Mutational Analyses of fs(1)Ya, an Essential, Developmentally Regulated, Nuclear Envelope Protein in Drosophila

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ABSTRACT

The fs(1)Ya protein (YA) is an essential, maternally encoded, nuclear lamina protein that is under both developmental and cell cycle control. A strong *Ya* mutation results in early arrest of embryos. To define the function of YA in the nuclear envelope during early embryonic development, we characterized the phenotypes of four *Ya* mutant alleles and determined their molecular lesions. *Ya* mutant embryos arrest with abnormal nuclear envelopes prior to the first mitotic division; a proportion of embryos from two leaky *Ya* mutants proceed beyond this but arrest after several abnormal divisions. *Ya* unfertilized eggs contain nuclei of different sizes and condensation states, apparently due to abnormal fusion of the meiotic products immediately after meiosis. Lamin is localized at the periphery of the uncondensed nuclei in these eggs. These results suggest that YA function is required during and after egg maturation to facilitate proper chromatin condensation, rather than to allow a lamin-containing nuclear envelope to form. Two leaky *Ya* alleles that partially complement have lesions at opposite ends of the YA protein, suggesting that the N- and C-termini are important for YA function and that YA might interact with itself either directly or indirectly.

THE nuclear envelope is a complex structure controlling all macromolecule exchange between nucleus and cytoplasm (reviewed in GERACE and FOISNER 1994). It has also been shown to play multiple roles in DNA replication and in the coordination of DNA replication and mitosis (reviewed in DINGLE and LASKEY 1992). One of the major components of the nuclear envelope is the nuclear lamina, which provides the structural framework for the nuclear envelope and anchoring sites for interphase chromosomes (reviewed in GERACE 1986). Developmental variations of the major lamina protein, lamin, have been reported in several vertebrate systems (KROHNE and BENAVENTE 1986; FURUKAWA and HOTTA 1993). For example, in Xenopus, both L_I and L_{II} lamins are present in many somatic cells, while germ cell specific lamins L_{III} and L_{IV} are only found in diplotene oocytes and spermatids, respectively. However, the mechanism by which the nuclear envelope might be modified to respond to developmental needs are not clear. One way to approach this is to study developmentally regulated nuclear envelope proteins that play essential roles. The Drosophila *fs(1)Ya* [*Young arrest* (*Ya*)] gene product is such a molecule. YA is the only example of a non-lamin-like protein in the nuclear lamina that is developmentally modulated, and its function is essential for initiating early embryonic mitotic divisions (LIN and WOLFNER 1991; LOPEZ *et al.* 1994).

The *Ya* gene encodes a novel protein that is found in only late stage oocytes, eggs, and 0–2 hr cleavage stage embryos (LIN and WOLFNER 1991; SONG 1994). In unfertilized eggs, the YA protein is located in the nuclear envelopes of the four meiotic products before they are condensed and fused (LOPEZ *et al.* 1994). It is recruited to the nuclear envelope of the male pronucleus upon fertilization (LOPEZ *et al.* 1994). During the rapid and synchronous cleavage divisions, YA is localized to the nuclear envelopes of both the cleavage nuclei and the nondividing yolk nuclei, and its localization to the cleavage nuclei is cell cycle stage dependent (LIN and WOLFNER 1991; LOPEZ *et al.* 1994). The nuclear events in eggs and early embryos have some special features. These include fusion of the polar bodies, coordination of the two pronuclei during the first mitotic division (gonomeric division), and coordination of the rapid and synchronous nuclear events in a syncytium during the cleavage divisions. YA might be required for these early nuclear events based on its nuclear envelope localization in eggs and early embryos.

Previously, we reported the characterization of one *Ya* mutant allele, Ya^2, which behaves like an apparent null mutation. The phenotype that we characterized was postfertilization. It showed that most embryos from homozygous Ya^2 females ("Ya^2 embryos") were arrested at the pronuclear stage, with a small proportion arrested with multiple abnormal nuclei. This suggested that YA function is required for the first and probably subsequent mitotic divisions (LIN and WOLFNER 1991).

