M. Zolan, unpublished data). The absence of tension resulting from these defects could trigger the spindle assembly checkpoint, leading to the observed metaphase arrest.

Similarities between *rad50-1* and *msh5-22*

In *C. cinerea*, genes that are coregulated with meiotic genes are expected to have meiotic roles, but genes involved in other processes, such as mushroom development, also are likely to be induced during meiosis. Because mushroom development proceeds normally in both *rad50-1* and *msh5-22*, genes that are not differentially expressed in the mutants compared with the wild type are not likely to be involved in meiosis or spore formation. The 206 genes that are in early clusters in wild type (Burns *et al.* 2010) and also have high expression at K+12 in *rad50-1* are those most likely to play interesting roles in early meiotic processes. Although genes in early clusters that are differentially expressed in the mutants at the end of meiosis tend to have elevated expression compared with the wild type, there is a group of 83 genes in early clusters that are underexpressed at K+9 in *rad50-1*, including the histones. Histones have high expression early in meiosis (Burns *et al.* 2010) but their synthesis is also necessary for post-meiotic DNA replication, suggesting that other genes in this category could similarly play both early and late roles. The 120 genes that are underexpressed in both *rad50-1* and *msh5-22* at K+12 are the strongest candidates for involvement in sporulation.

Recently, Nakazawa *et al.* (2011) created a *C. cinerea ku70* mutant and showed that in the absence of this non-homologous end joining protein, an exogenous copy of a gene introduced by transformation disrupts the genomic copy at a high frequency. When this strain is used, it would be feasible to knock out the candidate genes identified in our study. Alternatively, RNAi techniques are available to knock down gene expression in *C. cinerea* (Namekawa *et al.* 2005; Walti *et al.* 2006). Therefore, it would be straightforward knock out or knock down gene function to test the roles of candidate genes in the processes of meiosis and sporulation.

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LITERATURE CITED

Acharya, S. N., A. M. Many, A. P. Schroeder, F. M. Kennedy, O. P. Savytskyy *et al.*, 2008 *Coprinus cinereus rad50* mutants reveal an essential structural role for Rad50 in axial element and synaptonemal complex formation, homolog pairing and meiotic recombination. Genetics 180: 1889–1907.

Borde, V., A. S. Goldman, and M. Lichten, 2000 Direct coupling between meiotic DNA replication and recombination initiation. Science 290: 806–809.

Borner, G. V., N. Kleckner, and N. Hunter, 2004 Crossover/noncrossover differentiation, synaptonemal complex formation, and regulatory surveillance at the leptotene/zygotene transition of meiosis. Cell 117: 29–45.

Bousset, K., and J. F. Diffley, 1998 The Cdc7 protein kinase is required for origin firing during S phase. Genes Dev. 12: 480–490.

Broad Institute of Harvard and MIT. *Coprinopsis cinerea* Sequencing Project. Available at: http://www.broadinstitute.org/.

Burns, C., J. E. Stajich, A. Rechtsteiner, L. Casselton, S. E. Hanlon *et al.*, 2010 Analysis of the basidiomycete *Coprinopsis cinerea* reveals conservation of the core meiotic expression program over half a billion years of evolution. PLoS Genet. 6: pii: e1001135.

Chu, S., J. Derisi, M. Eisen, J. Mulholland, D. Botstein *et al.*, 1998 The transcriptional program of sporulation in budding yeast. Science 282: 699–705.

De Vries, S., E. Baart, M. Dekker, A. Siezen, D. De Rooij *et al.*, 1999 Mouse MutS-like protein Msh5 is required for proper chromosome synapsis in male and female meiosis. Genes Dev. 13: 523–531.

Dunkler, A., S. Jorde, and J. Wendland, 2008 An *Ashbya gossypii cts2* mutant deficient in a sporulation-specific chitinase can be complemented by *Candida albicans CHT4*. Microbiol. Res. 163: 701–710.

Elledge, S. J., Z. Zhou, J. B. Allen, and T. A. Navas, 1993 DNA damage and cell cycle regulation of ribonucleotide reductase. Bioessays 15: 333–339.

