Embryonic development begins with fertilization. For many animal species, however, fertilization takes place before the entire meiotic process is completed. That is, at fertilization, oocytes are still in a certain stage of meiosis, which is species-specific. For example, oocytes are in the middle of meiosis-II in Xenopus and mouse when fertilization occurs (5, 10), whereas in Caenorhabditis elegans they are at early meiosis-I (1). The remaining process of meiosis is resumed immediately after fertilization. MIWA et al. (7) identified nine genes required for embryogenesis by the analysis of temperature-sensitive embryonic arrest mutants in C. elegans. Among them, emb-1 and emb-3 genes are required very early in embryogenesis (7). The emb-1 (hc 57) mutant is abnormal in pronuclear reconstruction, and emb-3 (hc 59) forms an extraordinarily large polar body (8). These early abnormalities could arise from defects in meiosis either before or after fertilization. In order to understand the roles of emb-1 and emb-3 in embryonic development, we started a series of experiments, and here in this report we cytologically examined meiotic spindles and chromosomes in oocytes and embryos of the mutants by staining them with an anti-tubulin antibody and DAPI. We have found that both emb-1 and emb-3 gene functions are required for meiosis after fertilization, but that each gene plays a distinctly different role.

MATERIALS AND METHODS

Nematode strains: Handling of C. elegans was as described by BRENNER (2). The following strains were used: wild-type strain Caenorhabditis elegans ver. Bristol, which is conventionally termed N2 (2) and the temperature-sensitive embryonic lethal mutants emb-1 (hc 57) and emb-3 (hc 59) (7, 8).

Microscopy and Staining: For Nomarski microscopy to analyze mutant phenotypes, early embryos
were prepared by dissecting them out from parental hermaphrodites grown at 25°C (8). For immunofluorescence microscopy, embryos prepared as for Nomarski microscopy were fixed and stained according to STROME et al. (9) except for an incubation in secondary antibodies at 20°C for 1 hr. Diamidinophenylindole (DAPI) was used to stain DNA. A mouse anti-tubulin polyclonal antibody, E6B6, made by Gen MATSUMOTO was provided by Shahid SIDDIQUI. FITC-conjugated-anti-mouse IgG (Cappel) was used as the secondary antibody.

RESULTS

In C. elegans, oocytes rested at the diakinesis stage, which is the final stage of the meiosis-I prophase, resume the meiotic process upon fertilization and quickly enter metaphase. To visualize oocyte nuclei in the meiotic process, microtubules and chromosomes were stained by an anti-tubulin antibody and DAPI. In wild-type embryos, the meiotic spindle is spherical or barrel-shaped in both meiosis-I and -II, and the six bivalent chromosomes are arranged pentagonally with one of them in the center (Fig. 1 a-f). The meiosis-I spindle becomes detectable before completion of the egg shell formation. After producing the first and second polar bodies, both oocyte and sperm pronuclei are reconstructed (Fig. 1 g, h). In emb-1 (hc 57) embryos, the shape of the meiosis-I spindle and positioning of the six bivalents were apparently normal (Fig. 2 a, b). Quite differently from the wild-type embryo, however, the chromosomes began to decondensate or unfold to look like thin strings (Fig. 2 d, f). Similar chromosomal decondensation occurred in sperm chromosomes at a slightly later stage of meiosis than that seen in oocyte chromosomes (not shown). The spindles were deformed during chromosomal decondensation, and no meiosis-II spindles were formed (Fig. 2 c, e). The production of polar bodies was never observed in emb-1 (hc 57) embryos. About 40% (n=22) of emb-3 (hc 59) embryos displayed deformed meiosis-I spindles where the bivalents were mispositioned (Fig. 3 a, b). All or some of the chromosomes were extruded from oocytes as single large polar bodies (Fig. 3 c, d). Therefore, embryos often became aneuploid or haploid with only sperm-derived chromosomes. Many of such haploid embryos developed normally during early embryogenesis (Fig. 3 e), but eventually arrested as abnormal masses of several hundred cells.