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In this study, we extended our characterization in three ways. First, we characterized the molecular lesions and phenotypes of four *Ya* alleles (including Ya^2). Second, we used these alleles to examine more closely the postfertilization phenotype. We found that the Ya^2 embryos arrested with abnormal multiple nuclei are probably in the process of dying. However, among embryos with partially functional *Ya,* some proceed beyond pronuclear arrest and arrest prior to blastoderm stage. Finally, we examined the behavior of nuclei in *Ya* unfertilized eggs. We found that *Ya* mutant eggs start to show abnormalities immediately following meiosis. The defect in the mutants appeared to be due to a problem in the coordination of chromatin condensation states and fusion of the pronuclei or polar bodies, rather than to one in the formation of the lamin-containing nuclear envelope.

MATERIALS AND METHODS

Drosophila strains: All marker mutations and balancer stocks are described in LINDSLEY and ZIMM (1992). y^2 *fs(1)*Ya^2 w^{hf} *spl* sn^3/*FM6* and y^2 *fs(1)*Yb^1 w^{hf} *spl* sn^3/*FM6* flies (JUDD and YOUNG 1973) were gifts of B. JUDD. Marked strains carrying other *Ya* mutant alleles, Ya^{76}, Ya^{70}, Ya^{77} (MOHLER 1977; originally named *fs(1)*$M12^{13B\text{-}76}$, *fs(1)*$M12^{13\text{-}1970}$ and *fs(1)*$M12^{14\text{-}77}$/ respectively) were kindly provided by Drs. D. MOHLER and R. NAGOSHI. Oregon R P2, a strain unable to hold mature eggs (ALLIS *et al.* 1977), was obtained from Dr. R. J. MACINTYRE and used in all the experiments as wild-type controls. The scheme for testing whether Ya^{76} is null is the same as that described in LIN and WOLFNER (1991). The *Df(1)wrj1* deletion, which uncovers the 3A2–3C2 region, was used for testing Ya^{76}/*Df* embryos and Ya^2/*Df* eggs. Germlineless males were generated as the progeny of *tud*^{1} *bw sp* females (BOSWELL and MAHOWALD 1985) and were used to stimulate egg-laying in virgin females, as described in KALB *et al.* (1993). Each *Ya* mutant was tested for complementation of Ya^2, Ya^{76}, Ya^{70} and Ya^{77}; fertility was measured as described in LIN and WOLFNER (1991).

DNA sequencing, polymerase chain reactions (PCR): The following primers were synthesized at the Oligonucleotide Synthesis Facility at Cornell University and were used for all the sequencing and PCR analyses for identifying the lesions of *Ya* mutant alleles: the sense oligonucleotides KLP1 (CGTGCAAAGGTGTATGTCCATG, position 11–32), KLP3 (GAGCAGCCAACAGATGCGATCG, position 1452–1473), KLP5 (CGTGAATCCGTCCGAAACGATG, position 687–708), KLP7 (GCGAGAGCGTCGAGATCATACG, position 1322–1343), and the antisense oligonucleotides KLP2 (GCAGTGGTGTGGACGTCGAGCG, position 748–727), KLP4 (CTACTGGCGACGCATGCGCTCG, position 2151–2130), KLP6 (CCTGGCGATGACGACGCTCTGG, position 1558-1537), KLP8 (GTCGCATCATGGAACTCATCAG, position 1864–1843), KLP9 (GAAGACACGCTTGCAAATGTGG, position 132–111), and the M13 −20 universal primer. Positions of all the oligonucleotides are based on the numbering system for the *Ya* cDNA, as in LIN and WOLFNER (1991).

Genomic DNA sequences from −809 to +2130 were determined for cloned DNAs from Ya^2 and a Ya^+ chromosome from the same mutagenesis (carrying *fs(1)*Yb^1) (JUDD and YOUNG 1973). Genomic sequences from PCR-amplified *Ya* genes for Ya^{70}, Ya^{76}, and Ya^{77} were also determined using double stranded DNA sequencing. The PCR reactions were performed in a final volume of 50 μl, with slight modifications of the PCR protocol from Perkin-Elmer Cetus. The final concentration of $MgCl_2$ varied from 1.5 to 2.5 mM, depending on the specific sets of primers used. Amplification conditions were 1 min at 94°, 1 min at 50°, and 2 min at 72° for 10 cycles, and then 1 min at 94°, 1 min at 60°, and 2 min at 72° for 40 cycles, which was followed by 10 min at 72°. The amplification products were then made blunt with T4 DNA polymerase (BRL) and cloned into PUC19 for double-stranded DNA sequencing analyses. To eliminate errors from the PCR reaction, products from at least three different reactions were sequenced for each mutant and each set of primers. Since Ya^{70}, Ya^{76} and Ya^{77} were from the same genetic background, they served as controls for one another.

