The *Caenorhabditis elegans* maternal-effect sterile proteins, MES-2, MES-3, and MES-6, are associated in a complex in embryos

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The *Caenorhabditis elegans* maternal-effect sterile genes, *mes-2*, *mes-3*, *mes-4*, and *mes-6*, encode nuclear proteins that are essential for germ-line development. They are thought to be involved in a common process because their mutant phenotypes are similar. MES-2 and MES-6 are homologs of Enhancer of zeste and extra sex combs, both members of the Polycomb group of chromatin regulators in insects and vertebrates. MES-3 is a novel protein, and MES-4 is a SET-domain protein. To investigate whether the MES proteins interact and likely function as a complex, we performed biochemical analyses on *C. elegans* embryo extracts. Results of immunoprecipitation experiments indicate that MES-2, MES-3, and MES-6 are associated in a complex and that MES-4 is not associated with this complex. Based on *in vitro* binding assays, MES-2 and MES-6 interact directly, via the amino terminal portion of MES-2. Sucrose density gradient fractionation and gel filtration chromatography were performed to determine the Stokes radius and sedimentation coefficient of the MES-2/MES-3/MES-6 complex. Based on those two values, we estimate that the molecular mass of the complex is ≈255 kDa, close to the sum of the three known components. Our results suggest that the two *C. elegans* Polycomb group homologs (MES-2 and MES-6) associate with a novel partner (MES-3) to regulate germ-line development in *C. elegans*.

Germ cells give rise to gametes and offspring and thus are essential for the propagation of species. To identify maternally supplied gene products that are required for early germline development in *Caenorhabditis elegans*, Capowski *et al.* (1) screened for maternal-effect sterile (*mes*) mutants. Homozygous *mes* mutant hermaphrodites from heterozygous mothers are themselves fertile but produce sterile hermaphrodite progeny. Four *mes* genes, *mes-2*, *mes-3*, *mes-4*, and *mes-6*, are thought to be involved in a common process because their sterile phenotypes look similar: Sterility is caused by necrotic death of germ cells during larval development (2). MES-2 and MES-6 are homologs of the Polycomb group (PcG) proteins, Enhancer of zeste [E(Z)] and extra sex combs (ESC), respectively (3, 4). MES-3 is a novel protein with no recognizable motifs (2). MES-4 resembles MES-2 in having a SET domain, which is found in many chromatin-associated proteins (Y.F. and S.S., unpublished data).

PcG proteins are transcriptional repressors that are conserved among diverse species (3–6). In *Drosophila* their best-known targets are homeotic genes, which are involved in regulating anterior–posterior body patterning (7–9). Homeotic genes are expressed in spatially restricted domains along the anterior–posterior axis (10, 11). Their expression patterns are initially established by transiently expressed gap and pair-rule proteins, and later maintained by PcG and trithorax group (trxG) proteins: PcG proteins maintain repression of genes outside of their expressing domains, and trxG proteins maintain gene expression within the correct domains (5, 7, 12). This regulation is thought to be at the level of chromatin structure: The PcG promotes a repressed state, and the trxG promotes an active state (13, 14).

The *C. elegans* MES proteins also appear to function as transcriptional repressors, based on analyses of transgene expression in the germ line. Extrachromosomal arrays with a high copy number of transgenes are normally expressed in somatic cells but are silenced in the germ line of wild-type worms (15). This silencing is thought to result from the germ line packaging repetitive arrays into a transcriptionally silenced chromatin state. Strikingly, repetitive arrays are desilenced in the germ line of *mes* mutants (16). Desilencing of transgenes in the germ line also can be achieved by placing the transgenes in the context of complex genomic DNA to reduce the repetitive nature of the arrays (15). These findings suggest that the MES system participates in keeping at least some genes silenced in the germ line and that this is via an effect on chromatin state.

The *C. elegans* PcG differs from the PcG in insects and vertebrates in at least two important respects. First, the *C. elegans* genome contains recognizable homologs of only two of the 12 PcG genes thus far cloned from insects and vertebrates (3, 4, 6, 8, 17, 18). Second, the *C. elegans* PcG proteins, MES-2 and MES-6, serve essential roles only in germ-line development, whereas PcG proteins in insects and vertebrates function in somatic cells to regulate patterning of the body plan (1–4, 6–8). In flies, PcG proteins function in at least two distinct complexes, an E(Z)/ESC complex of ≈600 kDa (19–21) and a complex of 2–6 MDa containing Polycomb, polyhomeotic, and Posterior sex combs (13, 22). We report here that MES-2 and MES-6 also associate with each other *in vivo* and interact directly *in vitro*. MES-3 is associated with the MES-2/MES-6 complex, but MES-4 is not. The estimated size of the MES-2/MES-3/MES-6 complex (≈255 kDa) suggests that few, if any, additional components of the complex remain to be identified.

