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Research Paper

A Germline Clone Screen for Meiotic Mutants in *Drosophila melanogaster*

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ABSTRACT

Using an FLP/FRT-based method to create germline clones, we screened Drosophila chromosome arms *2L* and *3R* for new female meiotic mutants. The screen was designed to recover mutants with severe effects on meiotic exchange and/or segregation. This screen yielded 11 new mutants, including six alleles of previously known meiotic genes (*c(2)M* and *ald/mps1*). The remaining five mutants appear to define at least four new genes whose ablation results in severe meiotic defects. Three of the novel meiotic mutants were identified at the molecular level. Two of these, $mcm5^{A7}$ and $trem^{F9}$, define roles in meiotic recombination, while a third, $cona^{A12}$, is important for synaptonemal complex assembly. Surprisingly, five of the nine mutants for which the lesion has been identified at the molecular level are not the result of mutations characteristic of EMS mutagenesis, but rather due to the insertion of the transposable element *Doc*. This study demonstrates the utility of germline clone-based screens for the discovery of strong meiotic mutants, including mutations in essential genes, and the use of molecular genetic techniques to map the loci.

INTRODUCTION

Genetic screens have been instrumental for the identification of genes involved in meiosis in Drosophila females.[1-6] Screening for mutants that show meiotic defects has led to the discovery of numerous meiotic genes. Many of the meiotic mutants recovered through this type of approach appear to disrupt gene functions that are specific to meiosis (*nod*, *ord*, *mei-218*, *mei-S332*, *mei-S282*, *mei-P22*),[1-4] while others are alleles of genes that also function in cellular or developmental processes in other tissues (*mei-9, mei-41*, *mei-352*, *CycE*, *mei-P26, sub*).[2,4,6] In both cases, homozygous mutants are sufficiently viable and fertile for test crosses to reveal meiotic defects that are detected by analyzing the progeny that they produce. Most meiotic mutant screens used this strategy and thus required viable and fertile homozygous mutant females to screen for meiotic defects. Because of these requirements, the screens could not recover sterile or lethal mutants in genes whose products play essential roles in other tissues or developmental stages in addition to their roles in meiosis.

FLP recombinase-mediated mitotic recombination[7,8] has been used extensively in Drosophila to assess the functions of essential genes in both somatic and germline cells.[9-12] These techniques have also enabled genetic screens to reveal genes with functions that are otherwise masked by lethality earlier in development (reviewed in ref. 13). Screens of germline clones, in particular, have identified genes involved in oogenesis and embryonic pattern formation.[14-17] Fedorova and colleagues[5] performed a screen for meiotic mutants using a germline clone approach in females. However, only one of the recovered mutants was characterized for its effects on female meiosis.[5,18]

As described here, we further refined the process of screening for meiotic mutants using germline clones by both incorporating a Dominant Female Sterile (DFS) mutation into the screen, such that the only progeny that are recovered come from clones homozygous for the mutagenized arm (in our case *2L* or *3R*) and by mutagenizing chromosomes that carry the appropriate DNA polymorphisms to facilitate SNP mapping. The FLP-DFS system combines FLP/FRT with the use of the Dominant Female Sterile mutant ovo^{D1-18} to induce and select for mutant clones.[9,11] Mutant clones produced by FLP-DFS are screened for meiotic defects through crosses to males that carry a compound autosome, in which progeny only arise if a compensating autosomal nondisjunction event occurs in the

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female germline.[8] This cross scheme has the advantages of selecting for strong meiotic mutants that experience a high frequency of nondisjunction and allowing stocks bearing putative meiotic mutants to be established from the cross progeny, which eliminates the need to create and maintain thousands of fly lines carrying mutagenized chromosomes. This efficiency is complemented by the choice of parental chromosomes that have been characterized for DNA polymorphisms such as insertion/deletion markers (InDels) and single nucleotide polymorphisms (SNPs) that facilitate the mapping and identification of the recovered mutants.[19,20]

In screens of chromosome arms *2L* and *3R*, we isolated eleven female meiotic mutants that have been mapped using molecular methods. Five are novel meiotic mutants in at least four genes not previously known to be involved in meiosis, and six are new alleles of two previously studied meiotic genes (two in *c(2)M* and four in *ald/mps1*). Three of the novel meiotic mutants were identified at the molecular level. One of these, $mcm5^{A7}$, defines a role in meiotic recombination for a DNA replication protein Mcm5.[21] The second mutant, $trem^{F9}$, lies in a gene that encodes a previously uncharacterized zinc finger protein, while the third, $cona^{A12}$, identifies a gene whose wild-type product is required for synaptonemal complex assembly. Surprisingly, five of the nine mutants for which the molecular lesion has been identified are associated with *Doc* transposable element insertions. In the case of two *ald/mps1* alleles, the actual mutations were *Doc* insertions in the neighboring gene (*CG18212*) that may be exerting their effects through the cis-inactivation of *ald*.[22]

MATERIALS AND METHODS

Drosophila stocks. Drosophila stocks and crosses were maintained on a standard yeast/cornmeal/corn syrup/malt extract/agar medium at 25°C, unless indicated otherwise. Descriptions of genetic markers and chromosomes can be found at http://www.flybase.org/. *Df(3R)LEP* was constructed by FLP-mediated recombination essentially as described by Parks et al.[23] using FRT sequences in *PBac{RB}e04496* (FBti0041827) and *P{XP}d01968* (FBti0054690), inserted at coordinates 14,191,845 and 14,222,824, respectively, on the chromosome *3R* genome map (Release 5.1).

Alleles, deficiencies, transposon insertions, and aberrations used in this study:

ald^{1} (FBal0000408)
b (b^{1}; FBal0000858)
B (B^{1}; FBal0000817)
$c(2)M^{EP2115}$ (FBal0121689)
$c(3)G^{68}$ (FBal0001459)
car (car^{1}; FBal0001541)
$CG18212^{excision18}$
ci (ci^{1}; FBal0001644)
cu^{1} (FBal0002131)
cv (cv^{1}; FBal0002184)
e^{s} (FBal0003292)
ey^{R} (FBal0003934)
f (f^{1}; FBal0003944)
ncd^{1} (FBal0012910)
pr (pr^{1}; FBal0013947)
rec^{2} (FBal0014515)
Sb^{1} (FBal0015145)
sc (sc^{1}; FBal0015189)
st^{1} (FBal0016127)
Tb^{1} (FBal0016730)
v (v^{1}; FBal0017656)
w^{1118} (FBal0018186)
y (y^{1}; FBal0018607)
y^{d2} (FBal0117252)
y^{+} (FBal0190963)
Df(2L)r10 (FBab0001903)
Df(3R)AN6 (ref. 24)
Df(3R)BSC38 (FBab0036258)
Df(3R)Cha7 (FBab0002512)
Df(3R)DG2 (FBab0002519)
Df(3R)Dl-BX12 (FBab0002534)
Df(3R)Exel6159 (FBab0038214)
Df(3R)Exel6178 (FBab0038233)
Df(3R)Exel7305 (FBab0038299)
$Df(3R)fru^{w24}$ (FBab0026872)
Df(3R)H-B79 (FBab0002586)
P{70FLP}3F (FBti0002716)
$P\{ald^{+}\}$ (Ref. 25)
P{EP}EP381 (FBti0007809)
P{ey-FLP} (*P{ey-FLP.N}2*; FBti0015982)
P{gc(2)M-myc} (ref. 26)
$P\{ovo^{D1\text{-}18}\}2La$ (FBti0002108)
$P\{ovo^{D1\text{-}18}\}2Lb$ (FBti0002109)
$P\{ovo^{D1\text{-}18}\}3R$ (FBti0002111)
$P\{SUPor\text{-}P\}CG18212^{KG08013}$ (FBti0024197)
P{XP}d01968 (FBti0054690)
$P\{y^{+}\}TPN1$ (*P{Car20y}TPN1*; FBti0015795)
PBac{RB}e04496 (FBti0041827)
PBac{WH}f04903 (FBti0052057)
PBac{WH}f05981 (FBti0052677)
FRT40A (*P{neoFRT}40A*; FBti0002071)
FRT82B (*P{neoFRT}82B*; FBti0002074)
$B^{S}Y$ (FBab0029200)
C(3)EN (FBab0000131)
C(2)EN (FBab0000106)
C(4)RM (FBab0000159)
CyO (FBba0000025)
TM3 (FBba0000047)
TM6B (FBba0000057)
TM6C (FBba0000071)
$Y^{S}X \bullet Y^{L}$ (FBab0010396)

Alleles, deficiencies, and transposon insertions generated in this study:

ald^{A15} (FBal0190500)
ald^{B4}
ald^{B8}
ald^{C3}
$c(2)M^{8C}$ (FBal0191312)
$c(2)M^{10C}$ (FBal0191349)
$cona^{A12}$
$didy^{A6}$
$mcm5^{A7}$
$trem^{F9}$
Df(3R)LEP
$Doc\{\}CG18212^{A15}$
$Doc\{\}CG18212^{B8}$

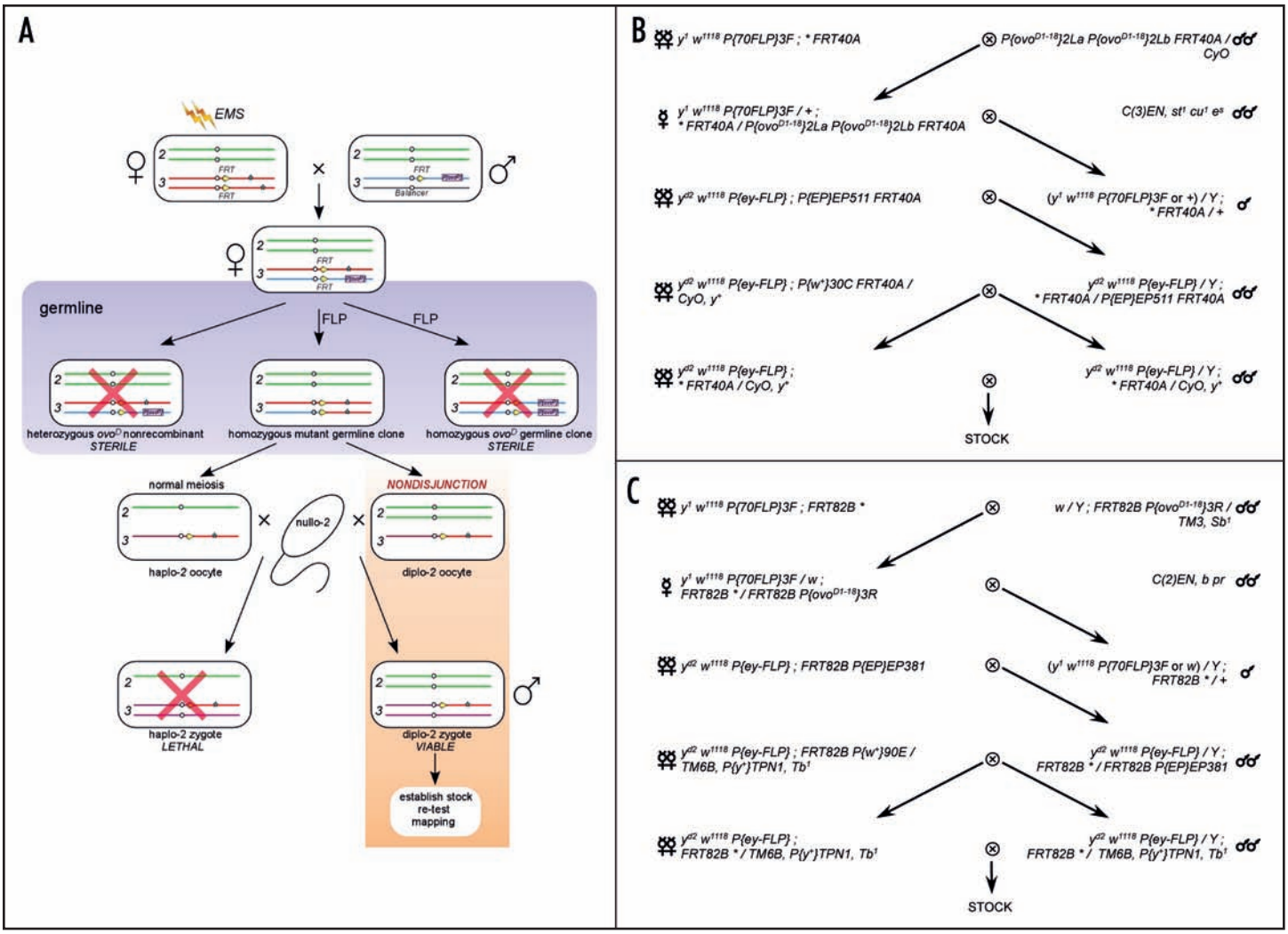

Figure 1. Germline clone screens for female meiotic mutants. (A) Schematic diagram of germline clone screen on chromosome arm *2L* for generating new EMS induced mutations and testing them for meiotic autosomal nondisjunction as germline clones. (B) Summary of crosses for the genetic screen to recover meiotic mutants on chromosome arm *2L*. The mutagenized *FRT40A* chromosome is represented by an asterisk (*). (C) Summary of crosses for the genetic screen to recover meiotic mutants on chromosome arm *3R*. The mutagenized *FRT82B* chromosome is represented by an asterisk (*).

