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mei-41 is required for precocious anaphase in *Drosophila* females

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Abstract. This paper reports on a new role for *mei-41* in cell cycle control during meiosis. This function is revealed by the requirement of *mei-41* for the precocious anaphase observed in crossover-defective mutants. Normally in *Drosophila* oocytes, tension on the meiotic spindle causes a metaphase I arrest. This tension results because crossovers, and the resulting chiasmata, hold homologs together that are being pulled by kinetochore microtobules toward opposite spindle poles. In the absence of tension, such as in a recombination-defective mutant, metaphase arrest is not observed and meiosis proceeds through the two divisions. Here we show that in some recombination-defective mutants, the precocious anaphase requires the *mei-41* gene product. For example, metaphase arrest is not observed in *mei-218* mutants because of the severe reduction in crossing over. In *mei-41 mei-218* double mutants, however, metaphase arrest was restored. The effect of *mei-41* is dependent on double-strand break formation. Thus, in mutants that fail to initiate meiotic recombination the absence of *mei-41* has no effect.

Introduction

Cell cycle control depends on checkpoint genes acting at specific times to monitor the completion of specific events. A classic example involves the response of the cell to DNA damage. There are specialized protein complexes that sense DNA damage, and then signal to other genes to arrest the cell cycle. This gives the cell time to repair the damage or in some cases allow for apoptosis to occur.

Much of our understanding of the checkpoint control of the cell cycle has come from studies in mitotic cells. In contrast, meiotic cells provide unique challenges to checkpoint controls. During meiotic prophase in *Saccharomyces cerevisiae*, double-strand breaks are created at several sites per chromosome to initiate recombination and crossing over (Lichten and Goldman 1995). A similar process probably occurs in other eukaryotes as well (Dernburg et al. 1998; McKim and Hayashi-Hagihara 1998). These breakage events are also the kind of event that would trigger a checkpoint response. Monitoring the repair of these breaks is done by many of the same signals and proteins that monitor mitotic cell cycle progression. Owing to the unique properties of a meiotic cell, such as the high frequency of breaks and their controlled resolution into either crossovers or gene conversions, there are also novel systems monitoring the cell cycle. Mutations that result in defective repair of meiotic double-strand breaks, such as in the *recA* homologs *RAD51* and *DMC1*, can cause cell cycle arrest during prophase (Bishop et al. 1992; Ghabrial et al. 1998). Monitoring the repair of meiotic double-strand breaks in *S. cerevisiae* involves the *MEC1, RAD17* and *RAD24* gene products (Lydall et al. 1996).

The MEC1 homolog in *Drosophila melanogaster* is encoded by the *mei-41* gene (Hari et al. 1995). There are at least three functions attributable to *mei-41*; it is required for normal levels of meiotic recombination, progression through the embryonic cell cycle, and DNA repair. Mutants carrying weaker fertile alleles have defects in meiotic recombination (Baker and Carpenter 1972), those carrying strong alleles cause maternal-effect embryonic lethality (Sibon et al. 1999), and both kinds of mutants are sensitive to DNA-damaging agents (Boyd et al. 1976; Banga et al. 1986). Probably as a consequence of the DNA repair function of *mei-41*, mutants defective in this gene also have elevated levels of mitotic recombination (Baker et al. 1978). *mei-41* and *MEC1* are members of a gene family that includes the human *ATM* and *ATR* genes. The carboxy-terminal segment of these proteins has a region with homology to the kinase domain of DNA-dependent protein kinase (DNA-PK). Thus it is possible that *mei-41* function is dependent on a kinase activity that is activated by association with double-strand breaks.

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Table 1. Classification of oocyte nuclei in single and double meiotic mutants. (*NS* only the presence of anaphase figures was scored)

Meiotic mutant	Metaphase I	Anaphase or later	Others
mei-9^{a} mei-41^{D18}/mei-9^{a} +	44	17	1
mei-9^{a} mei-41 ^{D18}	78[a]	1	4[b]
mei-41^{D18} mei-218^{1}	26	0	1[c]
mei-41^{D3}; mei-W68^{1} /Df(2R)LL5	2	14	20[c]
mei-41^{D18} ; mei-P22^{1}	NS	6	0
mei-41 ^{D18}; c(3)G^{68}	NS	3	0
grp	16	0	0
mei-9 ^{a}; grp	15	3	0
mei-9 ^{a}; grp/+	4	2	0