In the process of sequencing the *Ya* mutant genomic sequences, we discovered several substitutional errors and one single-base-pair insertion in the previously reported *Ya* cDNA sequence (LIN and WOLFNER 1991). The updated cDNA sequence is 2266 bp long and is in the Genbank database under the accession number M38442. The modified open reading frame for YA starts from the 61st base pair and differs from the originally reported sequence at the N-terminus in amino acids 1 to 26. It contains 696 amino acid residues, with a predicted molecular weight of 77 kD. The updated sequence still shows no significant similarity to sequences in the EMBL/Genbank/SwissProt/Pir databases using the FASTA program.

Immunofluorescence microscopy: Unfertilized eggs or embryos were collected from heterozygous or homozygous *Ya* mutant animals grown on yeast-glucose medium containing 30 μg/ml tetracycline (GLOVER *et al.* 1990). As in LIN and WOLFNER (1991), "*Ya* embryos/eggs" means embryos/eggs from homozygous *Ya* mothers. The eggs or embryos were then stained with DAPI or propidium iodide, affinity purified anti-YA (against the C-terminal 243 amino acids of the Ya protein; LIN and WOLFNER 1991) and/or anti-lamin antibodies (SMITH and FISHER 1989). They were then examined under a Zeiss Axioskop or by confocal microscopy, as detailed in LOPEZ *et al.* (1994). Unfertilized eggs were obtained from 0–15 min and 0–2 hr collections. To characterize the *Ya* mutant embryonic phenotype, we collected embryos from *Ya* mutant females in 0–15 min, 0–30 min, 0–2 hr collections. For some experiments described below, the embryos were then aged at room temperature. The 0–15 min and 0–30 min embryos were aged to 1 hr 45 min to 2 hr old or to 5–7 hr old, and the 0–2 hr embryos were aged to 5–7 hr old. At least 100 embryos or unfertilized eggs were stained and analyzed for each time point.

Western blotting analysis: YA protein in extracts of 0–2 hr embryos from Ore R P2 females or females homozygous for *Ya* mutant alleles was examined by Western blotting. Western blots were probed with affinity purified anti-YA antibodies as described in LIN and WOLFNER (1991). Bradford assays (Bio Rad) were performed to standardize loadings. Mouse anti-lamin monoclonal antibodies and affinity purified rabbit anti-lamin polyclonal antibodies (RISAU *et al.* 1981; SMITH and FISHER 1989), which were kind gifts of Drs. H. SAUMWEBER and P. FISHER, respectively, were also used to probe the Westerns as controls. For detection, the enhanced chemiluminescence (ECL) Western Blotting System (Amersham Corp.) was used as described in the instruction manual.

RESULTS

There are four existing mutant alleles of *Ya,* all induced by EMS: Ya^2 (JUDD and YOUNG 1973), Ya^{76}, Ya^{70}, and Ya^{77} (MOHLER 1977; MATERIALS AND METHODS). All four alleles are completely recessive, strict maternal effect mutations. Females homozygous for each mutation are completely sterile. Among the four alleles, Ya^2 and