Doc{}c(2)M^{8C}
Doc{}c(2)M^{10C}
Doc{}cona^{A12}

Germline clone screen for meiotic mutants. The same general strategy was used for screening chromosome arms *2L* and *3R* (Fig. 1). For *2L* (Fig. 1B), *y^{1} w^{1118} P{70FLP}3F/y^{1} w^{1118} P{70FLP}3F; FRT40A/FRT40A* females were mutagenized (see below) with ethylmethanesulfonate (EMS) and crossed to *P{ovo^{D1-18}}2La P{ovo^{D1-18}}2Lb FRT40A/CyO* males. Similarly, for *3R* (Fig. 1C), *y^{1} w^{1118} P{70FLP}3F / y^{1} w^{1118} P{70FLP}3F; FRT82B / FRT82B* females were mutagenized and crossed to *w/Y; FRT82B P{ovo^{D1-18}}3R/ TM3, Sb^{1}* males. The progeny of these crosses were heat shocked (see below) to induce FLP recombinase expression and germline clone formation. The resulting *FRT40A */P{ovo^{D1-18}}2La P{ovo^{D1-18}}2Lb FRT40A* and *FRT82B */FRT82B P{ovo^{D1-18}}3R* female progeny, which contain germline clones homozygous for the mutagenized chromosome arm (denoted by an asterisk [*]), were crossed to *+/Y; C(3)EN, st^{1} cu^{1} e^{s}* males when screening *2L*, or *+/Y; C(2)EN, b pr* males when screening *3R*. Males that carry a compound chromosome such as *C(2)EN* or *C(3)EN* produce sperm that are disomic or nullosomic for the corresponding autosome due to the attachment of the two autosome homologs in the compound chromosome.[27-29] If meiosis proceeds normally in the homozygous mutant clones, the resulting progeny will die as monosomic or trisomic embryos. Germline clones that are defective in meiosis and result in autosomal nondisjunction produce progeny that result from the fertilization of a disomic oocyte by a nullosomic sperm. Since they are heterozygous for the mutagenized chromosome, male progeny of this type were used to establish a stock containing the mutant chromosome for further analysis. Although not shown in Figure 1, fertilization of a nullosomic oocyte by a compound-bearing sperm will also result in progeny. However, these progeny were not used for stock establishment because the compound chromosome would prohibit further analysis of the mutant.

After the initial isolation of each mutant, individual stocks were assessed for homozygous lethality or viability of the putative meiotic mutants, which were then tested in a secondary screen to confirm the presence of a meiotic phenotype by analyzing nondisjunction frequency of the *X* and *4th* chromosomes. Those that were homozygous viable were assayed as homozygotes by crossing *y^{d2}*

Table 1 **Segregation in mutants recovered from a germline clone screen assayed as crosses of female homozygotes or homozygous germline clones by $Y^SX \bullet Y^L$, v f B/0; C(4)RM, ci ey^R/0 males**

Gamete Types											
Maternal	**Paternal**	***cona*^{A12}**	***ald*^{A15}**	***ald*^{B4}**	***ald*^{B8}**	***ald*^{C3}**	***mcm5*^{A7b}**	***trem*^{F9}**	***c(2)M*^{8C}**	***c(2)M*^{10C}**	***didy*^{A6}**
X; 4	*XY; 44*	170	83	23	316	78	49	24	33	158	17
X; 4	*0; 44*	178	109	28	309	93	41	26	19	170	38
X ndj											
0; 4	*XY; 44*	48	40	2	21	33	5	13	5	24	4
XX; 4	*0; 44*	45	39	4	6	17	10	10	5	18	3
4 ndj											
X; 0	*XY; 44*	14	13	0	3	12	4	3	nd	nd	1
X; 0	*0; 44*	14	5	2	3	10	1	0	nd	nd	0
X; 44	*XY; 0*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
X; 44	*0; 0*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
X;4 ndj											
0; 0	*XY; 44*	8	6	0	1	2	0	0	nd	nd	0
XX; 44	*0; 0*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
0; 44	*XY; 0*	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
XX; 0	*0; 44*	7	5	1	4	8	3	3	nd	nd	0
Total progeny		484	300	60	663	253	113	79	62	370	63
Adjusted total		592	390	67	695	313	131	105	72	412	70
% *X* nondisjunction		36.49	46.15	20.9	9.21	38.34	27.48	49.52	27.78	20.39	20
% *4* nondisjunction[a]		19.59	20.51	11.94	4.6	26.84	16.79	17.14	nd	nd	2.86
% nullo-*X*		18.92	23.59	5.97	6.33	22.36	7.63	24.76	13.89	11.65	11.43
% diplo-*X*		17.57	22.56	14.93	2.88	15.97	19.85	24.76	13.89	8.74	8.57
% nullo-*4*		9.8	10.26	5.97	2.3	13.42	8.4	8.57	nd	nd	1.43
% diplo-*4*		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Viability of mutation		viable	viable	lethal[c]	viable	lethal[c]	viable	viable	viable	viable	nd
No./Type of females assayed		6/hom	5/hom	6/glc	15/glc	7/glc	5/glc	10/glc	9/glc	7/hom	9/glc

Abbreviations: nd, not determined; hom, assayed in homozygous females; glc, assayed in homozygous female germline clones. [a]Because the females assayed were not homozygous for a recessive 4^{th} chromosome marker spa^{pol}, diplo-*4* progeny could not be scored, thus % *4* nondisjunction was estimated by doubling the frequency of nullo-*4* gametes. [b]Data from ref. 21. [c]Rare escapers observed.

w^{1118} *P{ey-FLP}; FRT82B */FRT82B ** virgin females obtained from the stocks to $Y^SX \bullet Y^L$, *v f B/0; C(4)RM, ci* ey^R males. Progeny from this cross were then scored for meiotic nondisjunction of the *X* and 4^{th} chromosomes (Table 1). For lethal mutants, we again generated females carrying homozygous mutant germline clones. For these mutants, y^{d2} w^{1118} *P{ey-FLP}; FRT82B */TM6B, P{*y^+*}TPN1,* Tb^1 virgin females were crossed to y^1 w^{1118} *P{70FLP}3F/Y; FRT82B* $P\{ovo^{D1\text{-}18}\}3R$*/TM6C,* Sb^1 males. The larval progeny were heat shocked (see below) to induce germline clone formation. Adult y^{d2} w^{1118} *P{ey-FLP}/*y^1 w^{1118} *P{70FLP}3F; FRT82B */FRT82B* $P\{ovo^{D1\text{-}18}\}3R$ female progeny carrying homozygous mutant germline clones were crossed to $Y^SX \bullet Y^L$, *v f B/0; C(4)RM, ci* ey^R males to produce progeny that were scored for meiotic nondisjunction (Table 1). Detailed methods for calculating *X* and 4^{th} chromosome nondisjunction have been published elsewhere.[30-32]

Mutagenesis procedure. Virgin female y^1 w^{1118} *P{70FLP}3F/*y^1 w^{1118} *P{70FLP}3F; FRT40A/FRT40A* or y^1 w^{1118} *P{70FLP}3F/*y^1 w^{1118} *P{70FLP}3F; FRT82B/FRT82B* flies were starved for four hours in bottles lacking medium. Four Whatman #3 circular filter papers were taped to the bottom of empty 8 oz (240 ml) round-bottom polypropylene Drosophila bottles (Applied Scientific) and 4 ml of 25–35 mM EMS (Sigma) in 1% sucrose was added to the bottles. After the EMS/sucrose solution was absorbed by the filter papers, the starved flies were added to the bottles at a density of approximately 60–100 flies per bottle. The flies were then allowed to ingest the EMS/sucrose mixture for 24 hrs. After 24 hrs, the flies were transferred to bottles containing standard fly medium for an additional 24 hrs. The EMS-treated females were then transferred to fresh food bottles just prior to mating with males. Mutagenesis was performed on a weekly basis, and therefore multiple bottles of EMS were used throughout the screening process.