[a] In two cases the two masses were connected by a thin thread of chromatin
[b] Three had very long spindles with an overstretched DNA mass; one had three chromosome masses
[c] Disorganized spindle and multiple chromatin masses

We have found a new role for the *mei-41* protein in meiotic cell cycle control. This function is revealed by the requirement of *mei-41* for the precocious anaphase observed in crossover-defective mutants. Normally in *Drosophila* oocytes, meiosis arrests during metaphase I. Previously we have shown that tension on the meiotic spindle is required for metaphase arrest (McKim et al. 1993; Jang et al. 1995). Crossovers, and the resulting chiasmata, hold homologs together, which facilitates bipolar attachment of each kinetochore to spindle microtobules. In a mature spindle, the microtubules attached to each kinetochore exert a poleward force on each chromosome, but owing to bipolar attachment, the chromosomes remain balanced at the metaphase plate. In the absence of tension, such as in a recombination-defective mutant, the metaphase arrest does not occur and meiosis proceeds precociously through the two divisions (McKim et al. 1993). In fact, in a nucleus with a single chiasma, metaphase arrest was observed, showing that the signal produced from a single pair of kinetochores under tension is sufficient to induce arrest. Here we show that in some recombination-defective mutants, precocious anaphase requires the *mei-41* gene product, and that the effect of *mei-41* is dependent on double-strand break formation.

Materials and methods

Confocal microscopy

Stage 14 oocytes were collected from 3–7 day old females and fixed as described previously (Theurkauf and Hawley 1992; McKim et al. 1993). Oocytes were stained for DNA with either fluorescent-conjugated anti-histone antibody (Chemicon), propidium iodide, Yo-Pro, or Oligreen (Molecular Probes), and for spindles with anti-tubulin (clone DM1A, Sigma) conjugated to either fluorescein isothiocyanate or rhodamine.

Results and discussion

Summary of meiotic progression in Drosophila *females and the effect of recombination-defective mutants on meiotic progression*

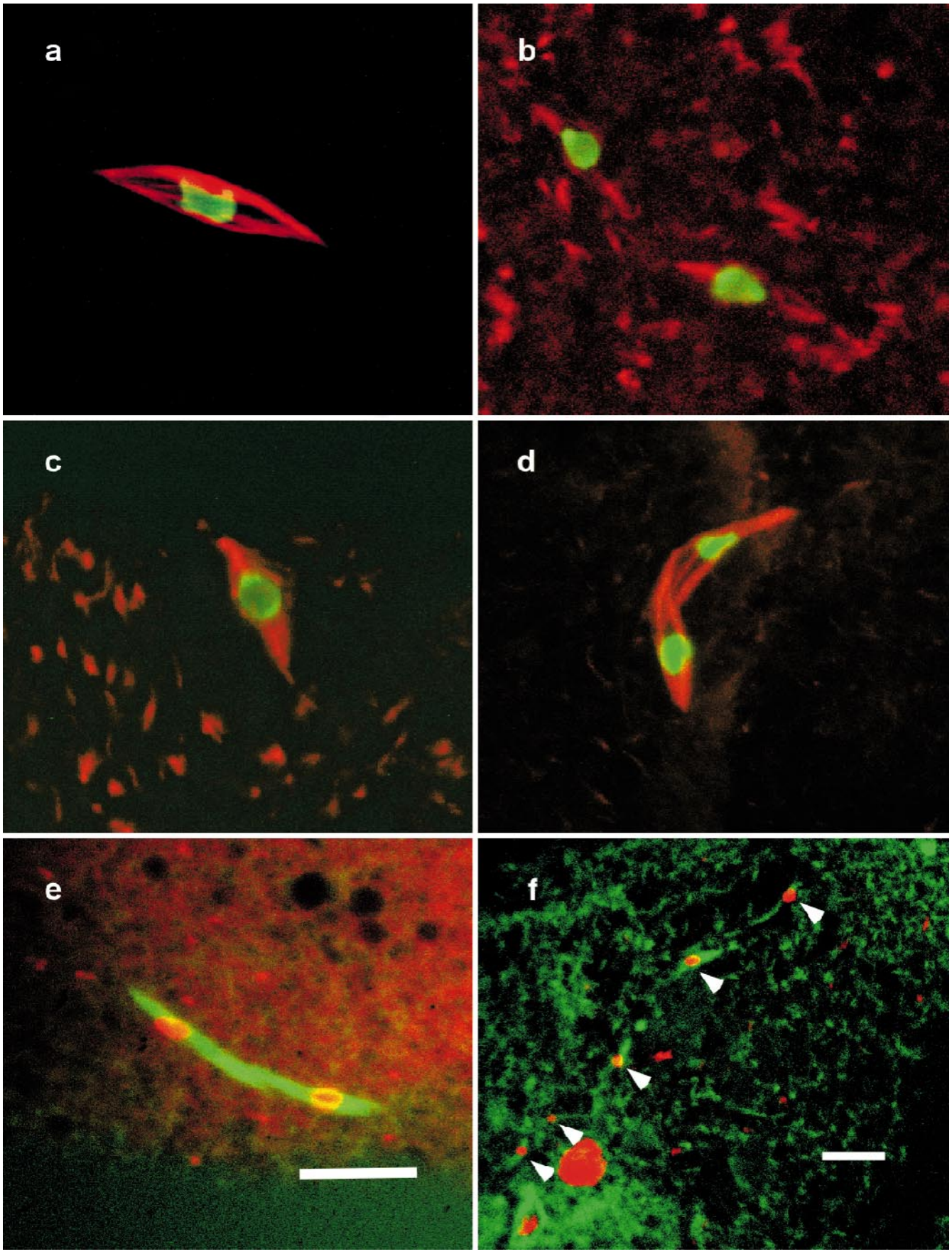
The *Drosophila* ovary is divided into two regions, the germarium, where mitotic divisions occur that produce the oocyte and accompanying nurse cells, and the vitellarium, where oocyte growth and differentiation occur. In the germarium, stem cells produce cystoblasts, which themselves go through four incomplete cell divisions to form a 16-cell cyst. One of the 16 cells becomes the oocyte, and the rest become nurse cells. Meiotic prophase occurs in the germarium and as the cyst enters the vitellarium, meiosis arrests and the chromosomes compact into the karyosome. The nucleus remains in this state until the oocyte is mature and ready for fertilization. At this time, the cell cycle resumes, the nuclear envelope breaks down and metaphase begins. Once again the cell cycle will arrest (Fig. 1a), this time awaiting passage through the oviduct and fertilization (King 1970; Theurkauf and Hawley 1992).

