Note

Multiple Subunits of the *Caenorhabditis elegans* Anaphase-Promoting Complex Are Required for Chromosome Segregation During Meiosis I

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ABSTRACT

Two genes, originally identified in genetic screens for *Caenorhabditis elegans* mutants that arrest in metaphase of meiosis I, prove to encode subunits of the anaphase-promoting complex or cyclosome (APC/C). RNA interference studies reveal that these and other APC/C subunits are essential for the segregation of chromosomal homologs during meiosis I. Further, chromosome segregation during meiosis I requires APC/C functions in addition to the release of sister chromatid cohesion.

IN mitotically dividing cells, loss of sister chromatid cohesion during the metaphase-to-anaphase transition triggers the segregation of sisters to opposite poles (reviewed in NASMYTH 1999). During meiosis I, reductional segregation of chromosomal homologs is achieved by restricting the loss of sister chromatid cohesion to chromatid arms. During meiosis II, equational segregation of sister chromatids occurs as the remaining centromeric cohesions are lost (reviewed in MIYAZAKI and ORR-WEAVER 1994). During mitosis, a multisubunit E3 ubiquitin ligase known as the anaphase-promoting complex or cyclosome (APC/C) facilitates both the loss of sister chromatid cohesion and mitotic exit by targeting several mitotic proteins for destruction (KING *et al.* 1996; ZACHARIAE and NASMYTH 1999). Among these targets is securin, whose destruction frees its normal binding partner, separin, to cleave the cohesion proteins that hold together sister chromatids (UHLMANN *et al.* 1999). Although the role of APC/C in separating sister chromatids during mitosis is thought to be universal, its role in separating chromosomal homologs during meiosis I remains controversial. Recently, genetic analysis of two different APC/C subunits in *Caenorhabditis elegans* revealed a requirement for APC/C in the meiosis I segregation of chromosomal homologs (GOLDEN *et al.* 2000; FURUTA *et al.* 2000). However, conflicting studies in Xenopus oocytes suggested that APC/C may be dispensable during meiosis I and required for the meiosis II segregation of sister chromatids only (PETER *et al.* 2001; TAIEB *et al.* 2001).

In previous studies, we identified 32 temperature-sensitive maternal-effect embryonic lethal mutants in *C. elegans* that arrest as fertilized one-cell embryos, which are blocked at metaphase of the oocyte's first meiotic division (GOLDEN *et al.* 2000). Seven of these mutants were new alleles of *emb-27* and *emb-30*, which proved to encode CDC-16(APC-6) (GOLDEN *et al.* 2000) and APC-4 (FURUTA *et al.* 2000), respectively. These findings not only demonstrated a role for several highly conserved APC/C subunits during meiosis I but also suggested that mutations in other APC/C subunits might be included within this phenotypic class. The remaining 25 mutants fell into three novel complementation groups, termed *mat* (*m*etaphase-to-*a*naphase *t*ransition defective): *mat-1*, *mat-2*, and *mat-3* (GOLDEN *et al.* 2000). Here, we show that *mat-2* and *mat-3* encode additional APC/C subunits and that RNA-mediated interference (RNAi) of most APC/C subunits results in a meiotic metaphase I arrest. In addition, we use *rec-8* RNAi to demonstrate that the loss of sister chromatid cohesion is insufficient to bypass a requirement for APC/C during meiosis I chromosome segregation, a striking result that implies that APC/C has additional, potentially novel, meiosis I targets.

The *mat-2* and *mat-3* genes encode subunits of the anaphase-promoting complex: The *mat-2* mutations mapped to a 0.25 map unit region of LG II (GOLDEN *et al.* 2000; see http://www.wormbase.org/db/gene/mapping_data?name=mat-2). In this region, one locus, W10C6.1, encodes a predicted ortholog of the *Saccharomyces cerevisiae*