Heat-shock procedure. For the generation of germline clones, five days after crossing adults, the adults were removed and the larvae were heat shocked to induce FLP expression. Heat shocks were applied to larvae in vials or bottles by immersion in a water bath set to 38°C for 1 hr. To ensure that larvae remained exposed to the water bath temperature, the rayon plug of each vial was pushed down to just above the surface of the medium, and the water level was adjusted to above the bottom of the plug. For bottles, the water level was adjusted up to the neck, and the bottle was held in place by a lead ring flask weight.

Molecular polymorphism mapping. Mutants recovered in the *3R* screen were mapped to smaller regions of the chromosome arm by a strategy outlined by Berger et al.[19] using molecular polymorphisms

(InDels and SNPs). For *2L*, a similar approach is available but was not used since both mutants recovered from *2L* were alleles of *c(2)M*.

For each mutant to be mapped, y^{d2} w^{1118} *P{ey-FLP}; FRT82B */TM6B, P{y+}TPN1, Tb*^{1} females were crossed to y^{d2} w^{1118} *P{ey-FLP}; P{EP}EP381* males to produce y^{d2} w^{1118} *P{ey-FLP}; FRT82B */P{EP}EP381* females. These females were crossed to y^{d2} w^{1118} *P{ey-FLP}; FRT82B* males to generate recombinant progeny. Male progeny carrying recombinants between the *FRT82B* * chromosome and *P{EP}EP381* were detected by pigmented eye clones resulting from FLP expression in the eye and the *mini-w*$^{+}$ gene carried by *P{EP}EP381*. Males carrying recombinant chromosomes were collected and separately crossed to establish stocks.

Lysates made from single *FRT82B P{EP}EP381 recombinant/ TM6B, P{y+}TPN1, Tb*^{1} flies[33] were subjected to PCR to genotype InDel markers using an amplification fragment length assay (Supplementary Material Table S1).[19-20] Further information on the molecular polymorphisms can be found at The FlySNP Project website (http://flysnp.imp.ac.at/index.php). For each marker, the reactions contained three oligonucleotide primers: one primer contained a T7 sequence at the 5' end and sequence specific to one side of the InDel at the 3' end, the second primer corresponded to sequence flanking the other side of the InDel, and the third primer was a T7 primer that was 5'-labeled with the fluorophore 6-FAM (Supplementary Material Table S1). After the reaction, PCR product sizes were detected using a 3730 DNA Analyzer and GeneMapper software (Applied Biosystems, Foster City, CA). Control reactions established the allele sizes on the parental *FRT82B* and *P{EP}EP381* chromosomes, as well as *TM6B, P{y+}TPN1, Tb*^{1} for each InDel.

Recombinant lines were genotyped for SNPs by sequencing. Lysate DNA was amplified by PCR using primers flanking the SNPs (Supplementary Material Table S2). The PCR products were then purified and sequenced from both ends using the amplification primers. The sequence data was analyzed using Mutation Surveyor software (Softgenetics, State College, PA) to determine SNP genotypes (Supplementary Material Table S3).

Selected recombinants were tested for presence of the mutation by a genetic assay for *X* chromosome nondisjunction. Recombinants that were homozygous viable were tested by crossing back to the original mutant stock to generate *recombinant / FRT* * females. Recombinants that were lethal were tested by crossing to y^{1} w^{1118} *P{70FLP}3F/Y; FRT82B P{ovo*$^{D1\text{-}18}$*}3R/TM3,* Sb^{1} males and generating germline clones. Comparison of genotypes with phenotype data allowed the meiotic mutant to be mapped.

In parallel with InDel/SNP mapping, several of the mutants were mapped by testing for complementation of the meiotic nondisjunction phenotype using deficiencies obtained from the Bloomington Drosophila Stock Center (http://flystocks.bio.indiana.edu/).

Immunolocalization. Immunofluorescence on ovarioles was performed essentially as described previously.[34] The primary antibody used was mouse monoclonal anti-C(3)G 1A8 (1:500).[35] The secondary antibody was Alexa 568 anti-mouse IgG (1:500) (Invitrogen, Carlsbad, CA). Microscopy was conducted using a DeltaVision microscopy system (Applied Precision, Issaquah, WA) equipped with an Olympus IX70 inverted microscope and CoolSnap CCD camera. Z-stacks of wide-field fluorescence images were collected at 0.2 μm intervals. Image stacks were deconvolved using the SoftWoRx v.2.5 software (Applied Precision).

RESULTS

A germline clone screen for meiotic mutations. We have used a strategy based on germline clones to screen for mutants in genes that function in female meiosis. This approach has the potential to identify genes that also have an essential role early in development and cause lethality when mutated. In the design of the screen, we generated females carrying homozygous mutant germline clones via the FLP-DFS technique.[9-11] As shown in Figure 1, only eggs that carry zero or two copies of a given autosome will survive when fertilized by sperm produced by compound autosome-bearing males. The ability of a given clone to produce a substantial number of such progeny is indicative of high levels of nondisjunction, as might be expected if that clone were homozygous for a strong meiotic mutant. In this scheme females carrying a second chromosome bearing an FRT site on *2L* were crossed to males bearing a compound *3* chromosome and females carrying a third chromosome bearing an FRT site on *3R* were crossed to males bearing a compound *2* chromosome.

Thus, fertile male progeny that did not carry the compound chromosome, but which were heterozygous for the mutagenized chromosome, were used to establish stocks, each of which were retested in search of a novel meiotic mutant. The screen was initially conducted on autosomal chromosome arms *2L* and *3R*, which together comprise approximately 42% of the Drosophila genome. In the *2L* screen, 20,817 chromosome arms were screened and 25,571 chromosome arms were screened for *3R*. We established 36 independent stocks of putative meiotic mutants on *2L* and 35 independent stocks of putative *3R* meiotic mutants.

The putative meiotic mutants were assessed for homozygous lethality or viability prior to testing in a secondary screen for meiotic nondisjunction to confirm the presence of a meiotic mutant. The majority of the putative mutants (59/71) showed low levels (less than 2%) or no *X* nondisjunction. These probably represented progeny from the original cross that arose through spontaneous nondisjunction events and did not carry a meiotic mutant. One of the remaining twelve lines did retest in terms of exhibiting elevated levels of nondisjunction, but repeated attempts to map the lesion causing this defect have been unsuccessful. We think it likely that this meiotic mutant is synthetic, and it was not characterized further. The eleven mutants detailed in this manuscript include two mutants on chromosome arm *2L* and nine mutants on *3R* that displayed elevated levels of *X* nondisjunction, ranging from 9.2 to 49.5% (Table 1). These most likely represent mutations affecting meiosis.