We have previously observed that, in the absence of crossing over, metaphase arrest does not occur, resulting in precocious anaphase (Fig. 1b, McKim et al. 1993). Metaphase arrest is caused by tension on the meiotic spindle, rather than a signal produced by the crossover events themselves (Jang et al. 1995).

The unique effects of mei-41 *on the meiotic cell cycle*

We failed to observe precocious anaphase in *mei-41^{D18}* (Table 1) or *mei-41^{D3}* (data not shown) mutants. This is surprising because *mei-41* mutants are crossover defective. While strong mutants are sterile, and therefore it is not known how severe the recombination defect is, it is reasonable to suppose, extrapolating from the weak alleles, that the defects in the strong alleles are similar to those of *mei-9* or *mei-218*. In double mutants with *mei-41*, and either *mei-9* (Figs. 1c, 2) or *mei-218* (data not shown), only oocytes in metaphase arrest were observed (Table 1). These epistasis results show that the absence of precocious anaphase results from an arrest triggered by the absence of *mei-41*, rather than there being sufficient crossing over to result in at least one chiasma per nucleus.

Carpenter (1984) showed that *mei-9* and *mei-218* mutants have normal levels of gene conversion. These results lead to the conclusion that these mutants are defective only in the production of crossovers, and that the initiation of recombination is normal. In contrast, genet-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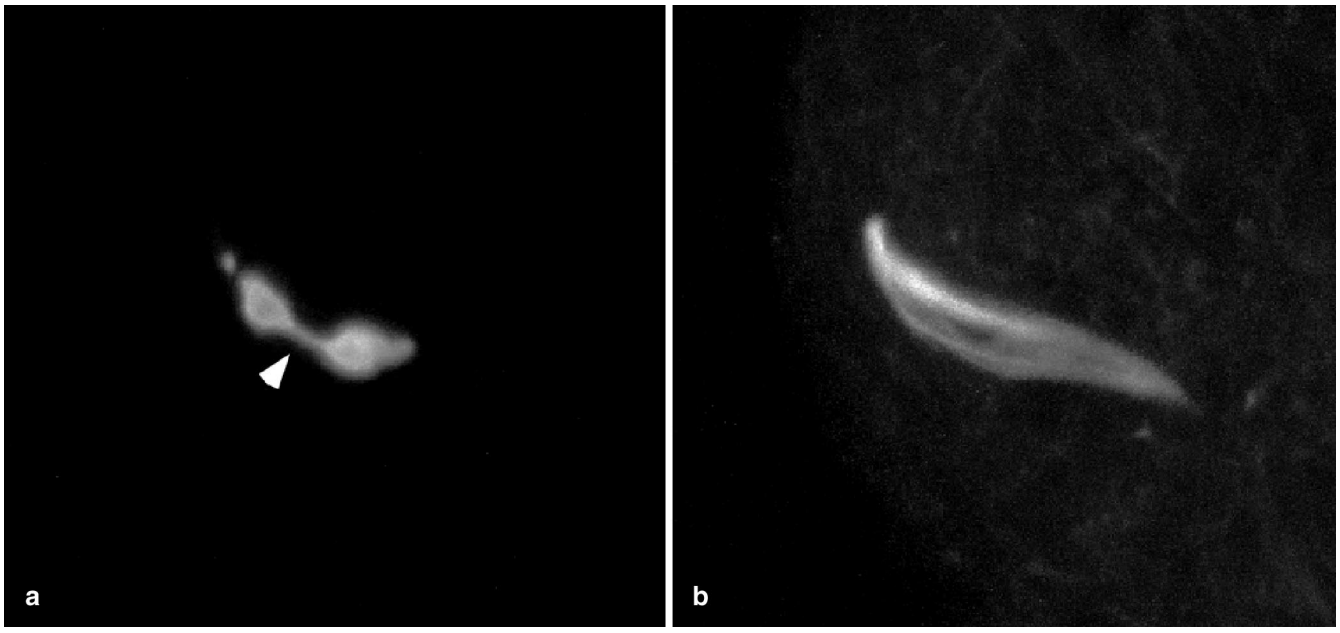

Fig. 2a, b. An example of a rare type of metaphase arrest in a *mei-9 mei-41* homozygote. The chromosome mass (*left panel*) has been stretched but remains connected by chromatin fibers (shown by an *arrowhead*), which is most likely a chiasma

Table 2. Chromosome loss in *mei-41* mutants

Female genotype	Genotype of progeny (oocyte genotype)[a]			