DISCUSSION

We observed barrel-like meiotic spindles in wild-type C. elegans fertilized eggs, as reported by ALBERTSON (1). Six bivalents were arranged pentagonally with one of them in the center. Because such a bivalent configuration is not observed in mature oocytes before fertilization, bivalents must be configured after fertilization during meiotic spindle formation. Both emb-1 and emb-3 mutations that affect early embryogenesis were found to cause defects in meiosis after fertilization. The emb-1 and emb-3 mutants show temperature-sensitive maternal effect, and their temperature-sensitive periods occur before the 1- or 2-cell stage and probably before fertilization (7). Conceivably, the products of these genes are provided from oocytes to promote meiosis. Although emb-1 and emb-3 are both required for meiosis, they play distinctly different roles. The emb-3 gene, on one hand, seems to be required to participate in the formation of meiosis-I spindles. The gene emb-1, on the other hand, appears to execute an essential function in producing polar bodies. Possibly, the functional expression of emb-3 precedes that for emb-1 during meiosis. The genes mei-1 and zyg-9 are also required for meiosis after fertilization (3, 6). mei-1 encodes a member of ATPases, and zyg-9 encodes a protein similar to microtubule associated proteins. Both of them are components of the meiotic spindles and poles (4, 6). Mutations in these genes disrupt meiosis-
Fig. 1. Meiosis in wild-type embryos. Anterior to the left. Left and right photos are of the same embryo stained, respectively, with DAPI and the anti-tubulin antibody. The outline of embryonic cell(s) is indicated by a white line (also in Figs. 2 and 3). Bar, 10μm. a to d: Embryos at metaphase of meiosis-I. Meiotic spindles are near the anterior pole: lateral view (a and b) and vertical view (c and d). The stained dot near the posterior pole is a cluster of condensed sperm chromosomes (b and d). e to f: An embryo at metaphase of meiosis-II. The first polar body is indicated (arrow). g to h: An embryo after completion of meiosis. The first and second polar bodies are indicated (arrow). Reconstituted oocyte and sperm pronuclei are visible (h). The staining other than that of polar bodies seen outside of the embryos in d and f is due to unrelated gonadal or sperm nuclei.
Fig. 2. Meiosis in *emb-1* (he 57) embryos. Embryos are shown as in Fig. 1. Bar, 10μm. a to b: An embryo at metaphase of meiosis-I. The meiotic spindle is normal. c to f: Spindles and chromosomes are gradually disorganized. Embryonic stages in (e) and (f) are later than those in (c) and (d).
Fig. 3. Meiosis in *emb-3* (*hec 59*) embryos. Embryos are shown as in Fig. 1 except for the bottom panels. Bar, 10µm. a to b: An embryo at meiosis-I. The meiotic spindle is disorganized. c to d: All the oocyte chromosomes are extruded as a single polar body (arrow) in this embryo. The sperm pronucleus is reconstituted (posterior pole). e: Four-cell stage embryo of a haploid *emb-3* mutant stained with DAPI. Only six chromosomes are visible in the dividing AB cells. f: A wild-type embryo of the equivalent cell stage as in (e). Twelve chromosomes are clearly visible in AB cells.
I spindles and show amorphous clouds of anti-tubulin staining surrounding chromosomes (4, 6). The anti-tubulin staining pattern for these mutations is quite different from that for emb-3 (Fig. 3 a). Although emb-3 spindles are disorganized, microtubules are still organized and spindle poles keep their morphology in clear shape. Therefore, unlike mei-1 and zyg-9, emb-3 does not appear to be required for spindle organization itself. Probably, emb-3 functions in the proper arrangement of the spindle parts, poles, microtubules, and/or chromosomes.

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LITERATURE CITED


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和文摘要

Caenorhabditis elegans の emb-1 と emb-3 遺伝子は
受精後の減数分裂に必要である

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温度感受性胚発生変異体 emb-1（hc 57）と emb-3（hc 59）における減数分裂を解析した。emb-1 変異胚では第一減数分裂糸錠体は正常に形成された。しかし糸錠体と染色体はその後分解し、極体の形成は見られなかった。emb-3 変異胚では第一減数分裂糸錠体の構造が崩れ、染色体の配置も異常であっ
た。全ての染色体が一個の大きな極体として放出されるのがしばしば見られた。これらの結果から、emb-3は第一減数分裂糸錠体の形成に必要であるが、極体の形成には必要でないこと、また emb-1 は
極体の形成に必要だが、第一減数分裂糸錠体の形成には必要でないことが推測できる。さらに発生過
程を考慮すると、遺伝子機能の発現は emb-3 が emb-1 に先行するであろうと予測される。