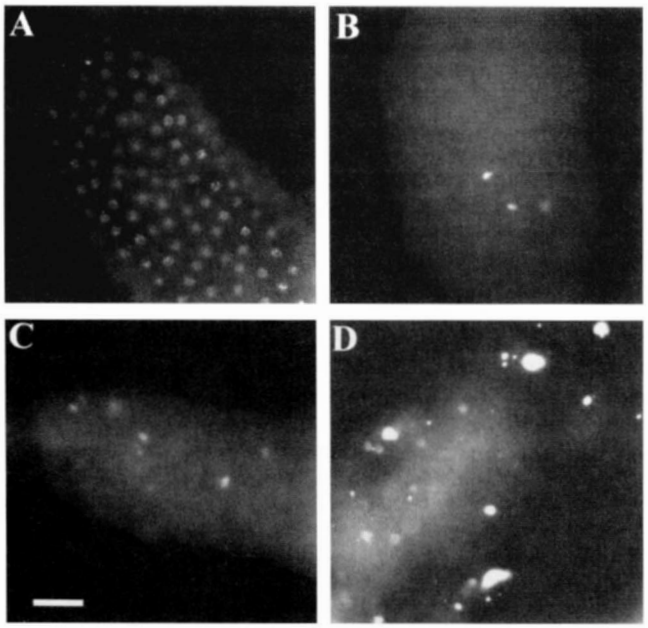

FIGURE 1.—Phenotypes of arrested *Ya* mutant embryos. Embryos (0–2 hr) from wild-type or *Ya* mutant alleles were stained with DAPI and examined. (A) A wild-type embryo in late cleavage stage. (B) A pronuclear arrested *Ya* mutant embryo from Ya^2. (C) A type II abnormal multiple-nuclear-arrested *Ya* embryo from Ya^{70} in which fewer than 16 nuclei were observed with different size and distribution. (D) A type I abnormal multiple-nuclear-arrested embryo from Ya^{77}, with very spotty and fragmented DNA. Bar, 120 μm and applies to all panels.

Ya^{76} produce embryos with the most severe phenotype and appear to be amorphic alleles (LIN and WOLFNER 1991 and below). Neither of these alleles complements any other *Ya* allele. Ya^{70} and Ya^{77} partially complement each other for fertility (MOHLER 1977 and below). To understand the function of YA in the nuclear envelope, we first characterized in detail the embryonic phenotype and molecular lesions of each *Ya* allele.

Distribution of nuclear phenotypes in embryos from *Ya* mutant alleles: Embryos collected in a 0–2 hr period from females homozygous for the mutant alleles were stained with DAPI, and their nuclear phenotypes were compared with those of control embryos (see MATERIALS AND METHODS). While the control embryos developed to late cleavage stages (Figures 1A and 2A), mutant embryos from all *Ya* alleles were arrested either with multiple abnormal nuclei or at what we call the "pronuclear stage," with fewer than four abnormally condensed nuclei presumably derived from the sperm and female meiotic products (Figure 1, B–D). This arrested phenotype is similar to that previously described for arrested Ya^2 embryos (LIN and WOLFNER 1991). However, the proportion of pronuclear-arrested and multiple-abnormal-nuclear-arrested embryos differed between alleles (Figure 2 and below).

Ya^2 and Ya^{76}: Embryos from Ya^{76} females arrested with a similar distribution of nuclear phenotypes to those of Ya^2 (Figure 2). In a 0–2 hr collection, 87% of the Ya^{76} embryos were pronuclear-arrested, and 13% had multiple abnormal nuclei. These percentages are similar to those previously reported for Ya^2 (LIN and WOLFNER 1991 and Figure 2), suggesting Ya^{76} is a nonfunctional allele. Consistent with this, a similar distribution of embryo phenotypes was observed for embryos from Ya^{76}/Df females as for Ya^{76}/Ya^{76} females (data not shown).

The embryos with multiple abnormal nuclei could result from either of two causes: they could be embryos that progressed beyond pronuclear arrest and then divided abnormally, or they could be embryos that arrested in the pronuclear stage and were in the process of degenerating. To distinguish between these two possibilities, we collected embryos for very short times (0–15 or 0–30 min) and then stained some of them. We aged the rest to 2 hr or to 5–7 hr and stained them. The results are summarized in Figure 2. Ya^2 and Ya^{76} gave identical results. The percentage of multiple-nuclear-arrested embryos increased when the embryos were aged longer. For example, only 3% of the Ya^2 embryos had multiple abnormal nuclei in the 0–15 min collection. When these embryos were aged to 2 hr, the percentage of multiple-nuclear-arrested embryos increased to 12%, and to 84% when aged to 5–7 hr (Figure 2B). In addition, all the embryos arrested with multiple abnormal nuclei had very spotty and fragmented DNA staining. The size of each abnormal nucleus varied dramatically and the number of the nuclei in these embryos were impossible to count. We called them type I multiple-nuclear-arrested embryos (Figure 1D). We believe they represent dying embryos that are in the process of degenerating after the pronuclear arrest.

Therefore, embryos from the apparent null *Ya* alleles were either arrested at the pronuclear stage, or appeared to be in the process of dying after pronuclear arrest.