	mei-41/+ (*X*)	*mei-41/Y* (*X*)	*mei-41/mei-41/Y* (*X/X*)	*+/O* (O)
$mei\text{-}41^{D18}/mei\text{-}41^{D18}$	599	174	2 (12)[b]	41
$mei\text{-}41^{D18}\ mei\text{-}218^{1}/mei\text{-}41^{D5}\ mei\text{-}218^{1}$	1013	432	4 (12)[b]	29
$mei\text{-}41^{D18}\ mei\text{-}9^{a}/mei\text{-}41^{D18}\ mei\text{-}9^{a}$	148	74	2 (17)[b]	34
$mei\text{-}41^{D18}/mei\text{-}41^{D18};\ mei\text{-}P22^{P22}/mei\text{-}P22^{P22}$	169	92	12 (19)[b]	35

[a] The number of *X* chromosomes in the oocyte that gave rise to each class of progeny is shown in parenthesis. *X/X* and O are the products of nondisjunction

[b] Owing to the relative inviability of *mei-41* progeny compared with wild type (compare the numbers of the two columns from normal oocytes: these are expected to be equal), in parentheses is the number of progeny expected if there were no viability defect

ic studies have shown that mutations in *c(3)G* (Carlson 1972), *mei-W68* and *mei-P22* (McKim et al. 1998) result in a failure to initiate meiotic recombination. In fact, the *mei-W68* gene encodes a Spo11 homolog (McKim and Hayashi-Hagihara 1998), a strong candidate for the enzyme that makes the meiotic double-strand break in *S. cerevisiae*. Precocious anaphase was observed in *mei-41; mei-W68* and *mei-41; mei-P22* and *mei-41; c(3)G* double mutants (Table 1, Fig. 1d, e). The only difference from single mutant oocytes (that is, carrying a wild-type allele of *mei-41*) was that in some of the *mei-41; mei-W68* double mutant oocytes we observed disorganized spindle and chromosomes (Fig. 1f). These results show that the effect of *mei-41* mutants on cell cycle progression is dependent on the initiation of meiotic recombination. We conclude that in *mei-41* mutants a recombination intermediate triggers a change in one or more proteins that regulate the metaphase to anaphase transition.