To ascertain whether we had recovered new alleles of previously characterized meiotic genes, we performed complementation tests with known meiotic mutants located on chromosome arms *2L* or *3R*. The mutants on *2L* were tested for complementation with *c(2)M*^{EP2115}, located at polytene band 35F7.[36] Mutants on *3R* were tested for complementation with *c(3)G*^{68} at 89A5,[34,37] *recombination-defective* (*rec*^{2}) at 89A5,[38,39] *altered disjunction* (*ald*^{1}) at 90C1,[25,40] and *non-claret disjunctional* (*ncd*^{1}) at 99C1-2.[41] Both mutants recovered in the *2L* screen failed to complement *c(2)M*^{EP2115} and four of the *3R* mutants failed to complement *ald*^{1}. For each of these six mutants we have characterized the mutation. These results indicated that this screen strategy can indeed recover mutants that affect genes involved in meiosis. In the case of *ald*, we recovered two lethal alleles, *ald*^{C3} and *ald*^{B4}, which would not have been found had the screen required testing viable homozygous mutant females.

Five of the mutants on *3R* fully complemented $c(3)G^{68}$, rec^{2}, ald^{1} and ncd^{1}, suggesting that they may identify novel genes whose products are required for meiosis. We refer to four of these mutants as *corona* (*cona*), *minichromosome maintenance 5* (*mcm5*), *trade embargo* (*trem*), and *disjunction dysfunction* (*didy*). Of these mutants, two ($cona^{A12}$ and $mcm5^{A7}$) were homozygous viable, and two ($trem^{F9}$ and $didy^{A6}$) carried unrelated lethals on *3R*. The one remaining meiotic mutant was phenotypically identical to *didy* (see below), and therefore will remain nameless until it is determined whether these two mutants are allelic. For $cona^{A12}$, $mcm5^{A7}$, and $trem^{F9}$, we have identified both the affected gene and the molecular lesion. Surprisingly, of the nine mutants recovered in the screen for which we have identified the mutation, five were shown to be the result of the insertion of a *Doc* retrotransposon.[42,43] Three of the remaining mutants carried point mutations and one was an intragenic deletion.

Mutants recovered on 3R. Four new alleles of *Ald*. We recovered four mutants (ald^{A15}, ald^{B4}, ald^{B8} and ald^{C3}) that fell into a single complementation group and caused from 9 to 46% *X* chromosome nondisjunction (Table 1). Using the molecular polymorphism mapping strategy (see Materials and Methods), all four mapped to intervals that overlapped between 90C1 and 90E1, which includes the Drosophila *mps1* homolog, *ald.* Each of the mutants failed to complement the meiotic phenotype of the ald^{1} allele and could be rescued by the presence of the $P\{ald^{+}\}$ construct (data not shown), which contains a functional copy of ald^{+}.[25]

Sequencing of ald^{B4} and ald^{C3} revealed a lesion in *ald* for each mutant; ald^{B4} was found to have an early C to T nonsense mutation that changes glutamine at position 48 to a stop codon, while ald^{C3} has a 27 bp deletion that deletes amino acids 369–377 (DLQADPQVV) from the kinase domain of the protein. ald^{C3} and ald^{B4} are lethal as homozygotes but permit rare escapers, a phenotype similar to other severe alleles of ald^{A4} that could also be rescued by the presence of $P\{ald^{+}\}$.

In contrast to ald^{B4} and ald^{C3}, sequencing of ald^{A15} and ald^{B8} showed no nucleotide changes in the *ald* transcription unit compared to the background stock. Further experiments revealed that a *Doc* non-LTR transposable element[42,43] had inserted in the neighboring gene (*CG18212*) downstream of *ald* in each of these two mutants. The insertion in ald^{A15}, $Doc\{\}CG18212^{A15}$, is located 1607 bp from the 3' end of the *ald* transcript and in ald^{B8}, $Doc\{\}CG18212^{B8}$ is located 2042 bp from the 3' end of the *ald* transcript. This is a paradoxical result in that ald^{A15} and ald^{B8} fail to complement ald^{1} and are rescued by the $P\{ald^{+}\}$ transgene, but the only lesions found are located in a neighboring gene (*CG18212*), outside the genomic region included in the $P\{ald^{+}\}$ transgene.[22]

Additional evidence strongly suggests that the phenotypes of ald^{A15} and ald^{B8} result from effects on the function of the *ald* gene rather than *CG18212*. $CG18212^{excision18}$, an imprecise excision of $P\{SUPor\text{-}P\}CG18212^{KG08013}$ that deletes ~90% of the *CG18212* coding sequence, including the insertion sites of both *Doc* elements, complements the meiotic nondisjunction phenotype of these alleles, indicating that loss of *CG18212* is not responsible for the meiotic phenotype. Conversely, a different imprecise excision of $P\{SUPor\text{-}P\}CG18212^{KG08013}$, *Df(3R)AN6,* which deletes *CG18212* and almost the entire *ald* locus, fails to complement ald^{A15} and ald^{B8} (data not shown). Thus, the *Doc* elements inserted in *CG18212* appear to specifically affect *ald* function. The *Doc* elements in ald^{A15} and ald^{B8} may be exerting effects in cis on the expression of the neighboring *ald* gene in the female germline.[22] The ability for transposable elements to exert cis-acting effects on the expression of neighboring genes is well known.[45-49] Moreover, fragments of non-LTR transposons closely related to *Doc* elements can mediate gene silencing in the female germline.[50] A cis-acting effect of the *Doc* elements on *ald* expression in the germline is consistent with the observation that ald^{A15} and ald^{B8} show meiotic defects but do not appear to affect the mitotic function of *ald*.[22]

A meiotic function for *mcm5*. Among the mutants recovered in the screen of *3R* was one that produced 27.48% *X* nondisjunction and 16.79% 4^{th} nondisjunction (Table 1). Molecular polymorphism mapping localized the mutation to the region 85B3-87C7. The mutant was further mapped to the interval 86C6-86C7 by non-complementation with the deficiencies *Df(3R)BSC38* (85F1;86C8), *Df(3R)Exel6159* (86C3;86C7) and *Df(3R)Exel7305* (86C6;86C7). Within the interval of approximately 17 kb defined by these deficiencies is the Drosophila *minichromosome maintenance 5* (*mcm5*) gene. Mcm5 is one of a conserved family of proteins that are required for DNA replication.[51] Sequencing of the *mcm5* gene in the mutant revealed a single missense A→T transversion (A2081T) in the first codon of the last exon, which results in the change of an aspartic acid residue to valine (D694V). To verify that the segregation defects observed were indeed the consequence of this mutation, we tested whether the phenotype could be rescued using a transgenic construct that expresses wild-type Mcm5 from its own genomic promoter or a construct expressing wild-type Mcm5 from a germline-specific promoter. Both of these constructs fully suppressed the meiotic phenotype of $mcm5^{A7}$.[21]