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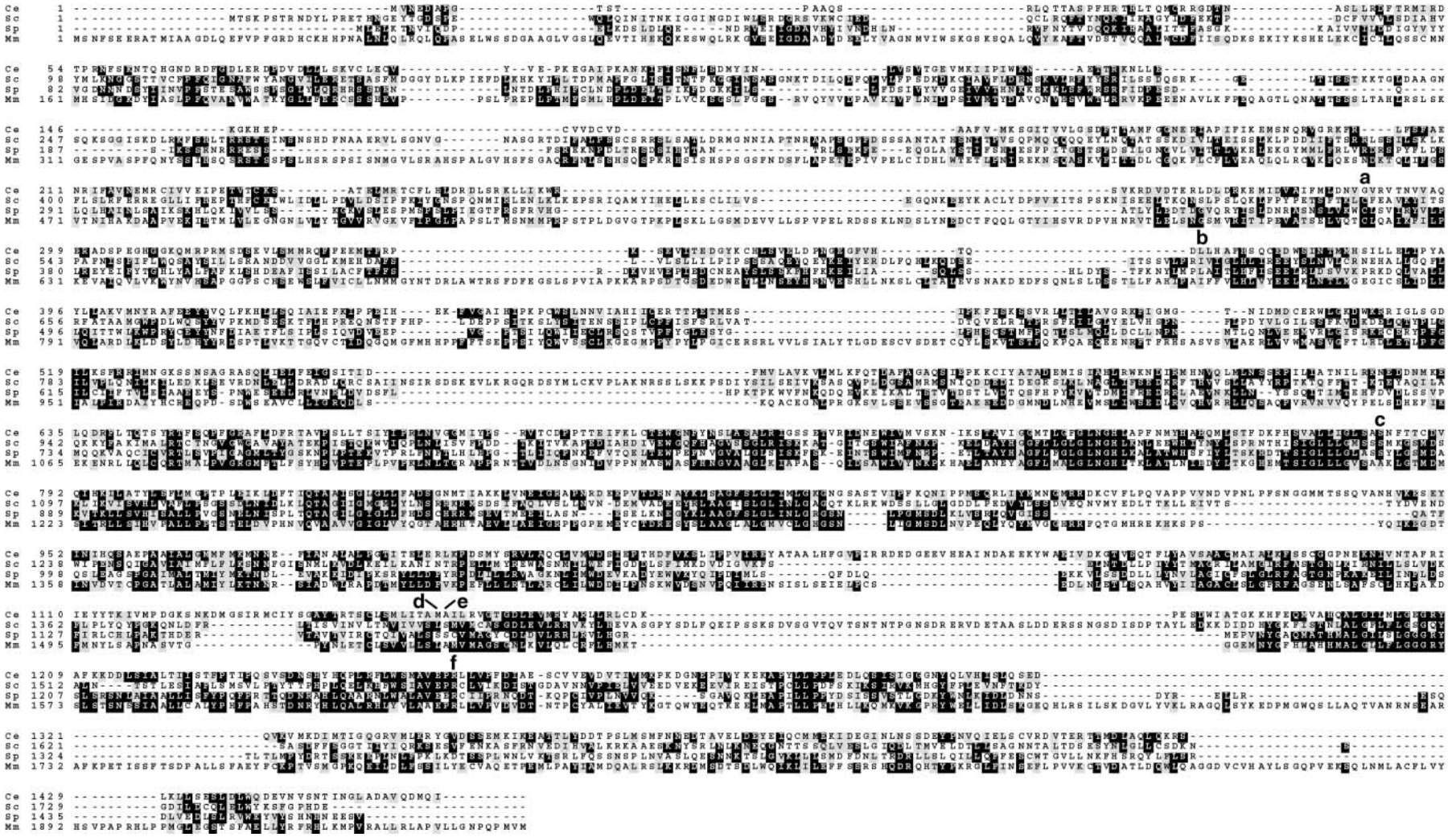


FIGURE 1.—Alignment of APC1 orthologs. Alignment of APC-1 orthologs from *C. elegans* [Ce; accession no. (AC) CAB16467], *S. cerevisiae* (Sc; AC NP_014227), *Schizosaccharomyces pombe* (Sp; AC T39266), and mouse (Mm; AC NM_008569). The *C. elegans apc-1* gene W10C6.1 has 10 exons, predicted by Genefinder and Intronerator (KENT and ZAHLER 2000a,b). The alignment was generated using CLUSTALW (THOMPSON *et al.* 1994) accessed from the BCM Search Launcher (http://searchlauncher.bcm.tmc.edu/). *mat-2/apc-1* allele positions are designated by letters above the *C. elegans* sequence: a, *ax102*; b, *ax143/or224*; c, *ax142*; d, *ax76*; e, *or170*; f, *ax78*. Residues shaded in black are identical; those in gray are similar.

TABLE 1

RNA interference of APC/C genes in *C. elegans*

APC/C component	ORF	Chromosome	Genetic mutant	Exon(s) used in RNAi	F_1 RNAi phenotype
APC-1	W10C6.1	II	*mat-2*[a,b]	exon 4[e]	Mei. 1-cell[b]
APC-2	K06H7.6	III		exons 5, 6[e,f]	Mei. 1-cell[b,h]
CDC-27(APC-3)	Y110A7A.17	I	*mat-1*[c]	exons 5-7[e,g]	Mei. 1-cell[c,i,j]
APC-4	F54C8.3	III	*emb-30*[d]	nd	Mei. 1-cell[d,h]
APC-5	M163.4	X		exons 5-7[e]	Multicellular embryos[b,k]
CDC-16(APC-6)	F10B5.6	II	*emb-27*[a]	exons 5-7[e]	Mei. 1-cell[a]
CDC-23(APC-8)	F10C5.1	III	*mat-3*[a,b]	exons 1-8[e,g]	Mei. 1-cell[b,h]
APC-10	F15H10.3	V		exons 1-5[e]	Multicellular embryos[b]
APC-11	F35G12.9	III		exon 1[e,f]	Mei. 1-cell[b,h]

Each of the genes is represented by at least one expressed sequence tag (EST), and most are represented by many ESTs. Mei. 1-cell, meiotic one-cell embryos arrested at metaphase of the first meiotic division, as described in Golden *et al.* (2000); ORF, open reading frame; multicellular embryos, a mixed population of dead and hatching multicellular embryos.

[a] Golden *et al.* (2000).