The failure to enter anaphase precociously in *mei-41* mutants is only a temporary arrest. Cytological analysis of activated oocytes and embryos from *mei-41* homozygous mothers shows that the oocytes eventually enter anaphase, probably during passage through the oviduct (data not shown and Sibon et al. 1999). This is also when wild-type oocytes are activated to complete meiosis.

Another unusual aspect of the *mei-41* mutants is a high frequency of chromosome loss. In most recombination-defective mutant females, there is approximately an

Fig. 1a–f. Confocal microscopy images of meiotic spindles from mature (stage 14) oocytes. **a** Metaphase arrest in wild type; **b** precocious anaphase in a recombination-defective mutant ($mei\text{-}9^{a}/mei\text{-}9^{a}\ mei\text{-}41^{D18}$); **c** metaphase arrest in a $mei\text{-}9^{a}\ mei\text{-}41^{D18}$ homozygote; **d** precocious anaphase in a *mei-41; c(3)G* homozygote; **e** precocious anaphase in $mei\text{-}41^{D3};\ mei\text{-}W68^{1}/\ Df(2R)LL5$ oocytes. *LL5* is a deficiency for the *mei-W68* locus. **f** A more disorganized precocious anaphase typical of thosefound in some $mei\text{-}41^{D3};\ mei\text{-}W68^{1}/\ Df(2R)LL5$ oocytes. This panel is at a lower magnification than the others, as shown by the *bar*, which represents 10 µm (compare with **e**). The *arrowheads* indicate five chromosome masses on a highly fragmented spindle; the other *red signals* are nonspecific staining. In **a–d**, the chromosomes are stained *green*, and the spindle is stained *red*. In **e** and **f**, the chromosomes are stained *red*, and the spindle is stained *green*

equal number of eggs produced with either two *X* chromosomes (diplo-*X*) or none (nullo-*X*). In weak *mei-41* mutants, however, there is often a higher frequency of nullo-*X* gametes compared with diplo-*X* (Table 2). This effect is not suppressed by the crossover-defective mutants, but is partially suppressed by the recombination-initiation mutants. These results parallel the cytological results and are consistent with the idea that in *mei-41* mutants the presence of double-strand breaks has consequences for progression through meiosis and the stability of chromosomes.

In mitotic cells, one function of *mei-41* is to control the cell cycle via the *chk1* protein (*grapes*) (Sibon et al. 1999). To determine whether the activity of *mei-41* required for precocious anaphase acts through the *chk1* protein, we observed meiosis in *mei-9; grapes* double mutants. The fact that we did observe precocious anaphase in these oocytes (Table 1) shows that the pathway during meiosis is distinct from that in mitotic cells which involves *mei-41* signaling to the *grapes* protein. We suggest that there are least two pathways through which *mei-41* signals during meiosis. As described above, the first is in response to unrepaired double-strand breaks, and may signal through the *vasa* protein to arrest oocyte development. The second, evidence for which is presented here, is a double strand break-dependent control of the metaphase to anaphase transition. This latter function may be related to its role in meiotic recombination, while the former function might represent a more typical DNA repair pathway. In mitotic cells as well, it has been proposed that *ATM* homologs signal through different pathways at different times of the cell cycle (Martinho et al. 1998).

The cell cycle effects of mei-41 *and* spnB *mutants are distinct*

Ghabrial et al. (1998) discovered a class of *Drosophila* meiotic mutant that causes sterility because a temporary arrest during meiotic prophase disrupts subsequent developmental events such as oocyte polarity determination. These genes encode double-strand break repair proteins such as Rad54 (*okra*) and another most like the Rad51 family member XRCC3 (*spnB*). The cell cycle arrest in these mutants is probably due to the presence of unrepaired double-strand breaks. Two lines of evidence suggest that the cell cycle defects in *mei-41* and the *spnB/okra* mutants are different. First, *mei-41* mutants suppress the cell cycle defect in these mutants (Ghabrial and Schupbach 1999). Second, we observed precocious anaphase in *spnB* mutants (Fig. 3). In summary, the effect of *spnB* mutants is to delay cell cycle progression during prophase. In *mei-41* mutants there are two cell