Ya^{70} and Ya^{77}: The Ya^{70} and Ya^{77} embryos had phenotypes that appeared to be leakier than those of Ya^2 and Ya^{76}. In a 0–2 hr collection, only 69% of Ya^{70} and 67% of Ya^{77} embryos arrested with pronuclear-arrested phenotype. The rest arrested with multiple abnormal nuclei. When these embryos were examined after aging of short collections, the percentage of multiple-abnormal-nuclear-arrested embryos also increased (Figure 2C). However, there were two types of embryos with multiple abnormal nuclei for these two alleles. The first (type I) had the same phenotype as the multiple-nuclear-arrested embryos from Ya^2 and Ya^{76}, which are likely to be dying embryos as mentioned above. A second type (type II) of multiple-nuclear-arrested embryos was seen only in Ya^{70} and Ya^{77}. Type II embryos (Figure 1C) had fewer than 16 nuclei, which were not fragmented and were countable. Even in long collections we did not observe more nuclei. However, the nuclei were of different sizes and their distribution in the embryos was irreg-

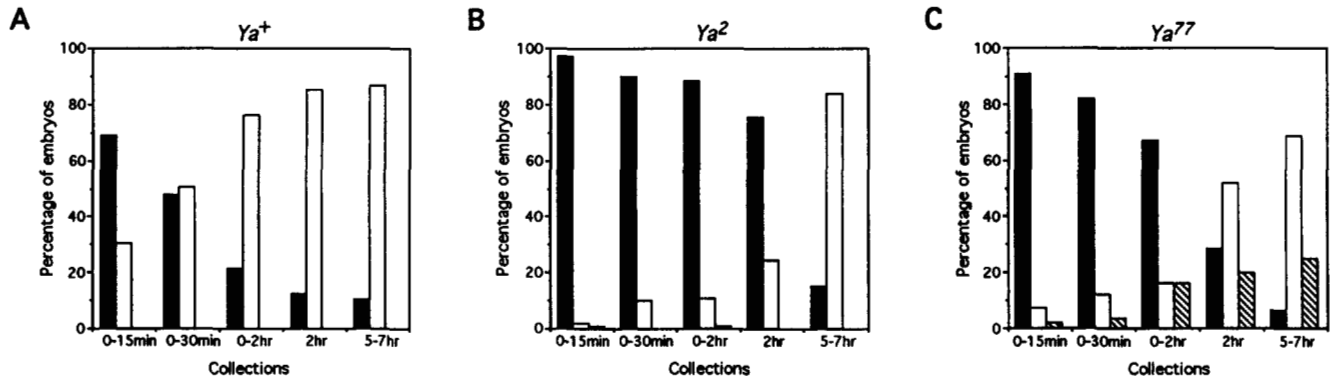

FIGURE 2.—Changes in nuclear phenotype distribution of wild-type and *Ya* mutant embryos when aged. As described in MATERIALS AND METHODS, 0–15 min, 0–30 min, 0–2 hr embryos were collected from females heterozygous or homozygous for *Ya* alleles. Half of the embryos were fixed and stained with DAPI. The other half were aged either to 2 hr or 5–7 hr and then fixed and stained with DAPI. At each time point, the numbers of embryos arrested at each stage were counted. Data from several collections were pooled and graphed as percentage of embryos arrested at each stage. For wild-type controls, embryos with fewer than four nuclei were considered as being in the pronuclear stage (■), and the ones at late cleavage (aged to 2 hr) or gastrulation (aged to 5–7 hr) stages were scored as having multiple nuclei (□). For mutants, ■ represents pronuclear arrested embryos; □ and ▨ represent embryos arrested with type I and type II abnormal multiple nuclei, respectively. For wild-type controls, embryos with fewer than four nuclei were counted as normal and being in the pronuclear stage, while the ones with multiple nuclei are also normal and are at late cleavage stages (aged to 2 hr) or gastrulation (aged to 5–7 hr). Ya^{76} embryos have a similar distribution of embryo phenotypes to that of Ya^{2}, and Ya^{70}, similar to Ya^{77}, except for the proportion of embryos in each class (see RESULTS).

ular. These embryos never develop to blastoderm stage. We believe that type II embryos are ones that progress beyond pronuclear arrest and have abnormal subsequent mitotic divisions. Under this interpretation, Ya^{70}, with a higher proportion of type II embryos (21.7%), retains more YA function than Ya^{77} (16.2% of type II embryos), which in turn has more YA function than Ya^{2} and Ya^{76}. The presence of type II embryos suggests that YA function might also be required for subsequent mitotic divisions.