Materials and Methods

Preparation of Embryo Extracts. *C. elegans* embryos of mixed stages were harvested from gravid adults by standard procedures (23) and resuspended in 3–4 vol of 15 mM Hepes (pH 7.6), 10 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM EGTA, 0.35 M sucrose, 1 mM DTT, 0.1% aprotinin, 0.2 mM PMSF, 1 mM benzamidine, and 1 mM sodium metabisulfite. The embryo suspension was frozen and ground under liquid nitrogen to break the cells. The released components were centrifuged at ≈7,800 × *g* for 10 min, and the supernatant was further centrifuged at ≈78,600 × *g* for 30 min. The final supernatant was dialyzed against 50 mM KCl, 1 mM DTT, 0.2 mM EDTA, 5% glycerol, and 20 mM Tris·HCl (pH 8.0), frozen in liquid nitrogen,

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Abbreviations: ESC, extra sex combs; PcG, Polycomb group; E(Z), Enhancer of zeste.

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and stored at −80°C. All biochemical experiments were performed at 4°C, unless otherwise indicated.

Immunoprecipitations and Western Blots. In most cases, 200 μl of affinity-purified rabbit anti-MES-2 antibody (3) or 5 μl of rabbit anti-MES-6 crude serum (4) was coupled to Protein A-agarose and added to ≈200 μl of embryo extract to precipitate MES-2 or MES-6 protein, respectively. After an overnight incubation, the mixture was spun briefly. The pellets were washed with HEMK buffer (25 mM Hepes/0.2 M KCl/12.5 mM $MgCl_2$/0.1 mM EDTA/10% glycerol/0.2 mM PMSF/0.1% Nonidet P-40, pH 7.6) followed by HEMK buffer containing 0.4 M KCl instead of 0.2 M KCl.

After immunoprecipitation, 5% of the supernatant (S) and 5% of the pellet (P) were electrophoresed on an 8% SDS/polyacrylamide gel and transferred to nitrocellulose membrane (24). Primary antibodies used for immunodetection of proteins were the same rabbit anti-MES-2 (1:200) and anti-MES-6 (1:5,000) as used in the immunoprecipitation, affinity-purified rat anti-MES-3 (1:50), and affinity-purified rat anti-MES-4 (1:25). The specificity of the anti-MES-3 and anti-MES-4 antibodies was determined by immunostaining and Western blots. Each antibody recognizes MES-3 or MES-4 protein only in wild-type worms but not in *mes-3* or *mes-4* homozygous mutants (unpublished data). The secondary antibodies used in the Western blots were horseradish peroxidase-conjugated goat anti-rabbit (1:10,000) and goat anti-rat (1:2,000) antibodies (The Jackson Laboratory). Protein bands were detected by using a chemiluminescence detection kit (ECL; Amersham Pharmacia).

To detect MES-6 protein (which has a molecular mass similar to the IgG heavy chain) in the anti-MES-2 immunoprecipitation reaction, the anti-MES-2 antibody was crosslinked to Protein A-agarose via dimethylpimelimidate before the precipitation, following the protocol in ref. 25. After immunoprecipitation, proteins attached to the Protein A-agarose beads noncovalently were eluted by using 0.2 M Gly-HCl (pH 2.5). 50% of the eluted material (P) and 5% of the supernatant (S) were analyzed by Western blot as described above.

***In Vitro* Binding Assays.** The TNT T7 Quick Couple Transcription/Translation System from Promega was used to synthesize MES-2, MES-3, MES-4, and MES-6 proteins *in vitro* as described (20). A reaction was assembled by mixing 12.5 μl of TNT lysate, 1 μl of TNT buffer, 0.5 μl of T3 or T7 RNA polymerase, 0.5 μl of amino acid mix minus methionine, 1 μl Redivue L-[^{35}S]methionine (Amersham Pharmacia), 1 μg of plasmid DNA purified by Qiagen (Chatsworth, CA) plasmid purification column, and H_2O to achieve a final volume of 25 μl. The mixture was incubated at 30°C for 90–120 min and stored at 4°C.

Approximately 4 μl of each *in vitro*-translated protein were mixed together and incubated at 30°C for 2 h. One-fourth volume of 5× embryo extract buffer (250 mM KCl/5 mM DTT/1 mM EDTA/25% glycerol/100 mM Tris·HCl, pH 7.4) was added to the mixture and incubated at 4°C for 12 h. One-fourth volume of appropriate antibodies was added to the mixture and incubated at 4°C for 12 h. The reaction mixture was then added to 30 μl of a 50% slurry of Protein A- or Protein G-agarose. After incubation at 4°C overnight, beads were washed four times with 150 μl of HEMK buffer. Bound proteins were eluted with 15 μl of 2× SDS sample buffer and analyzed by SDS/PAGE and autoradiography.

Gel Filtration Chromatography. One milliliter of embryo extract in elution buffer (EB) (25 mM Hepes/200 mM KCl/12.5 mM $MgCl_2$/0.1 mM EDTA/0.1% Tween-20, pH 7.6) was applied to a 16/60 Sephacryl S-300 column (Amersham Pharmacia) pre-equilibrated with EB buffer. The sample was eluted by using the same buffer at 0.1 ml/min. One-milliliter fractions were collected. Proteins of known Stokes radii (2.01 nm carbonic anhydrase/3.61 nm BSA/4.55 nm alcohol dehydrogenase/6.1 nm apoferritin/8.5 nm thyroglobulin; Sigma) were used as standards and were detected by measuring the absorbance of each collected fraction at 280 nm. To monitor the elution profile of MES proteins, fractions were precipitated by 2 vol of ethanol and subjected to Western blot analysis as described above.