[b] This report.

[c] J. Schumacher, M. Abdolurasulnia, D. Shakes and A. Golden, unpublished results.

[d] Furuta *et al.* (2000).

[e] Injection method of RNAi.

[f] Soaking method of RNAi.

[g] Feeding method of RNAi (see Figure 3 legend).

[h] Previously subjected to RNAi and classified as defective in ability to progress through meiotic divisions (Gonczy *et al.* 2000).

[i] Previously subjected to RNAi and classified as early embryonic arrest (Fraser *et al.* 2000).

[j] Previously subjected to RNAi and classified as 1-cell arrest with a terminal phenotype as 1- to 12-cell arrest (Zipperlen *et al.* 2001).

[k] Previously subjected to RNAi and classified as wild type (Maeda *et al.* 2001).

Apc1p protein (Figure 1). While *S. cerevisiae* Apc1p is required for the metaphase-to-anaphase transition (Zachariae *et al.* 1996), and APC-1 orthologs are present in many eukaryotic organisms (Starborg *et al.* 1994; Peters *et al.* 1996; Yamashita *et al.* 1996; Jorgensen *et al.* 2001), the protein lacks any obvious structural motifs that might indicate its biochemical function.

To test whether W10C6.1 was a *mat-2* candidate, its *in vivo* function was analyzed using RNAi. Wild-type hermaphrodites were injected with double-stranded RNA (dsRNA) corresponding to exon 4 of W10C6.1. Under these conditions, dsRNA often depletes the maternal levels of the corresponding mRNA (Fire *et al.* 1998), and mothers receiving a specific dsRNA usually produce embryos with strong loss-of-function phenotypes (Guo and Kemphues 1996). Mothers injected with W10C6.1 dsRNA produced embryos that arrested at the meiotic one-cell stage with a phenotype similar to that of *mat-2* (Table 1).

DNA sequencing of the *apc-1* gene from all seven *mat-2* strains revealed that each contained a single nucleotide substitution that altered the predicted amino acid sequence (Table 2). The *mat-2* mutations were generally scattered throughout the protein sequence. However, the lesions in *ax76* and *or170* were in adjacent residues, and *ax143* and *or224* carried identical mutations. Each change occurred in a residue that was conserved in APC-1 orthologs from at least one other species (Figure 1). Therefore, *mat-2* encodes the *C. elegans* APC-1. We hereafter refer to this gene as *mat-2/apc-1*.

Similarly, *mat-3* mapped to the left arm of LG III (Golden *et al.* 2000; see http://www.wormbase.org/db/gene/mapping_data?name=mat-3), where one locus, F10C5.1, encodes an ortholog of Cdc23p(APC8), an APC/C subunit in *S. cerevisiae* (Figure 2A; Doi and Doi 1990; Sikorski *et al.* 1990; Zachariae *et al.* 1996) and many other eukaryotic organisms (Figure 2A; Yu *et al.* 1998; Zhao *et al.* 1998; Yamashita *et al.* 1999).

As do all other CDC-23 orthologs, *C. elegans* CDC-23 contains nine 34-amino-acid degenerate repeats known as tetratricopeptide repeats (TPRs). TPRs were first described in *S. cerevisiae* Cdc23p (Sikorski *et al.* 1990), but have since been discovered in many proteins with diverse functions (reviewed in Blatch and Lassle 1999). TPRs share little primary sequence identity but are predicted to form amphipathic α-helices (Sikorski *et al.* 1990) that mediate protein-protein interactions (Lamb *et al.* 1994; Blatch and Lassle 1999).

mat-3 proved to encode an ortholog of CDC-23. Specifically, RNAi of F10C5.1 caused mothers to produce meiotic one-cell arrested embryos (Figure 3; Table 1), and DNA sequencing of the *cdc-23* gene from the 12

TABLE 2

List of *mat-2/apc-1* and *mat-3/cdc-23* alleles and corresponding amino acid changes

mat allele	Amino acid change
mat-2/apc-1, class 1 (Mel)	
ax76	M1152K
ax78	R1253K
or170	A1153V
mat-2/apc-1, class 2 (Ste)	
ax102	G289R
ax142	S784F
ax143, *or224*	L367F
mat-3/cdc-23, class 1 (Mel)	
ax148	A531T
or172, *or180*, *or187*	S505F
mat-3/cdc-23, class 2 (Ste)	
ax68	E164K
ax70	A454V
ax71	G535D
ax77	A342L
ax79, *ax136*	E404K
ax82	E240K
or192	E73K

For each mutation, the first letter refers to the amino acid as found in wild type (N2); the number following corresponds to the amino acid position within the protein; the second letter refers to the amino acid in the mutant (*i.e.*, M1152K). When L1 larvae are shifted to 25°, class 1 alleles display maternal-effect embryonic lethal (Mel) phenotypes, whereas class 2 alleles display sterile (Ste) phenotypes as previously described (Golden *et al.* 2000). Genomic DNA was prepared from homozygous animals grown at the permissive temperature. Gene-specific primers were used to PCR amplify all coding sequences and splice sites. PCR fragments were sequenced directly or cloned into pCRII (Invitrogen, Carlsbad, CA) and sequenced using the dRhodamine Dye Terminator Cycle Sequencing kits (PE Biosystems, Warrington, England) on an ABI 310 Capillary Genetic Analyzer. All PCR and sequencing was performed in duplicate.