Ya^{70} and Ya^{77} partially complement: Homozygous females carrying either the Ya^{70} or the Ya^{77} mutations are completely sterile; however, the sterility is partially restored when they are in heteroallelic combination (MOHLER 1977 and this work). In 0–2 hr collections of embryos from Ya^{70}/Ya^{77} females, 15% of the embryos developed to cleavage stages and eventually into adults. The rest of the embryos remained arrested, with 50% arrested at the pronuclear stage and 35% arrested with type II abnormal multiple nuclei. Therefore, Ya^{70} and Ya^{77} show partial intragenic complementation.

Identification of the molecular lesions in *Ya* mutant alleles: Analyses of the embryonic phenotype of the four *Ya* alleles indicated that two alleles (Ya^{2} and Ya^{76}) are nonfunctional and the other two (Ya^{70} and Ya^{77}) are partially functional. We wished to identify the lesions that resulted in the impairment of *Ya* function in these mutants. Genomic Southern blotting indicated that all four EMS-induced *Ya* alleles showed no gross alterations of *Ya* DNA (G. HUTCHESON, J. LIU and M. F. WOLFNER, data not shown). Northern blot analyses also indicated that all four mutants made *Ya* RNAs of the normal size and amount (M. PARK, G. HUTCHESON and M. F. WOLFNER, data not shown). These results suggested that the mutations were small lesions, possibly single base pair mutations. We therefore compared the sequence of each *Ya* mutant allele with *Ya* sequences from the chromosome in which it was induced (see MATERIALS AND METHODS). We also probed Western blots for mutant YA proteins and compared them with wild-type YA protein. In wild type, YA protein is present in 0–2 hr embryos and unfertilized eggs, and has a mobility of 96 and 98 kD on SDS-PAGE gels (Figure 3; SONG 1994).

In addition to identifying the lesions of the alleles, our sequence analyses revealed several features of the *Ya* gene. First, *Ya* contains a single 57-bp intron between position 102 and 103 of the cDNA (Figure 3). The intron is flanked by consensus splice donor and acceptor sites and contains two sites that match the consensus for a branch site (MOUNT 1982; SHARP 1987). There are several cryptic splice donor sites upstream of the intron, suggesting that the *Ya* mRNA could be differentially spliced. Second, Motifs searches of the updated sequence identified two potential Cysteine 2-Histidine 2-type zinc fingers at the N-terminus. Such Cys2-His2-type zinc fingers are known motifs for DNA and/or RNA binding (for reviews, see BERG 1990; COLEMAN 1992). Third, we found several apparent silent amino acid differences between the *Ya* cDNA and the genomic DNA from the mutant alleles (Figure 3 legend).

The DNA sequence of each allele revealed that each mutation is indeed a point mutation. The only lesion in Ya^{2}, a putative null allele (LIN and WOLFNER 1991), is an A to T transversion at position 1450, which creates a stop codon (TAG) in place of a lysine (AAG) of amino acid 464 (Figure 3). The YA protein encoded by Ya^{2} would be expected to lack its terminal 233 amino acids,