Sucrose Density Gradient Centrifugation. Centrifugations were carried out by using a Beckman SW41 Ti rotor in a Beckman (model L80-M) ultracentrifuge. A 7–47% linear sucrose gradient (12 ml of total volume) was formed in an Ultra-Clear tube (Beckman Coulter). Then 200 μl of embryo extract was layered on top of the gradient and centrifuged at ≈197,600 × *g* for 15 h at 4°C. Fractions (500-μl) were collected and precipitated by 2 vol of ethanol. Proteins with known sedimentation coefficients (3.2S carbonic anhydrase/4.5S BSA/8.9S β-amylase/17.6S apoferritin/19.4S thyroglobulin; Sigma) were used as references and detected by Bradford assays. The migration profiles of MES proteins were determined as described above.

Determining the Hydrodynamic Properties of the MES-2/MES-3/MES-6 Complex. The molecular mass of the MES-2/MES-3/MES-6 complex was calculated from the Stokes radius (R_s) and sedimentation coefficient ($s_{20,w}$) by using the equation $M = 6\pi\eta N s R_s/(1 - \upsilon\rho)$ (26); where M, molecular mass; η, viscosity of the medium ($\eta = 1$); υ, partial specific volume ($\upsilon = 0.725$ ml/g); ρ, density of the medium ($\rho = 1$ g/ml); and N, Avogadro's number.

The peak of the MES-2/MES-3/MES-6 complex in sucrose density gradients coincided with the peak of β-amylase ($n = 2$) and thus was judged to have the same sedimentation coefficient (8.9S). The Stokes radius (R_s) of the complex was calculated from gel filtration chromatography experiments ($n = 2$). The distribution coefficient, K_{av}, was calculated from the equation $K_{av} = (V_e - V_0)/(V_t - V_0)$, where V_e is the elution volume of each reference protein and the MES complex, V_t is the total volume of the column (120 ml, provided by the manufacturer), and V_0 is the void volume, which was determined by the elution volume of blue dextran ($V_0 = 46.8$ ml). The Stokes radius of the MES-2/MES-3/MES-6 complex was determined by interpolation using a calibration curve, which was constructed by plotting Stokes radii of reference proteins vs. $(-\log K_{av})^{1/2}$ according to the relationship $(-\log K_{av})^{1/2} = \alpha(\beta + R_s)$ (27).

Results

MES-2, MES-3, and MES-6 Are Associated in *C. elegans* Embryos. To determine whether the two PcG homologs, MES-2 and MES-6, are associated in a complex in *C. elegans* embryo extracts, we performed coimmunoprecipitation analyses by using anti-MES-2 and anti-MES-6 antibodies. The levels of MES-2 and MES-6 in the precipitate and in the supernatant were determined by Western blot analysis, using the same antibodies. Because MES-6 is similar in size to IgG heavy chain, it was necessary to covalently crosslink the anti-MES-2 antibodies to protein A-agarose beads via dimethylpimelimidate. After immunoprecipitation, proteins bound to the anti-MES-2 antibodies were released by 0.2 M Gly-HCl (pH 2.5), but the IgG was not (see *Materials and Methods*). As shown in Fig. 1*A*, anti-MES-2 antibodies precipitated MES-2 (lanes 1 and 2) and coprecipitated MES-6 (lanes 3 and 4). Conversely, anti-MES-6 antibodies coprecipitated MES-2 (Fig. 1*C*, lanes 1 and 2). These results indicate that at least a portion of MES-2 and MES-6 are associated in embryo extracts. Furthermore, both anti-MES-2 and anti-MES-6 antibodies coprecipitated MES-3 (Fig. 1*B*, lanes 3 and 4, and Fig. 1*C*, lanes 3 and 4), but not MES-4 (Fig. 1*B*, lanes 5 and 6, and Fig. 1*C*, lanes 5 and 6). These results suggest that MES-2, MES-3, and MES-6 are associated in a complex in *C.*