Foiani, M., C. Santocanale, P. Plevani, and G. Lucchini, 1989 A single essential gene, *PRI2*, encodes the large subunit of DNA primase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. 9: 3081–3087.

Freedman, R. B., 1989 Protein disulfide isomerase: multiple roles in the modification of nascent secretory proteins. Cell 57: 1069–1072.

Hochwagen, A., and A. Amon, 2006 Checking your breaks: Surveillance mechanisms of meiotic recombination. Curr. Biol. 16: R217–R228.

Hopfner, K. P., L. Craig, G. Moncalian, R. A. Zinkel, T. Usui *et al.*, 2002 The Rad50 zinc-hook is a structure joining Mre11 complexes in DNA recombination and repair. Nature 418: 562–566.

Huang, M., Z. Zhou, and S. J. Elledge, 1998 The DNA replication and damage checkpoint pathways induce transcription by inhibition of the Crt1 repressor. Cell 94: 595–605.

Kamada, T., S. Miyazaki, and T. Takemaru, 1976 Quantitative changes of DNA, RNA and protein during basidiocarp maturation in *Coprinus macrorhizus*. Trans. Mycol. Soc. Jpn. 17: 451–460.

Kanda, T., H. Arakawa, Y. Yasuda, and T. Takemaru, 1990 Basidiospore formation in a mutant of incompatibility factors and in mutants that arrest at meta-anaphase I in *Coprinus cinereus*. Exp. Mycol. 14: 218–226.

Kues, U., 2000 Life history and developmental processes in the basidiomycete *Coprinus cinereus*. Microbiol. Mol. Biol. Rev. 64: 316–353.

Kugou, K., H. Sasanuma, K. Matsumoto, K. Shirahige, and K. Ohta, 2007 Mre11 mediates gene regulation in yeast spore development. Genes Genet. Syst. 82: 21–33.

Longhese, M. P., P. Plevani, and G. Lucchini, 1994 Replication factor A is required *in vivo* for DNA replication, repair, and recombination. Mol. Cell. Biol. 14: 7884–7890.

Lu, B. C., and D. Y. Jeng, 1975 Meiosis in Coprinus VII. The prekaryogamy S-phase and the postkaryogamy DNA replication in *C. lagopus*. J. Cell Sci. 17: 461–470.

Lu, B. C., N. Gallo, and U. Kues, 2003 White-cap mutants and meiotic apoptosis in the basidiomycete *Coprinus cinereus*. Fungal Genet. Biol. 39: 82–93.

Lucchini, G., A. Biraghi, M. L. Carbone, A. De Scrilli, and G. E. Magni, 1978 Effect of mutation in the aromatic amino acid pathway on sporulation of *Saccharomyces cerevisiae*. J. Bacteriol. 136: 55–62.

Lynn, A., R. Soucek, and G. Borner, 2007 ZMM proteins during meiosis: crossover artists at work. Chromosome Res. 15: 591–605.

Marzluff, W., and R. Duronio, 2002 Histone mRNA expression: multiple levels of cell cycle regulation and important developmental consequences. Curr. Opin. Cell Biol. 14: 692–699.

Mata, J., R. Lyne, G. Burns, and J. Bahler, 2002 The transcriptional program of meiosis and sporulation in fission yeast. Nat. Genet. 32: 143–147.

Murakami, H., and P. Nurse, 2001 Regulation of premeiotic S phase and recombination-related double-strand DNA breaks during meiosis in fission yeast. Nat. Genet. 28: 290–293.

Nakazawa, T., Y. Ando, K. Kitaaki, K. Nakahori, and T. Kamada, 2011 Efficient gene targeting in *ΔCc.ku70* or *ΔCc.lig4* mutants of the agaricomycete *Coprinopsis cinerea*. Fungal Genet. Biol. 48: 939–946.

Namekawa, S. H., K. Iwabata, H. Sugawara, F. N. Hamada, A. Koshiyama *et al.*, 2005 Knockdown of *LIM15/DMC1* in the mushroom *Coprinus cinereus* by double-stranded RNA-mediated gene silencing. Microbiology 151: 3669–3678.