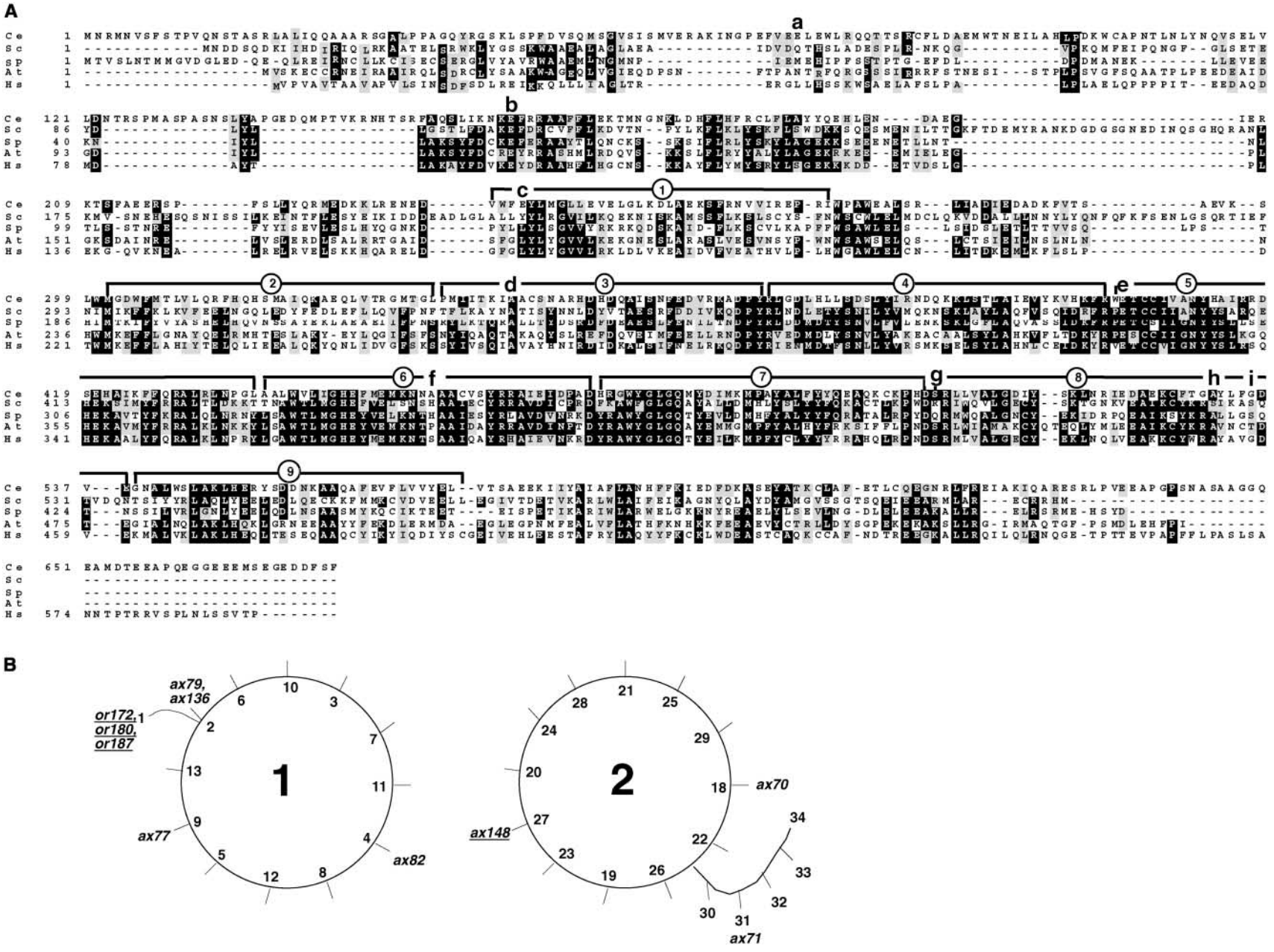
mat-3 strains revealed that each contained a single nucleotide substitution (Table 2). We hereafter refer to this gene as *mat-3/cdc-23*. *mat-3/cdc-23* lesions occurred throughout the CDC-23 protein sequence (Figure 2A), but, of the 12 mutations, only 9 are unique (Table 2). Seven of the 9 unique mutations occurred within TPRs (Figure 2, A and B), 3 within TPR8.

In addition to the oocyte meiotic defects shared by all *mat* alleles (Golden *et al.* 2000), some *mat-3/cdc-23* alleles exhibit defects in germline proliferation (Table 2; Golden *et al.* 2000). Interestingly, the four mutants with glutamic acid to lysine changes all exhibit germline defects, suggesting that the CDC-23 protein is sensitive to charge alterations. Furthermore, the E404K substitution in *ax79* and *ax136* is identical to the change found in the *cdc23-37* temperature-sensitive mutant in *S. cerevisiae* (Sikorski *et al.* 1993).