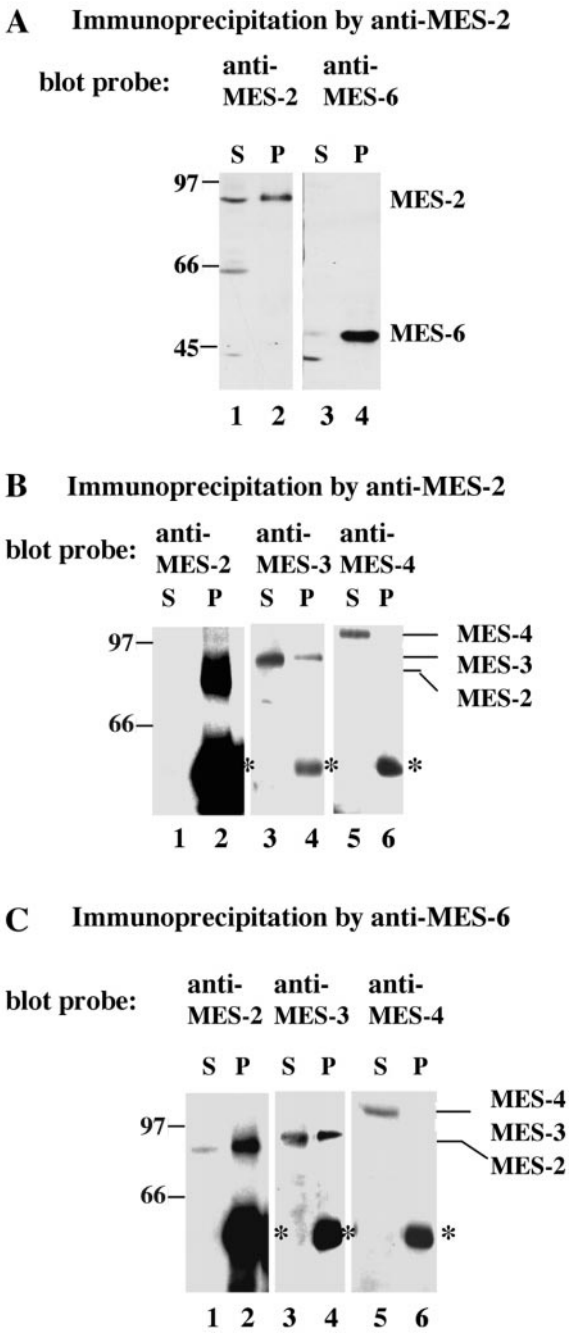


Fig. 1. Coimmunoprecipitation of MES-2, MES-3, and MES-6 from *C. elegans* embryo extracts. Proteins were immunoprecipitated from embryo extracts with either rabbit anti-MES-2 (*A* and *B*) or rabbit anti-MES-6 (*C*). (*A*) The rabbit anti-MES-2 antibodies were crosslinked to Protein A-agarose beads. (*B* and *C*) Equal amounts of immunoprecipitates (P) and supernatants (S) were analyzed by SDS/PAGE and Western blot analysis by using rabbit anti-MES-2, rat anti-MES-3, rat anti-MES-4, or rabbit anti-MES-6 as indicated (see *Materials and Methods*). Signals indicated by * are due to cross-reactivity between the secondary antibodies (goat anti-rabbit or goat anti-rat) and the heavy chain of the antibodies used for immunoprecipitations. Numbers to the left are kDa.

elegans embryo extracts and that MES-4 is not a partner in the MES-2/MES-3/MES-6 complex.

MES-2 and MES-6 Interact with Each Other Directly *in Vitro*. *In vitro* binding assays were performed to test for direct interactions between MES-2 and MES-6. Full-length MES-2 and MES-6 proteins were translated and radiolabeled *in vitro*. The two proteins were incubated together and then with anti-MES-2 or anti-MES-6 antibodies attached to Protein A-agarose beads. After extensive washing, binding between MES-2 and MES-6 was assessed by SDS/PAGE of the Protein-A-bound samples. As shown in Fig. 2*A*, MES-2 and MES-6 were coprecipitated by either anti-MES-2 or anti-MES-6 (lanes b and d), indicating that MES-2 and MES-6 are able to bind to each other directly *in vitro*. As a control, anti-MES-6 antibodies did not precipitate MES-2 in the absence of MES-6, and anti-MES-2 antibodies did not precipitate MES-6 in the absence of MES-2 (lanes a and c).

Similar binding assays were performed to test for interactions between MES-2 and MES-3, and between MES-6 and MES-3. To distinguish between MES-2 and MES-3, which have similar sizes, nonradiolabeled MES-2 (cold MES-2) and radiolabeled MES-3 and MES-6 were incubated together and then with anti-MES-2 or anti-MES-6. As shown in Fig. 2*B*, MES-3 protein did not coprecipitate with MES-2 (lane c) or with MES-6 (lane f) or with both MES-2 and MES-6 (lanes d and g). Thus, MES-3 does not bind directly to either MES-2 or MES-6 under our *in vitro* assay conditions. As a positive control, radiolabeled MES-6 was precipitated by anti-MES-2 in the presence of cold MES-2 (lanes a and d).

Different combinations of MES-4 and other MES proteins were tested for interaction by similar *in vitro* binding assays. As shown in Fig. 2*C*, *in vitro*-translated MES-4 protein did not coprecipitate with MES-2 (lane b) or MES-6 (lane d), and MES-3 did not coprecipitate with MES-4 (lane f). Thus, MES-4 also does not interact directly with any of the other three MES proteins in this *in vitro* assay.