Nicolette, M. L., K. Lee, Z. Guo, M. Rani, J. M. Chow *et al.*, 2010 Mre11-Rad50-Xrs2 and Sae2 promote 5′ strand resection of DNA double-strand breaks. Nat. Struct. Mol. Biol. 17: 1478–1485.

Popolo, L., M. Vai, E. Gatti, S. Porello, P. Bonfante *et al.*, 1993 Physiological analysis of mutants indicates involvement of the *Saccharomyces cerevisiae* GPI-anchored protein gp115 in morphogenesis and cell separation. J. Bacteriol. 175: 1879–1885.

Pramila, T., W. Wu, S. Miles, W. S. Noble, and L. L. Breeden, 2006 The Forkhead transcription factor Hcm1 regulates chromosome segregation genes and fills the S-phase gap in the transcriptional circuitry of the cell cycle. Genes Dev. 20: 2266–2278.

Primig, M., R. Williams, E. Winzeler, G. Tevzadze, A. Conway *et al.*, 2000 The core meiotic transcriptome in budding yeasts. Nat. Genet. 26: 415–423.

Ramesh, M. A., and M. E. Zolan, 1995 Chromosome dynamics in *rad12* mutants of Coprinus cinereus. Chromosoma 104: 189–202.

Redhead, S. A., R. Vilgalys, J. M. Moncalvo, J. Johnson, and J. S. Hopple, 2001 *Coprinus* Pers. and the disposition of *Coprinus* species *sensu lato*. Taxon 50: 203–241.

Schlecht, U., and M. Primig, 2003 Mining meiosis and gametogenesis with DNA microarrays. Reproduction 125: 447–456.

Stajich, J. E., S. K. Wilke, D. Ahren, C. H. Au, B. W. Birren *et al.*, 2010 Insights into evolution of multicellular fungi from the assembled chromosomes of the mushroom *Coprinopsis cinerea* (*Coprinus cinereus*). Proc. Natl. Acad. Sci. USA 107: 11889–11894.

Strahl-Bolsinger, S., T. Immervoll, R. Deutzmann, and W. Tanner, 1993 *PMT1*, the gene for a key enzyme of protein O-glycosylation in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA 90: 8164–8168.

Tonami, Y., H. Murakami, K. Shirahige, and M. Nakanishi, 2005 A checkpoint control linking meiotic S phase and recombination initiation in fission yeast. Proc. Natl. Acad. Sci. USA 102: 5797–5801.

Trombetta, E. S., J. F. Simons, and A. Helenius, 1996 Endoplasmic reticulum glucosidase II is composed of a catalytic subunit, conserved from yeast to mammals, and a tightly bound noncatalytic HDEL-containing subunit. J. Biol. Chem. 271: 27509–27516.

Tye, B. K., 1999 MCM proteins in DNA replication. Annu. Rev. Biochem. 68: 649–686.

Usui, T., H. Ogawa, and J. H. Petrini, 2001 A DNA damage response pathway controlled by Tel1 and the Mre11 complex. Mol. Cell 7: 1255–1266.

Valentine, G., Y. J. Wallace, F. R. Turner, and M. E. Zolan, 1995 Pathway analysis of radiation-sensitive meiotic mutants of *Coprinus cinereus*. Mol. Gen. Genet. 247: 169–179.

Vazquez De Aldana, C. R., J. Correa, P. San Segundo, A. Bueno, A. R. Nebreda *et al.*, 1991 Nucleotide sequence of the exo-1,3-beta-glucanase-encoding gene, *EXG1*, of the yeast *Saccharomyces cerevisiae*. Gene 97: 173–182.

Walti, M. A., C. Villalba, R. M. Buser, A. Grunler, M. Aebi *et al.*, 2006 Targeted gene silencing in the model mushroom *Coprinopsis cinerea* (*Coprinus cinereus*) by expression of homologous hairpin RNAs. Eukaryot. Cell 5: 732–744.

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