Molecular identification of additional APC/C genes in *C. elegans*: As it became apparent that *mat* genes could encode APC/C subunits (Table 1), we asked whether additional APC/C subunits function during the metaphase-to-anaphase transition of *C. elegans* meiosis I. Of the 8–12 known APC/C subunits in yeast and vertebrates, orthologs of several have been predicted on the basis of *C. elegans* genomic sequence data (Table 1). In particular, K06H7.6 and F35G12.9 were predicted as orthologs of the subunits APC-2 (Yu *et al.* 1998; Zachariae *et al.* 1998) and APC-11 (Ohta *et al.* 1999), respectively. We confirmed the gene predictions of the *C. elegans* Sequencing Consortium by sequencing the cDNAs yk268d10 (*apc-2*), yk566h7 (*apc-11*), and a reverse transcription-PCR clone of *apc-11* (data not shown). The amino acid sequence of APC-2 exhibits 32% identity with *S. cerevisiae* Apc2p within the critical cullin domain but shows little similarity along the rest of the protein. Conversely, APC-11, the key catalytic subunit of the APC/C (Leverson *et al.* 2000), exhibits 44% identity along the entire protein. M163.4 was reported to encode APC-5 (Zachariae *et al.* 1998) and has 11% identity and 20% similarity with *S. cerevisiae* Apc5p. BLAST searches revealed F15H10.3 as a convincing ortholog for APC-10 (27% identity, 45% similarity).

Other APC/C subunits required for the metaphase-to-anaphase transition of meiosis I: In a series of RNAi experiments, mothers injected with either *apc-2* or *apc-11* dsRNA produced embryos that arrested uniformly at the meiotic one-cell stage within 12–18 hr postinjection (Figure 3, c–e; Table 1). In contrast, moth-

Figure 2.—Alignment of CDC-23 orthologs. (A) Alignment of CDC-23 orthologs from *C. elegans* (Ce; AC AAK68876), *S. cerevisiae* (Sc; AC NP_012036), *S. pombe* (Sp; AC CAB11101), *Arabidopsis thaliana* (At; AC T13004), and human (Hs; AC XP_039328). The *C. elegans cdc-23* gene F10C5.1 has eight exons and is likely the second gene of a two-gene operon, as predicted by Genefinder and Intronerator (Kent and Zahler 2000a,b). *mat-3/cdc-23* allele positions are designated by letters above the *C. elegans* sequence: a, *or192*; b, *ax68*; c, *ax82*; d, *ax77*; e, *ax79/ax136*; f, *ax70*; g, *or172/or180/or187*; h, *ax148*; i, *ax71*. The nine TPR domains are numbered and indicated by brackets above the *C. elegans* sequence. Residues shaded in black are identical; those in gray are similar. (B) Schematic of a canonical TPR repeat with positions of each *mat-3/cdc-23* mutation indicated. Predicted helical wheel (Sikorski *et al.* 1990) represents each of the nine TPRs in *C. elegans* CDC-23. Domains 1 and 2 are indicated, with the typical hydrophobic faces on the inside, facing each other as described (Sikorski *et al.* 1990). Numbers indicate the predicted relative position of the 34 residues of each TPR. Residues 30–34 are generally not part of the amphipathic α-helices. Alleles are divided into two classes on the basis of L1 larval upshift experiments (Golden *et al.* 2000). When shifted to 25° as L1 larvae, alleles that are underlined exhibit a maternal-effect embryonic lethal (Mel) phenotype and produce all meiotic one-cell embryos. The other alleles display a Sterile (Ste) phenotype.