The N-terminal Portion of MES-2 Interacts with MES-6 *in Vitro*. In *Drosophila*, the portion of E(Z) that binds to ESC is contained within amino acids 34–66 at its N terminus (19). Six residues in this region are conserved between fly E(Z) and its mammalian homologs. In mammals, the portion of the E(Z) homolog (mENX-1) that interacts with the murine ESC homolog is also in its N terminus, but in a region (amino acids 132–160) that has greater sequence identity between flies and mammals (28). The N terminus of the *C. elegans* homolog, MES-2, is considerably more diverged in amino acid sequence (Fig. 3*A*). To test whether it nevertheless is responsible for binding to the ESC homolog, MES-6, the N-terminal 194 aa and the remaining C-terminal portion of MES-2 were separately *in vitro* translated and tested for *in vitro* interactions with MES-6. As shown in Fig. 3*B*, the N-terminal 194 aa of MES-2 was coprecipitated with MES-6 by anti-MES-6 antibody (*Upper*, lane b). The remaining 579 aa of MES-2 (amino acids 195–773) was not coprecipitated with MES-6 by anti-MES-6 antibody (*Lower*, lane b). These data demonstrate that *in vitro*, the N-terminal 194 aa of MES-2 are both necessary and sufficient for its binding to MES-6.

MES-2 and MES-6 Remain Associated in the Presence of High Salt. To test whether the interaction between MES-2 and MES-6 is stable under high salt conditions, we washed the pellet precipitated by anti-MES-2 antibodies with buffers containing different concentrations of KCl. MES-2 and MES-6 proteins appear to remain associated even after being washed by 2 M KCl (Fig. 4), suggesting that the interaction between MES-2 and MES-6 remains stable in the presence of high salt and therefore is likely to involve hydrophobic forces.

MES-2, MES-3, and MES-6 Comigrate in Sucrose Density Gradient and Gel Filtration Analyses. The sum of the molecular masses of MES-2 (≈89 kDa), MES-3 (≈90 kDa), and MES-6 (≈50 kDa) is ≈230 kDa. Is the MES-2/MES-3/MES-6 complex likely to include other factors? To address this question, we determined the molecular mass of the MES-2/MES-3/MES-6 complex present

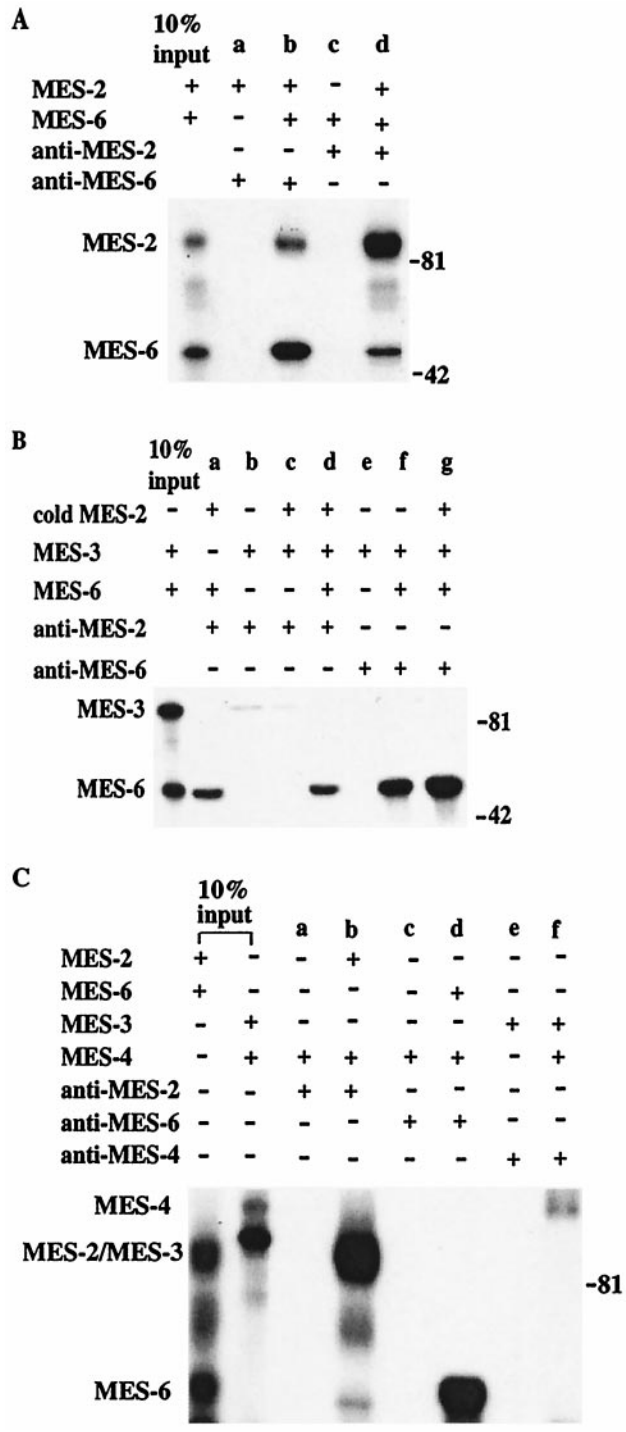


Fig. 2. MES-2 and MES-6 directly interact *in vitro*. ^{35}S-labeled MES-2, MES-3, MES-4, and MES-6 proteins were synthesized *in vitro* (see *Materials and Methods*). Equal amounts of MES proteins were mixed and incubated together and then with anti-MES antibodies coupled to Protein A-agarose or Protein G-agarose beads. After extensive washing, proteins in the pellets were analyzed by SDS/PAGE and autoradiography. Tests for interactions were of radiolabeled MES-2 and MES-6 (*A*), nonradiolabeled (cold) MES-2 and radiolabeled MES-3 and MES-6 (*B*), and different combinations of radiolabeled MES-4 and MES-2, MES-3, or MES-6 (*C*). Numbers to the right are kDa.