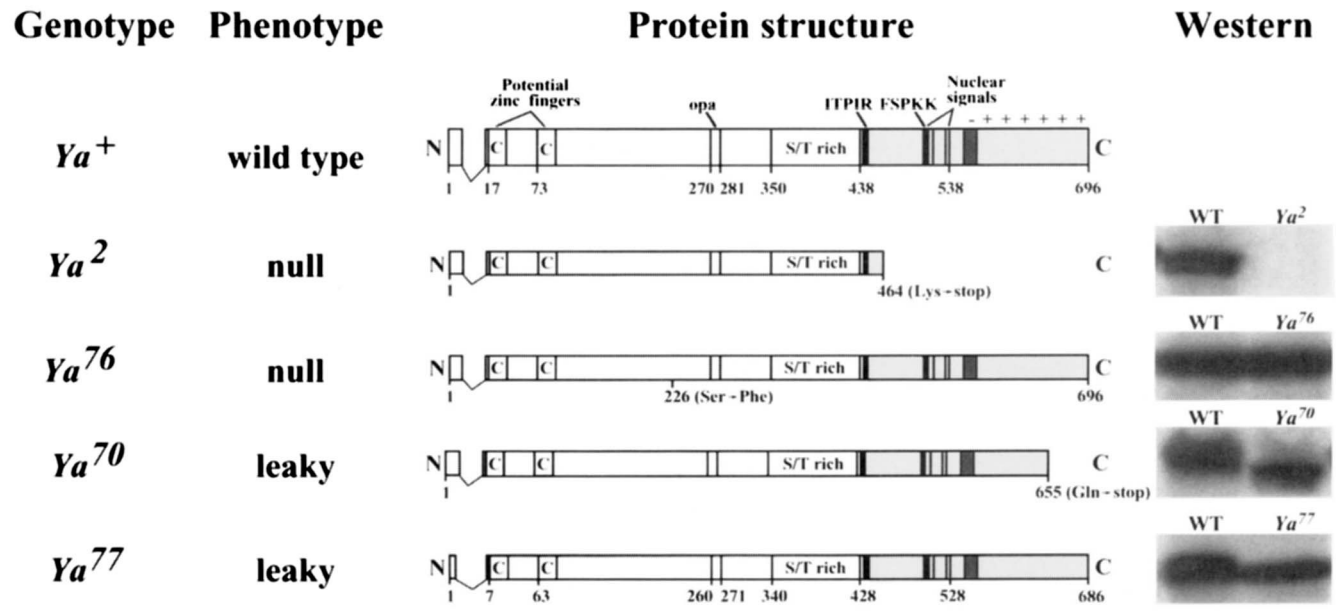

FIGURE 3.—A schematic presentation of the wild-type and mutant YA protein sequences and their expression pattern. The YA protein is encoded by two exons, with a 57-bp intron in between symbolized by "\/." The intron sequence is: GTAGGCTCCAAACGCATCCAAACGTTCCATCCGTAGTGACTCCACCTTGATTTCCAG, with the 5′ and 3′ splice sites and the branch sites underlined. The positions of the *Ya* mutants are indicated as cartoons of the predicted YA proteins and amino acid numbers are below the sequence. "C"s stand for two potential Cys2-His2 type zinc fingers. "opa" indicates the Gln rich region. "S/T rich" represents the Ser/Thr rich region. "ITPIR" and "FSPKK" are two potential MPF phosphorylation target sites, which are indicated as solid bars. "-" represents a region rich in acidic amino acids, and the "+"s represent the highly positively charged C-terminal region. The shaded area is the part of the protein (the C-terminal 243 amino acids) used to generate anti-YA antibodies. Western blots showing YA proteins from wild-type and mutant 0–2 hr embryos are also shown. The mutation of Ya^{77} is at the 5′ splice donor site. The predicted protein structure shown here is a hypothetical one using a cryptic upstream splicing donor site, thus resulting in a 10 amino acid deletion at the N-terminus of the YA^{77} protein. Several base pair changes relative to the cDNA sequence were observed in the course of sequencing the four mutant alleles. At positions 522, 714, 969, and 1203, respectively, there are A to C, G to A, A to G, and A to G changes in the Mohler mutants relative to the cDNA sequence. At positions 1465 and 1471, there are C to G and A to G changes, respectively, from the Judd lab strains, relative to the cDNA sequence. None of these changes alter the encoded amino acids. At position 1294, all the mutant strains have a G, whereas the cDNA, which makes a functional protein (LOPEZ *et al.* 1994), has an A. This results in a Lys to Glu substitution at amino acid 412. At position 1313, the functional YA protein from the Judd lab strain has a T whereas the other sequences have an A; the Judd strain thus has a Phe substitution for a Tyr at amino acid 418. These changes appear to be due to genetic background and have no effect on YA function, since they are present in both the mutant and the control genomic sequences.

including the potential nuclear localization signals and the polar C-terminus. Consistent with this, the YA^2 protein was not detected using our antibodies (Figure 3), which are directed against the C-terminal 243 amino acids of the YA protein (LIN and WOLFNER 1991). We suspect this is because the last 10 amino acids in the YA^2 protein do not confer strong antigenicity, though it is also possible that the YA^2 protein is not stable and might have been degraded.