We reasoned that a mutation in *mcm5* could possibly affect meiotic recombination. Assays for meiotic crossing over in $mcm5^{A7}$ homozygotes revealed a 90% decrease in crossover frequency (Table 2), although C(3)G localization appears normal.[21] These results indicate that $mcm5^{A7}$ is defective in the pathway of meiotic recombination. A fuller description of this mutant, in the context of a thorough genetic dissection of the *mcm5* gene has recently been published.[21]

Corona, a novel mutant that disrupts crossing over. We recovered a single allele ($cona^{A12}$) of a novel gene required for proper synaptonemal complex formation that we have named *corona* (*cona*). The $cona^{A12}$ mutant is viable and displayed frequencies of *X* and 4^{th} chromosomal nondisjunction of 36.5% and 19.6%, respectively. The $cona^{A12}$ mutant was shown by molecular polymorphism mapping to lie between InDel marker 3R092 and SNP marker 3R118, a region spanning 89F3 to 91B2. Deficiencies within the genetically mapped interval, *Df(3R)DG2* and *Df(3R)Cha7*, failed to complement $cona^{A12}$, thus further refining the position of cona to the interval 90F1-91B2. This mapping was further refined to 91A1-91A5 by non-complementation of $cona^{A12}$ with *Df(3R)Exel6178* and $Df(3R)fru^{w24}$, and finally to a molecularly defined 31 kb interval by failure to complement a newly constructed deficiency, *Df(3R)LEP*.

One transposon insertion in the 31 kb interval, *PBac{WH}f04903*, failed to complement $cona^{A12}$, and thus represents a second allele, called $cona^{f04903}$. *PBac{WH}f04903* is a *PiggyBac* insertion located in the region flanking the 5' end of the gene *CG7676*, which suggested that a mutation in *CG7676* could be present in $cona^{A12}$. The *CG7676* locus on the $cona^{A12}$ chromosome was sequenced, revealing no base changes within the protein-coding region. However, an insertional mutation was detected in the 3' untranslated region of *CG7676* between the stop codon and the polyA signal. Inverse

Table 2 **Results of crosses of females of the genotype *y sc cv v f · y*$^{+}$/ *y* $^{+}$ $^{+}$ $^{+}$ $^{+}$*; 3/3 carrying the indicated chromosomes 3 by y sc cv v f car/B*^{S}*Y males*[a]

	X Chromosome Interval						
	sc-cv	*cv-v*	*v-f*	*f-y*$^{+}$	Total Map Length (cM)	Frequency of Oocytes that Did Not Undergo *X* Exchange (E_o)	N
wild-type	14.6	20.2	21.8	14.1	70.7	0.07	2505
mcm5^{A7}[b]	1.0	1.3	1.5	2.5	6.9	0.88	875
trem^{F9}	0.7	3.4	1.7	1.4	7.2	0.86	2297
cona^{A12}	0.0	0.0	0.0	0.4	0.4	0.99	821
cona^{f04903}	0.1	0.1	0.1	1.1	1.4	0.97	1174
didy^{A6}	10.4	9.0	26.0	14.0	59.4	0.08	299

[a]Female progeny were scored.[34] [b]Data from ref. 21.

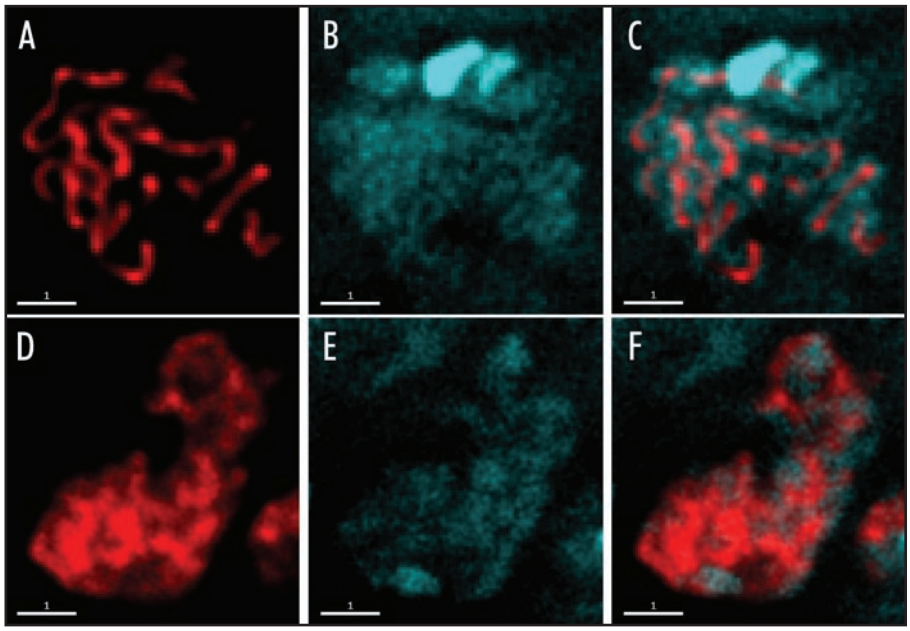

Figure 2. *corona* is required for the proper localization of the synaptonemal complex protein C(3)G. (A–C) C(3)G localizes in a thread-like pattern in wild type. (A) C(3)G (red), (B) DAPI (cyan), (C) merge of (A) and (B). (D–F) C(3)G localization is abnormal in *cona*^{f04903} homozygotes, with an amorphous, plaque-like appearance that is excluded from concentrations of chromatin. An identical phenotype is observed in *cona*^{A12} homozygotes (not shown). (D) C(3)G (red), (E) DAPI (cyan), (F) merge of (D and E). All panels are single deconvolved optical sections. Scale bar, 1 μm.

PCR experiments led to the identification of the insertion as a *Doc* element that was not present on the FRT82B chromosome used in the screen. The presence of the insertion within the *CG7676* 3' untranslated region in *cona*^{A12}, and the failure of *PBac{WH}f04903* to complement *cona*^{A12} strongly implicates *CG7676* as the defective gene resulting in the *cona* meiotic phenotype. The *CG7676*/*cona* gene is comprised of four exons separated by short introns. Based on cDNA sequencing, *cona* encodes a previously uncharacterized 207 amino acid protein. The Cona protein shows no strong homologies to other proteins or protein motifs except for orthologs in other Drosophila species.