in wild-type *C. elegans* embryo extracts (see *Materials and Methods*). Two key parameters are required for this calculation: the sedimentation coefficient and the Stokes radius of the complex.

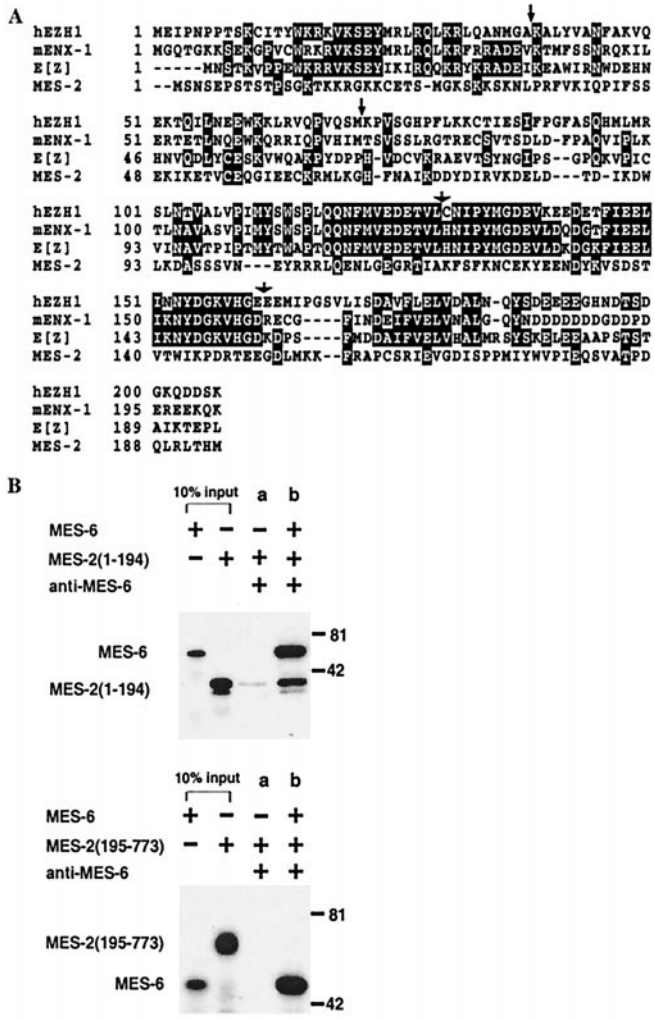


Fig. 3. The N terminus of MES-2 binds MES-6 *in vitro*. (*A*) Alignment of *C. elegans* MES-2_{1-194} with the N termini of *Drosophila* E(Z), its mouse homolog (mENX-1), and its human homolog (hEZH1). The domain in E(Z) that interacts directly with ESC *in vitro* is flanked by narrow arrows (amino acids 34–66), and the domain in mENX-1 that interacts with the mammalian ESC homolog is flanked by wide arrows (amino acids 132–160). The alignment was done by San Diego Supercomputer Center BIOLOGY WORKBENCH software (http://workbench.sdsc.edu). Residues identical to those in E(Z) are highlighted in black. (*B*) Radiolabeled N-terminal 194 aa (*Upper*) and C-terminal 579 aa (*Lower*) of MES-2 were tested for their ability to bind MES-6. Numbers to the right are kDa.

We performed sucrose density gradient analyses to determine the sedimentation coefficient of the MES-2/MES-3/MES-6 complex. Western blot analysis of the fractions collected after centrifugation showed that MES-2 and MES-3 (and MES-6; data not shown) cosedimented with a peak sedimentation coefficient of 8.9S ($n = 2$) (Fig. 5*A*). We performed gel filtration analyses to determine the Stokes radius of the MES-2/MES-3/MES-6 complex. MES-2 and MES-3 (and MES-6; data not shown) were eluted in a peak with a calculated Stokes radius of 6.87 and 6.99 nm ($n = 2$) (Fig. 5*B*). Based on these results, the hydrodynamic properties of the MES-2/MES-3/MES-6 complex were derived and are summarized in Fig. 5*C*. The molecular mass of this complex was calculated to be ≈255 kDa.