A
B
ax79,
ax136
or172,
or180,
or187
ax77
ax82
ax148
ax70
ax71
1
2

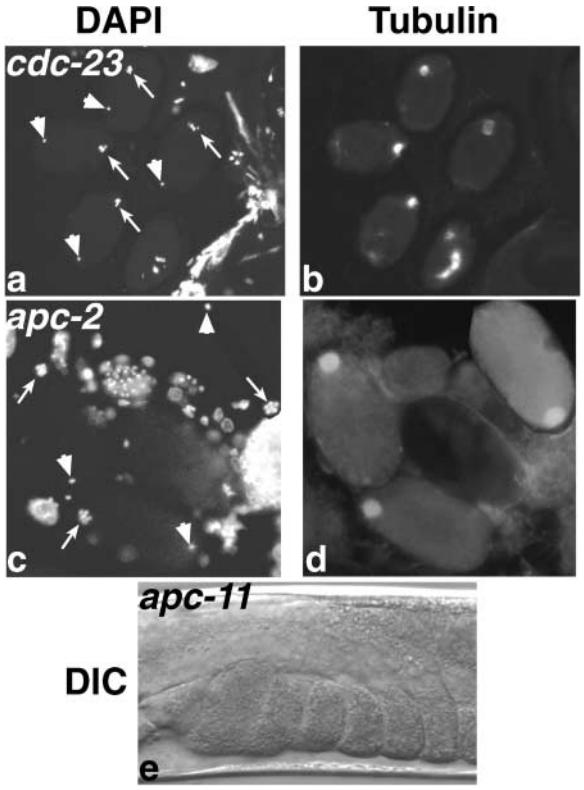


Figure 3.—RNAi of APC/C genes results in a meiotic one-cell arrest. DAPI (a and c) and anti-α-tubulin (b and d) images of embryos from hermaphrodites into which had been introduced *cdc-23* (a and b) and *apc-2* (c and d) dsRNAs. Arrows point to maternal chromatin. Arrowheads point to paternal chromatin. (e) Nomarski/DIC image of a whole-mount hermaphrodite following injection with *apc-11* dsRNA. Since *mat* mutants also affect spermatocyte meiosis, all temperature shifts and RNAi were performed after the completion of spermatogenesis in hermaphrodites; we could therefore analyze the oocyte meiosis defects specifically. For *cdc-23* RNAi, a 2018-bp fragment of a cDNA clone was inserted into the L4440 feeding vector and transformed into bacteria according to a published protocol (Timmons *et al.* 2001). Animals that ingested these bacteria were dissected for embryo isolation and staining. For other APC/C genes, 300- to 600-bp exon-specific fragments were PCR amplified from *C. elegans* genomic DNA using custom primers (Gene Link, Thornwood, NY; Life Technologies, Rockville, MD) that contained either T3 or T7 promoters at their 5′ ends. PCR amplification was carried out with gene-specific primer pairs corresponding to the following exons: *apc-1* (exon 4), *apc-2* (exons 5 and 6), *apc-5* (exons 5–7), *apc-10* (exons 1–5), and *apc-11* (exon 1 or exons 1–3). Double-stranded RNA was synthesized *in vitro* using T3 and T7 Megascript kits (Ambion, Austin, TX) according to manufacturer's instructions. For the analysis of RNAi embryos, wild-type hermaphrodites were either injected, soaked overnight (Tabara *et al.* 1998), or fed (Timmons *et al.* 2001) with gene-specific dsRNA and incubated at either 20° or 25° (all genes were tested by injection to confirm soaking or feeding RNAi phenotypes; more extreme phenotypes did not occur after dsRNA injections). *In utero* embryos were isolated for analysis 12–24 hr after their parental hermaphrodite had been either injected or removed from soaking solution or 72 hr after transfer to bacteria expressing dsRNA. For immunocytochemical analysis of early embryos, hermaphrodites were dissected and stained as previously described (Golden *et al.* 2000), using a 1:100 dilution of FITC direct-labeled anti-α-tubulin antibody (DM1A; Sigma, St. Louis; Blose *et al.* 1984) and DAPI (1 μg/ml).

ers injected with either the *apc-5* or *apc-10* candidate dsRNAs, during the same time interval and/or at a higher incubation temperature, failed to produce meiotic one-cell arrested embryos; rather, they produced a reduced clutch of viable and inviable embryos (Table 1). However, after longer time intervals, they exhibited severe germline maintenance defects and became sterile. Importantly, earlier analysis of sterility in *mat* mutants indicated that they were associated with defects in the mitotic divisions of the germline nuclei (Golden *et al.* 2000). In the current studies, mothers injected with dsRNA corresponding to other APC/C genes exhibited similar sterility defects after producing an initial small clutch of one-cell arrested embryos (data not shown). Although these results raise the possibility that *apc-5* and *apc-10* do not function as part of a meiotic APC/C, it is also possible that the rapid onset of RNAi-associated germline defects is masking subsequent defects in oocyte meiosis.

To distinguish whether these one-cell embryos were arresting in metaphase of meiosis I, meiosis II, or mitosis, the structures of the DNA and spindles were examined by 4′,6-diamidino-2-phenylindole (DAPI) staining and tubulin immunofluorescence. Under these conditions, the arrested embryos exhibited four critical features: (1) The oocyte chromosomes were aligned in a pentagonal array on a morphologically normal, barrel-shaped meiotic spindle; (2) meiotic polar bodies were absent; (3) the sperm chromatin mass remained highly condensed (Figure 3c); and (4) the embryos possess incompletely hardened, osmotically sensitive eggshells. These four features define an arrest in metaphase of meiosis I (Albertson and Thomson 1993; Golden *et al.* 2000). Importantly, the same RNAi phenotype has been observed for not only *apc-1*, *apc-2*, *cdc-23*, and *apc-11* (this study), but also *cdc-16* (Golden *et al.* 2000), *apc-4* (Furuta *et al.* 2000), and *cdc-27* (J. Schumacher, M. Abdolurasulnia, D. Shakes and A. Golden, unpublished results). Thus, with the possible exception of the *apc-5* and *apc-10* candidates, most of the APC/C subunits, which are essential for mitotic anaphase in other organisms, are also required to separate chromosomal homologs during meiosis I in *C. elegans*. Given that these predicted APC/C orthologs not only share a common RNAi phenotype but also are the only *C. elegans* genes with any significant similarity to their corresponding APC/C subunits in biochemically analyzed systems, they too are likely to function together in a complex.