To further investigate the meiotic phenotype of *cona*, we analyzed crossing over and SC formation in females homozygous for the original *cona*^{A12} allele or a second allele, *cona*^{f04903}. In wild-type controls, the *X* chromosome genetic map length from *sc* to the centromere was 70.7 cM (n = 2505). In contrast, crossover frequency is reduced 50-200 fold in *cona* homozygotes, to 0.4 cM for *cona*^{A12} (n = 821) and 1.4 cM for *cona*^{f04903} (n = 1174) (Table 2). This severe effect on meiotic recombination could be due to a deficiency of a recombination-specific function or result from a defect in SC formation. To test whether Cona could be important for the formation and/or function of the SC, we investigated whether C(3)G is able to localize normally in the absence of wild-type *cona*. The C(3)G protein is a component of the transverse filaments of the SC[34,35] and forms a thread-like pattern of immunolocalization within pro-oocyte nuclei (Fig. 2A–C). In *cona*^{f04903} or *cona*^{A12} homozygotes, however, this pattern is severely disrupted (Fig. 2D–F). In contrast to the thread-like signals that are associated with concentrations of chromatin in wild-type pro-oocytes, C(3)G is observed in amorphous plaque-like structures that tend to be excluded from concentrations of chromatin within the nucleus, suggesting that the SC is not formed correctly. These data demonstrate that Cona provides a function crucial for proper SC formation and meiotic recombination. Further studies of the function of Cona will be described in a future manuscript.

Trade embargo, a novel recombination defective meiotic mutant. One of the strongest mutants isolated in the screen, which we have named *trade embargo* (*trem*), demonstrated 49.5% *X* nondisjunction upon initial testing in germline clones among a low number of progeny. Molecular polymorphism mapping using InDel and SNP markers localized the *trem*^{F9} mutation to between markers 3R130 and 3R160 (92A2–93E4). Deficiencies in this region were then used to perform complementation tests, which revealed that the overlapping deficiencies *Df(3R)Dl-BX12* (91F1-2;92D3-6) and *Df(3R)H-B79* (92B3; 92F13) uncovered the mutation. This narrowed the interval containing *trem*^{F9} to the region of overlap between these deficiencies, 92B3-92D6. Among a number of insertion mutations that map within this region, *PBac{WH}f05981* failed to complement *trem*^{F9}. *PBac{WH}f05981* is inserted 55 bp upstream of the start codon of *CG4413*, thus implicating *CG4413* as being mutated in *trem*^{F9}. Sequence analysis of *CG4413* in *trem*^{F9} showed a C to T point mutation that was not present on the parental chromosome.

The protein encoded by *CG4413* contains a zinc finger-associated C4DM domain (ZAD) and a C-terminal series of five C2H2 zinc fingers.[52] The *trem*^{F9} mutation changes proline 299 to a leucine residue in the conserved linker sequence between the first and second zinc fingers. Zinc finger linker sequences are well conserved but their exact role in protein function is not fully known.[53] However, mutational analyses have revealed a role for linker sequences in

Table 3 **Nondisjunction phenotype[a] and phenotypic rescue of new alleles of *c(2)M***

Genotype[b]	% *X* Nondisjunction	Adjusted Total
$c(2)M^{8C}/c(2)M^{8C}$ [c]	27.78	72
$c(2)M^{8C}/c(2)M^{EP2115}$	22.02	981
$c(2)M^{8C}/Df(2L)r10$	33.15	1047
$c(2)M^{8C}/c(2)M^{8C}$; *P{gc(2)M-myc}/+* [c]	0.0	520
$c(2)M^{8C}/c(2)M^{10C}$	20.70	860
$c(2)M^{10C}/c(2)M^{10C}$	20.39	412
$c(2)M^{10C}/c(2)M^{EP2115}$	22.15	1616
$c(2)M^{10C}/Df(2L)r10$	32.92	565
$c(2)M^{10C}/c(2)M^{10C}$; *P{gc(2)M-myc}/+*	0.38	2099
$c(2)M^{10C}/c(2)M^{10C}$	20.39	412

[a]Results of crosses of females of the indicated genotypes by $Y_SX \cdot Y_L$, *v f B/0; C(4)RM, ci* $ey^R/0$ males. [b]Full genotype not shown. [c]Nondisjunction assayed by generating germline clones of the genotype shown in females crossed by $Y_SX \cdot Y_L$, *v f B/0 ; C(4)RM, ci* $ey^R/0$ males.

promoting DNA binding in at least some zinc finger proteins.[53-58] Many zinc finger proteins are able to bind DNA and act as transcription factors, although it is intriguing to speculate that Trem could interact with DNA during the process of recombination. We therefore tested the frequency of recombination of $trem^{F9}$ homozygotes. $trem^{F9}$ displays a 90-fold decrease in the total frequency of crossing over (Table 2). This decrease in recombination was not due to the failure to form proper SC, in that C(3)G localization appeared normal in $trem^{F9}$ homozygotes (data not shown). The function of Trem and the affect it has on recombination will be discussed in a future manuscript.

Disjunction dysfunction (*didy*), a novel meiotic mutant that does not alter SC formation or recombination frequency. Germline clones homozygous for the $didy^{A6}$ mutant produce few progeny, but demonstrate a rate of 20% *X* nondisjunction (Table 1). Mapping of $didy^{A6}$ using molecular polymorphisms places it within the interval 94C1 to 99E1. Although the gene has not yet been identified, we have analyzed SC formation and meiotic recombination in $didy^{A6}$ germline clones. We did not detect a defect in SC formation (data not shown) or an altered recombination frequency (Table 2).

Another mutant recovered from the screen was phenotypically similar to $didy^{A6}$. This mutant maps to the same interval (94C1-99E1), displays poor fertility, normal SC and had no affect on recombination (data not shown). However, it displays a lower frequency of *X* nondisjunction than $didy^{A6}$, at approximately 11%. In addition, both chromosomes apparently bear noncomplementing lethal mutations, and therefore, we could not assess the meiotic phenotype in trans-heterozygotes. For these reasons, it remains possible that these two mutants define the same gene.

Mutants recovered on *2L*. New alleles of *c(2)M*. Two meiotic mutants were recovered from the screen of chromosome arm *2L* and both are alleles of *c(2)M*, called $c(2)M^{8C}$ and $c(2)M^{10C}$. As a homozygote, $c(2)M^{10C}$ is viable and displays 20.39% *X* nondisjunction. $c(2)M^{8C}$ carries an unlinked lethal mutation on chromosome *2*. When assayed in germline clones, $c(2)M^{8C}$ shows 27.78% *X* nondisjunction. The $c(2)M^{8C}$ and $c(2)M^{10C}$ alleles fail to complement each other and fail to complement both $c(2)M^{EP2115}$ and a deficiency for the *c(2)M* locus, *Df(2L)r10*. We attempted to determine whether the *c(2)M* gene carried mutations in $c(2)M^{8C}$ and $c(2)M^{10C}$ by amplifying and sequencing *c(2)M*. Each allele contained a segment of the *c(2)M* coding region that repeatedly failed to amplify from template DNA isolated from homozygous or hemizygous individuals. Inverse PCR was then employed to determine whether a DNA rearrangement had occurred. In both alleles, a *Doc* retrotransposon was found to have inserted into the *c(2)M* gene. The *Doc* element in $c(2)M^{8C}$ was inserted in exon 2 in the opposite 5'–3'orientation compared to the gene. In $c(2)M^{10C}$, a *Doc* element was located in exon 4 in the same 5'-3' orientation as the gene. Both insertions were into protein coding sequence and thus would be expected to severely disrupt the production of functional C(2)M protein.