Discussion

A MES-2/MES-6 Partnership Is Conserved Across Species. Twelve PcG proteins have been cloned so far in *Drosophila* (8, 17, 18), but

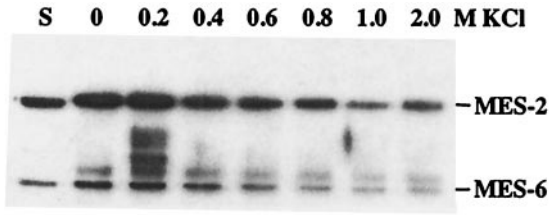


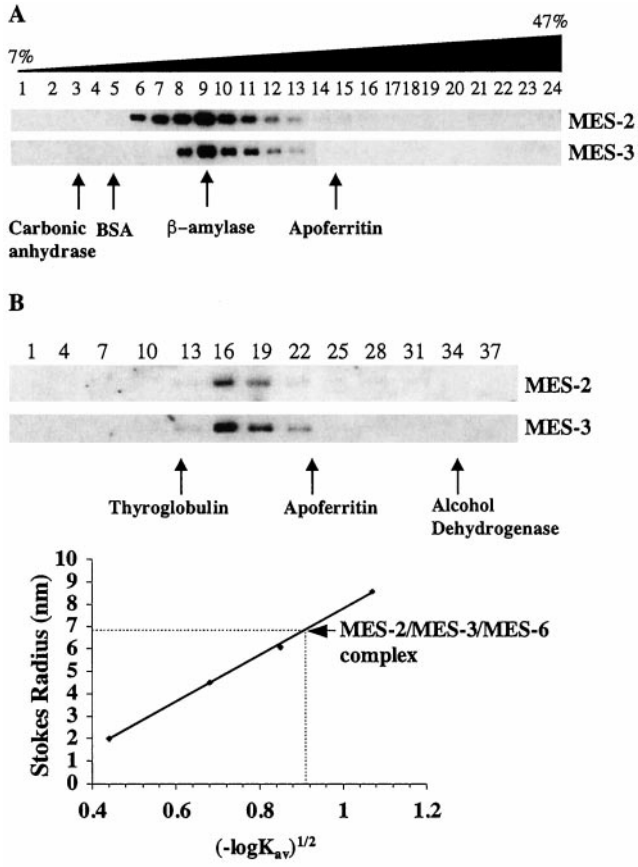
Fig. 4. The MES-2/MES-6 complex is resistant to high KCl concentrations. Proteins were immunoprecipitated from embryo extracts with anti-MES-2 antibodies. The immunoprecipitates were washed with increasing concentrations of KCl. Equivalent amounts of the initial supernatant (S) and the precipitate after each wash were analyzed by SDS/PAGE and Western blot analysis by using a mixture of rabbit anti-MES-2 and rabbit anti-MES-6 antibodies.

only two of them, E(Z) and ESC, are conserved in worms (3, 4) and also in plants (29, 30). These data suggest that these two proteins might be distinctive PcG members, which function independently of the other PcG proteins. In *Drosophila*, E(Z) and ESC are associated in a complex *in vivo* and interact with each other directly *in vitro* (19, 20). This association is conserved for their mammalian homologs, ENX and EED (28, 31–33), and here we report also for their worm homologs, MES-2 and MES-6.

The portion of MES-2 that is important for its interaction with MES-6 *in vitro* is the N-terminal 194 aa. Similarly, sequences within the N-terminal region of fly E(Z) (amino acids 34–66) and the mouse E(Z) homolog (amino acids 132–160) are responsible for their interactions with ESC homologs (19, 28). Although there is very little sequence similarity between the N termini of MES-2 and E(Z) homologs, their tertiary structure and the nature of the interaction between the partners might be conserved. The ESC-binding region of E(Z) is predicted to include a long stretch of helix (20), and the N-terminal 194 aa of MES-2 is predicted to form multiple long helices.

The conservation of the interaction between E(Z) and ESC among worms, flies, and mammals suggests that the molecular mechanism by which these protein partners function has been maintained throughout evolution. The MES-2/MES-6 complex therefore might regulate gene expression in *C. elegans* by the same mechanism used by the E(Z)/ESC complex in *Drosophila*. Indeed, Kelly and Fire (16) found that MES-2 and MES-6 participate in repressing gene expression in *C. elegans*, as E(Z) and ESC are known to do in *Drosophila*.

MES-3 Is a Member of the MES-2/MES-6 Complex in *C. elegans*. Although the interaction between E(Z) and ESC is conserved, E(Z)/ESC-related complexes are involved in different developmental processes in different organisms. In worms, MES-2 and MES-6 are expressed predominantly in the germ line, and *mes-2* and *mes-6* mutants display a maternal-effect sterile phenotype (1, 3, 4). In contrast, in flies, E(Z) and ESC are expressed ubiquitously in the soma (34, 35), and mutations in *E(z)* or *esc* cause a zygotic lethal or maternal-effect lethal phenotype, respectively (36, 37). This difference in developmental roles between organisms is likely to be due to different sites of expression and also to different cofactors that E(Z)/ESC-related complexes possess. Known cofactors are histone deacetylase in flies and mammals and the histone-binding protein p55 in flies (38, 39). In this paper, we have shown that in *C. elegans* embryos MES-3 is a cofactor with MES-2 and MES-6. MES-3 does not resemble any other known proteins and does not contain recognizable motifs (2). This suggests that MES-3 may be unique to *C. elegans* and may contribute to the germ line-specific role of the MES-2/MES-6 complex.