APC/C is required for anaphase I functions beyond the separation of homologs: In mitotically dividing yeast, the loss of cohesion between sister chromatids is sufficient to trigger the metaphase-to-anaphase transition. In fact, some of the first cohesin mutants were isolated as suppressors of temperature-sensitive APC/C mutants; in the absence of sister chromatid cohesion, mitotically dividing double mutants bypass the APC/C-associated metaphase block and instead arrest with two

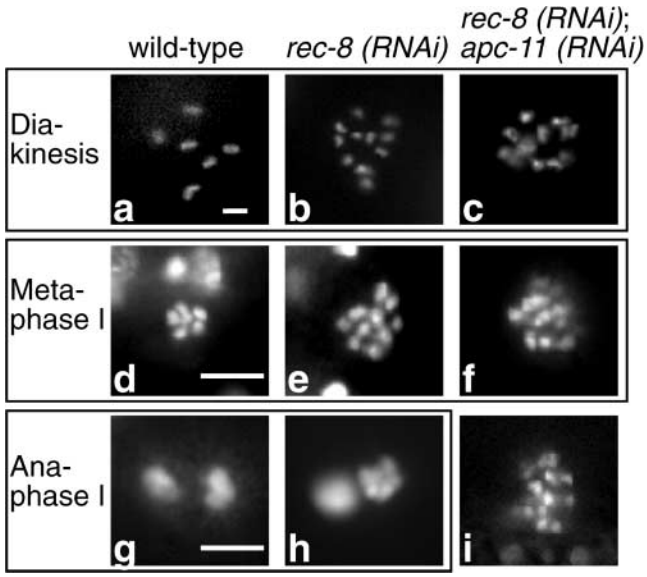


Figure 4.—Loss of cohesion does not bypass metaphase I arrest. DAPI images of chromosomes from oocytes (a–c) and meiotic one-cell embryos (d–i). Bar, 10 μm. In all images except a, it was impossible to capture all of the chromosomes in a single focal plane. Sperm nuclei can be seen at the periphery of d and e. The stained chromosomes are from wild-type (a, d, and g) hermaphrodites or hermaphrodites that have been introduced with either *rec-8* dsRNAs (b, e, and h) or a combination of *rec-8* and *apc-11* dsRNAs (c, f, and i). *apc-11(RNAi)* controls (data not shown) are indistinguishable from the wild-type images seen in a and d. Consistent with our earlier findings (Figure 3 and Table 1), *apc-11(RNAi)* embryos analyzed as controls for the *rec-8* study arrest in metaphase of meiosis I. Young adult worms were microinjected with *rec-8* dsRNA (800-bp fragment of exon 5) by standard methodology. Surviving animals were allowed to recover for 24 hr and were then transferred to new plates. F_1 embryos laid in the ensuing 48 hr were grown to the L4 larval-young adult stage and soaked 18 hr in either *apc-11* dsRNA (all 3 exons) or water. Survivors of the soaking procedure were allowed to recover for 7 hr and were then transferred to plates with N2 or *him-8* males for 11 hr to ensure that the embryos were fathered by wild-type sperm. All incubations were at 20°. The gravid hermaphrodites were dissected, fixed in methanol, and stained with FITC-conjugated anti-α-tubulin monoclonal antibody (Sigma) and DAPI as described (Golden *et al.* 2000).

distinct masses of DNA (Michaelis *et al.* 1997). Could the disruption of sister chromatid cohesion likewise bypass the meiosis I metaphase arrest of APC/C(RNAi) embryos or is the degradation of additional factors required for the segregation of paired, homologous chromosomes? Or, more specifically, do specialized features of either the meiotically telocentric chromosomes of *C. elegans* or its anastral, acentriolar spindle (Albertson *et al.* 1997) require regulation by additional APC/C substrates?

To address these questions, we used *rec-8* RNAi to disrupt sister chromatid cohesion. Rec8 is a meiosis-specific cohesion protein, first discovered in fission yeast, whose absence leads to aberrant, equational division of chromosomes during meiosis I (Molnar *et al.* 1995; Krawchuk *et al.* 1999). Notably, in fission yeast, *rec8* mutants complete both meiotic divisions and form four, albeit abnormal, spores. In *C. elegans*, the F_1 progeny of mothers injected with *rec-8* dsRNA exhibit striking defects in their diakinetic oocytes (Pasierbek *et al.* 2001). While diakinetic oocytes from wild-type mothers contain six DAPI-staining structures corresponding to the six paired homologs (Figure 4a), both sister and homolog cohesion is disrupted in *rec-8(RNAi)* oocytes (Figure 4b). In our hands, two-thirds of F_1 *rec-8(RNAi)* hermaphrodites contained oocytes with 6 frayed DAPI-staining entities. The remaining one-third contained oocytes with 12 or more DAPI-staining entities, suggesting that the disruption of homolog cohesion was complete but that, as previously reported (Pasierbek *et al.* 2001), sister chromatids remained loosely associated (Figure 4b). Following the fertilization of these more severely affected oocytes, further detachment of the sister chromatids occurred as the chromosomes attempted to align in an aberrant metaphase I plate. Such plates lacked the normal pentagonal organization characteristic of wild-type embryos (compare Figure 4d to 4e), but, during anaphase, the affected chromosomes nonetheless segregated into two apparently equal-sized masses (Figure 4h). Importantly, fertilized *rec-8(RNAi)* oocytes not only completed meiosis, albeit abnormally, but formed multicellular, morphogenetic embryos. In fact, quantitative analysis of all embryos that were laid on growth plates revealed that 26% ($n = 1415$) hatched into larvae.