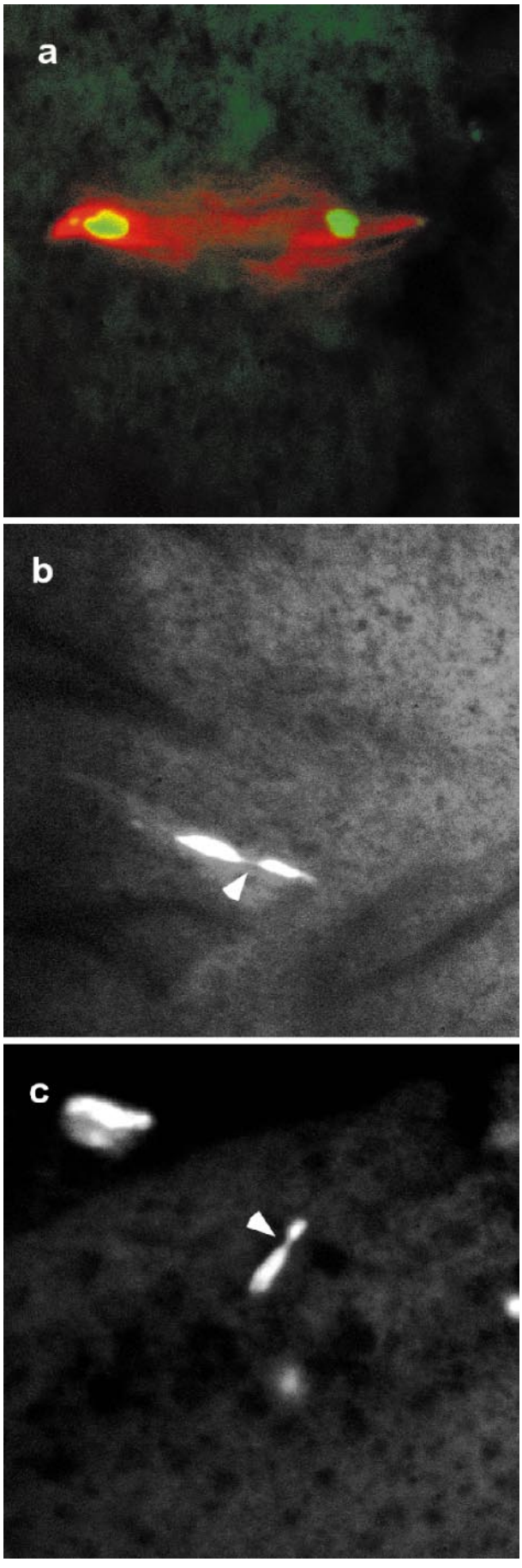

Fig. 3. a Meiotic anaphase I spindle in $spnB^{BU}$ homozygous females. Chromosomes and spindles labeled as in Fig. 1. **b, c** In some oocytes, separating chromatin masses are linked by a thin thread of chromatin (shown by an *arrowhead*). For clarity, only the signal from the DNA channel is shown

cycle events that do not occur: the prophase delay in *spnB* mutants, and the precocious anaphase observed in crossover-defective mutants.

It is not known whether the double-strand breaks that persist in *spnB* mutants are repaired in time for metaphase I. We have observed in some *spnB* oocytes threads of chromatin between the separating masses (Fig. 3b, c), suggesting that there might be types of damage in a *spnB* mutant that are not repaired prior to the completion of meiosis.

A function for mei-41 *early in the recombination pathway has effects on the metaphase to anaphase transition*

mei-41 and its homolog in *S. cerevisiae MEC1* are required for the meiotic-arrest phenotype observed in some meiotic recombination mutants (Lydall et al. 1996; Ghabrial and Schupbach 1999). In both *Drosophila* and *S. cerevisiae* these genes are involved in the repair of double-strand breaks. Our data show a second role for *mei-41* in meiotic progression. As suggested by Sekelsky et al. (1998), the initiation of meiotic recombination may induce a signal that inhibits anaphase. Once the double-strand break is repaired, this signal is turned off, in a process that requires the *mei-41* gene. In order to explain the eventual occurrence of anaphase in *mei-41* mutants, this inhibitory signal must be overridden by the oviduct-activating signal. This model implies that the function of *mei-41* is to monitor repair of the double-strand breaks. An alternative to this checkpoint function, however, is that *mei-41* may have a direct role in the repair of double-strand breaks, perhaps by the activation of other repair genes such as *spnB.* Unlike, *spnB* mutants, a pachytene arrest would not be expected because *mei-41* is required for this event. The persisting damage in a *mei-41* mutant may activate a checkpoint that can inhibit the metaphase to anaphase transition. In this case, the precocious anaphase observed in *spnB* mutants suggests the double-strand breaks have been repaired and the cell cycle has resumed prior to oocyte maturation. This repair function for *mei-41* might also explain the high level of chromosome loss observed in mutants, since broken chromosomes would be lost during the meiotic divisions.

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