C

Hydrodynamic properties of the MES-2/MES-3/MES-6 complex

Sedimentation coefficient ($s_{20,w}$)	8.9 S
Stokes radius (R_s)	6.87, 6.99 nm
Molecular mass of the complex	252, 257 kD

Fig. 5. Analysis of the MES-2/MES-3/MES-6 complex by sucrose density gradient centrifugation and gel filtration. Embryo extract was layered on a 7–47% linear sucrose gradient and centrifuged (*A*) or fractionated by Sephacryl S-300 chromatography (*B*). Fractions were analyzed by SDS/PAGE and Western blot analysis using anti-MES-2 and anti-MES-3. The fraction numbers are indicated at the top. The peaks of migration of various protein standards are indicated by arrows. The calibration curve was constructed by plotting Stokes radii of reference proteins vs. $(-\log K_{av})^{1/2}$. The Stokes radius of the MES-2/MES-3/MES-6 complex was determined by interpolation using this curve. For details see *Materials and Methods*. (*C*) Summary of the hydrodynamic properties of the MES-2/MES-3/MES-6 complex. The complex comigrated with β-amylase (8.9S) in two separate sucrose gradient experiments. The results of two different gel filtration experiments are shown, along with the resulting molecular mass estimates.

The estimated mass of the MES-2/MES-3/MES-6 complex is ≈255 kDa. This estimate suggests that MES-2, MES-3, and MES-6, whose molecular masses add up to ≈230 kDa, are the sole components of the complex. Our *in vitro* binding assay did not detect a direct interaction between MES-3 and MES-2 or MES-6. If there is a direct association, it apparently requires conditions or protein modifications that were not achieved *in vitro*. Alternatively, another factor may mediate the interaction between MES-3 and MES-2 or MES-6. An additional factor could be accommodated by the estimated weight of the complex and could contribute, along with MES-3, to the germ line-specific function of the MES-2/MES-6 partnership.

This paper describes analysis of the MES complex obtained from *C. elegans* embryos. We do not know whether germ-line tissue contains similar or different complexes. Previous epistasis results (3, 4) showed that the normal distributions of MES-2 and MES-6 depend on each other at all stages of development. Interestingly, their distributions depend on MES-3 in embryos, but not in the adult germ line. Thus, there may be heterogeneity in the composition of MES-2/MES-6 complexes in different cells and/or at different stages. Epistasis results suggest that MES-4 is unlikely to be a component of the complex at any stage.

MES Proteins Function in Different Complexes. The four MES proteins are thought to be involved in a common process. Their sterile mutant phenotypes are similar (1–4, 40), and all four genes are involved in repressing germ-line expression of transgenes (16). Yet MES-4 appears to operate separately from the MES-2/MES-3/MES-6 complex. Similarly, in *Drosophila*, PcG proteins are thought to function in a similar process because they display similar mutant phenotypes and genetically interact. But they form at least two different complexes, an E(Z)/ESC complex and a Polycomb/polyhomeotic/Posterior sex combs complex, which are distinct from each other by both biochemical and immunolocalization criteria (13, 19–22). Immunolocalization and chromatin immunoprecipitation results suggest that the two complexes have some gene targets in common and some different targets (41, 42).

The targets of MES regulation in *C. elegans* are not yet identified. We think that at least some of the targets are on the X chromosomes. This prediction is based on the observation that XX (hermaphrodite) progeny from homozygous *mes* mothers are sterile, whereas their XO (male) sibling progeny are generally fertile (40). Consequently, a current model invokes that MES proteins repress the expression of certain genes on the X chromosomes, and that *C. elegans* germ-line development is highly sensitive to the levels of these gene products. In *mes* mutant germ lines, X-linked gene products are elevated to deleterious levels in XX animals, but not in XO animals. Nevertheless, there is no evidence to date for preferential accumulation of any of the MES proteins on the X chromosomes. More detailed experiments, such as DNA microarray analyses, need to be done to identify the targets of MES protein regulation and to address whether the MES-2/MES-3/MES-6 complex and MES-4 have different targets.

In conclusion, E(Z) and ESC appear to be ancient components of a protein pair, which have coevolved to serve different roles in different organisms. In *Drosophila*, E(Z) and ESC homologs operate as part of a ≈600-kDa complex and in concert with numerous other PcG proteins to serve essential roles in somatic development. In *C. elegans*, E(Z) and ESC homologs operate in a smaller complex, with MES-3 and perhaps one other component, to serve essential roles in germ-line development. Notably, *Arabidopsis* resembles *C. elegans* in containing E(Z) and ESC homologs but no other recognizable PcG homologs, and in requiring its E(Z) and ESC homologs for reproductive development (29, 30, 43, 44). Ultimately, identification and analysis of other components of E(Z)-ESC-related complexes across species will shed light on how different protein partners contribute to the specialized functions of these complexes.

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