To analyze the double mutant phenotype for evidence of suppression, F_1 *rec-8(RNAi)* mothers were soaked in *apc-11* dsRNA. Such animals produced oocytes with the expected *rec-8(RNAi)* defects (Figure 4c), and, following fertilization, the disjoined chromosomes aligned in an aberrant metaphase plate (Figure 4f). However, unlike *rec-8(RNAi)* embryos, *rec-8(RNAi); apc-11(RNAi)* embryos failed to segregate their chromosomes into two distinct chromatin masses (Figure 4i) that would have indicated progression to or through anaphase and suppression of the *apc-11(RNAi)* metaphase I arrest. Instead, these embryos ($n = 105$) remained arrested with their chromosomes locked in a meiosis I metaphase-like state (Figure 4i), and, except for having disjoined sister chromatids, they were otherwise indistinguishable from the *apc-11(RNAi)* controls. Importantly, some *rec-8(RNAi); apc-11(RNAi)* embryos exhibited complete detachment of sister chromatids into >24 DAPI-staining chromatids and chromatid fragments during their prolonged metaphase arrest (data not shown). Notably, these one-cell arrested embryos also lacked several key indicators of meiotic progression including the decondensation of the sperm chromatin mass, the formation of polar bodies, and the formation of an impermeable eggshell. In parallel quantitative studies, only 0.5% of embryos ($n = 1123$) laid on growth plates from pooled *rec-8(RNAi);*

apc-11(RNAi) mothers ever hatched into larvae. Similar qualitative and quantitative results were obtained in our analysis of *mat-3(or180ts); rec-8(RNAi)* embryos grown at the restrictive temperature (data not shown).

Understanding the composition and function of the APC/C during meiosis: Using forward and reverse genetics in *C. elegans*, we have demonstrated that several APC/C subunits, known to play essential mitotic roles in other organisms, are also required for the metaphase-to-anaphase transition during oocyte meiosis I. Because of the mitotic requirements for APC/C, this analysis was carried out using a combination of conditional alleles and RNAi. Null mutants in all of these genes are expected to develop into sterile adult hermaphrodites with highly reduced germlines as well as protruding or everted vulvae, as is the case for null alleles of *emb-30/apc-4* (Furuta *et al.* 2000) and *mat-3/cdc-23* (D. Garbe and M. Sundaram, personal communication). In the absence of conditional alleles, RNAi proved to be the best method for examining the role of *C. elegans* maternal products in the completion of the oocyte meiotic divisions following fertilization and the only viable approach for studying the potential meiotic role of *apc-2* and *apc-11*. Importantly, the meiotic defects displayed by the APC/C(*RNAi*) embryos proved to be no more severe than those of our non-null *mat* mutants, a result that indicates that, because of either dosage or tissue specificity, these temperature-sensitive mutants behave as nulls during oocyte meiosis I.

Recent genome-wide RNAi screens have expanded the number of genes required for meiotic progression. In addition to APC/C subunits, this class includes numerous subunits of the proteosome (Gonczy *et al.* 2000; Zipperlen *et al.* 2001), the protein complex that degrades proteins ubiquitinated by the APC/C. Taken together, these genetic and RNAi studies suggest that, in *C. elegans*, the APC/C drives the meiosis I segregation of homologs by degrading one or more maternal products. Importantly, the RNAi and genetic approaches used in this study can be used to effectively deplete functional APC/C levels during the earliest stages of meiosis. Thus, an alternative explanation for seemingly contradictory studies in Xenopus (Peter *et al.* 2001; Taieb *et al.* 2001) is that, by the time Xenopus oocytes reach late prophase, the meiosis I functions of their preassembled and localized APC/Cs are protected from disruption by APC/C inhibitors.

In *rec-8(RNAi); apc-11(RNAi)* studies, the loss of sister chromatid cohesion proved insufficient to bypass the metaphase I block of APC/C-depleted embryos. This result indicates that, in *C. elegans*, the metaphase-to-anaphase transition during meiosis I requires an early function for APC/C beyond the separation of sister chromatids. Although the nature of these additional APC/C targets awaits further investigation, intriguing possibilities include both cyclin A (Parry and O'Farrell 2001) and chromokinesin (Funabiki and Murray 2000) as well as potentially novel, meiotic-specific APC/C substrates.

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Note added in proof: As first noted by David Greenstein (personal communication), the *mat-2* alleles fail to complement the previously identified mutant *evl-22* (*ar104*), which is sterile and has an everted vulva (G. Seydoux, C. Savage and I. Greenwald, 1993, Isolation and characterization of mutations causing abnormal eversion of the vulva in *Caenorhabditis elegans*. Dev. Biol. **157:** 423–436). Our sequencing of this allele revealed that the mutation in *ar104* results in a Phe-to-Leu change at amino acid 931 of APC-1.

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