To confirm that the lesions in *c(2)M* were responsible for the meiotic phenotype observed in these alleles, we asked whether the defects in $c(2)M^{8C}$ and $c(2)M^{10C}$ could be rescued by a wild-type copy of the *c(2)M* gene. For these experiments, we utilized *P{gc(2)M-myc}*, a transgene that produces myc epitope-tagged C(2)M under the control of the *c(2)M* promoter.[26] These experiments demonstrated that the presence of *P{gc(2)M-myc}* could ameliorate the nondisjunction phenotypes of both $c(2)M^{8C}$ and $c(2)M^{10C}$ (Table 3).

Mutation of *c(2)M* is known to disrupt the localization of the synaptonemal complex protein C(3)G.[36] C(3)G protein localization in $c(2)M^{10C}$ homozygous ovaries was found to be defective, with only punctate sites of localization along chromosomes in meiotic prophase nuclei (data not shown), which resembles the phenotype of $c(2)M^{EP2115}$.[36]

DISCUSSION

Screening for meiotic mutants in germline clones. This study demonstrates the utility of germline clone screens to isolate novel mutants affecting female meiosis and also the efficacy of molecular mapping methods based on SNP and InDel polymorphisms to quickly identify the genes defined by those mutations. We have taken a clonal approach to identify novel mutants affecting female meiosis because it allows the successful recovery of both lethal and viable mutants. This differs from previous screens, which could only recover mutations in genes that are not essential for viability.

Our approach combines three key features to facilitate the rapid isolation and identification of new meiotic mutants. First, the FLP-DFS technique[9,11] is used for the generation of germline clones homozygous for entire chromosome arms while simultaneously blocking oogenesis in nonrecombinant and twin-spot clones using the dominant female sterile ovo^{D1-18} mutation carried on *P* element insertions.[59] Second, females bearing germline clones are mated with males carrying either *C(2)EN* or *C(3)EN*. The generation of nullo-*2* or nullo-*3* sperm by these males[27-29] allows oocytes in which nondisjunction of the corresponding autosome has occurred during female meiosis to survive. Moreover, crossing these females to compound autosome-bearing males also eliminates normal meiotic products as lethal monosomic or trisomic zygotes. Third, we utilized InDels and SNPs to rapidly map the recovered mutants.[19,20] This genetic mapping approach allows the meiotic phenotype to be assayed, whereas lethals, if also present, would confound mapping based only on deficiencies. Overall, the screen is quite stringent, requiring several conditions to be met for mutant recovery (germline clone formation, nondisjunction, gametic complementation), which results in selection for strong meiotic phenotypes, such as those observed in $cona^{A12}$, $trem^{F9}$ and ald^{C3} (Table 1).

Our design contains several advantages over a previously published FLP/FRT-based screen of chromosome arm *3L* carried out specifically for the discovery of meiotic mutants.[5,18] Similar to our screen, the FLP/FRT system was used to generate clones of gamma-irradiated chromosomes *3L* in females that were crossed to *C(2)EN*-bearing males. However, the screen in this study utilizes molecular polymorphism mapping to rapidly map and identify the gene affected. Also, in the *3L* screen, germline clones were generated in a wild-type background, so nonrecombinant and twin spot clones were not suppressed, whereas the FLP-DFS technique in our screen prevents non-mutant cells from competing with the mutant clones during oogenesis. Finally, by using EMS rather than gamma irradiation to induce mutations, we are more likely to avoid the generation of chromosome rearrangements, which can mimic true meiotic mutations.

***Doc* element-induced mutations.** Generally, EMS mutagenesis is expected to produce point mutations.[13,60] Surprisingly, five of the nine mutations that have been identified at the molecular level were associated with new insertions of *Doc* retrotransposons, one was a small deletion, and only three were point mutations. In two cases ($c(2)M^{8C}$ and $c(2)M^{10C}$), *Doc* elements were inserted into protein coding sequence of the meiotic gene affected, and in a third case ($cona^{A12}$), the insertion occurred in the 3' untranslated region. The remaining two cases (ald^{A15} and ald^{B8}) were *Doc* insertions that affect the *ald* gene despite being located in a neighboring gene (*CG18212*) over 1.5 kb away, possibly through the cis-inactivation of *ald* in the female germline.[22]

Doc elements are approximately 4.7 kb long non-LTR retrotransposons that have been estimated to be present in 20 to 55 copies in the genomes of Drosophila stocks.[42,43,61,62] One possible cause for the recovery of several *Doc* element insertions in the screen is the fortuitous use of stocks in which *Doc* elements were unstable. The transposition of *Doc* elements within Drosophila stocks has been documented[63] and if transposition was occurring within the stocks used in the mutagenesis, the subsequent screen could have selected for new *Doc* insertions associated with a meiotic phenotype. DNA damage induced by EMS exposure could also have promoted transposition by leading to single-strand nicks in chromosomal DNA, which have been suggested to serve as sites of non-LTR retrotransposon insertion.[64]

The limits of the screen. While our finding that only two of eleven new mutants were lethal or semi-lethal does not support the idea that a substantial fraction of meiotic mutants have been missed in previous screens as a consequence of post-zygotic lethality, our results demonstrate that this screen allowed us to identify meiotic mutants that were either lethal on their own (such as ald^{C3} and ald^{B4}), as well as mutants on chromosomes that carried unrelated lethal mutations (for example, $trem^{F9}$). However, we note that a number of mutants with strong meiotic defects, such as *nhk1*, *grauzone* and *cortex* have been identified by cytological analysis of female sterile mutants,[65,66] thus the possibility remains that mutants may have been lost as female steriles. It is important to note that certain types of female sterile mutants would have escaped recovery, including any mutant that defined a function that is also required within the female germline to produce viable eggs. Such mutations might have indicated genes involved in early cystoblast divisions, oocyte differentiation or growth, or that are required for early embryonic survival. To identify mutants that are also essential for the early stages of oogenesis, we suspect that additional methods will likely be required, such as screening for dominant enhancers of known meiotic mutants or screening for conditional (i.e., temperature-sensitive) meiotic mutations.

The mutants we didn't get. Although five of the 11 mutants identified in this study define at least four new meiotic genes, it is curious that we did not recover mutants in other well characterized meiotic genes on chromosome *3R*, such as *c(3)G, rec*, and *ncd*. We do, however, note that a previous *P* element screen for new autosomal meiotic mutants also failed to identify novel mutants in either *c(3)G* or *rec*.[4] Both the absence of new mutants in these genes from either screen and the finding of numerous previously unidentified meiotic loci suggest that the screens are far from saturating and that many new meiotic mutants remain to be identified. It is obviously imperative to extend this study to the remaining sixty percent of the genome (*X*, *2R*, *3L*). This can be done with relative ease because of the availability of chromosomes bearing FRT sites, corresponding dominant female sterile $P\{ovo^{D1\text{-}18}\}$ insertions, and the multitude of SNP and InDel polymorphisms that have now been characterized.[19,20]

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