The other putative null allele, Ya^{76}, is a missense mutation. A transition of C to T at position 737 caused a serine to a phenylalanine change at amino acid 226 (Figure 3). The size and the abundance of the YA^{76} protein from the mutant embryos are normal (Figure 3).

One of the leaky alleles, Ya^{70}, is a nonsense mutation, due to a C to T transition at position 2023. This change from a codon encoding glutamine to a stop codon at amino acid 655 would cause a 41 amino acid truncation of the mutant protein at the C terminus (Figure 3). As predicted from its sequence, the YA^{70} protein is 4 kD smaller than wild-type YA protein. It runs as a doublet of molecular weight of 92 and 94 kD (Figure 3) and is also of normal abundance.

The mutation in Ya^{77}, the other leaky allele, is an apparent change in the 5′ splice donor site. The consensus 5′ splice donor site GT is changed to AT in Ya^{77} (Figure 3). This would potentially allow an upstream cryptic splice donor site to be used, and thus change the N-terminal amino acids of the YA protein. On Western blots of proteins prepared from Ya^{77} mutant embryos, only a single YA protein band of 96 kD was seen (Figure 3). This suggests that alternative splicing might be the cause of the presence of two forms of YA protein in wild-type embryos.

Since Ya^{70} and Ya^{77} complement each other, the phenotypic and sequence analyses of these alleles suggest that N-terminal mutations can complement C-terminal mutations. Consistent with this, a nonfunctional N-terminal epitope-tagged YA construct was able to partially complement a nonfunctional C-terminal tagged one (J.

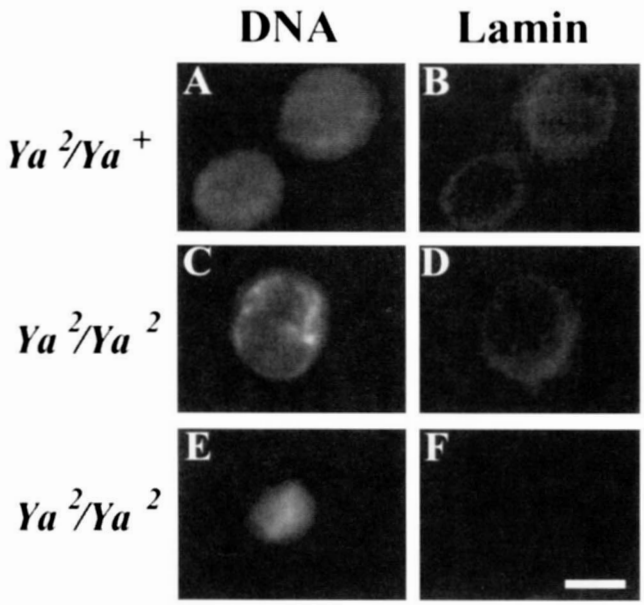

FIGURE 4.—Immunofluorescence staining of Ya^2 unfertilized eggs. Unfertilized eggs (0–15 min) from females homozygous or heterozygous for Ya^2 were collected and stained with DAPI and Rhodamine-conjugated anti-lamin. DAPI staining is shown in A, C, and E; B, D, and F show anti-lamin antibody staining. Two out of four meiotic products on the same focal plane from Ya^+/Ya^2 eggs are shown in A and B. Lamin is in the nuclear envelope of all four meiotic products. Two nuclei from Ya^2/Ya^2 eggs are shown in C–F. One of the nuclei is bigger and uncondensed, and it has lamin staining in the nuclear envelope (C and D). The DNA staining of this nucleus appears brighter in our photographs since it is near the surface of the egg. The other one is smaller and without nuclear envelope lamin staining (E and F). Bar, 10 μm.

LIU and M. F. WOLFNER, unpublished results), further supporting the idea that YA might function by interacting with itself either directly or indirectly.

***Ya* mutants show abnormalities in unfertilized eggs:** With the above detailed characterization of the four mutant alleles, we could start to use these alleles to ask further how YA functions in the nuclear envelope. Characterization of *Ya* mutant embryos (presented above) suggests that *Ya* mutations affect the first and probably subsequent mitotic divisions. We therefore decided to look at unfertilized *Ya* eggs to find out when the effect of lacking *Ya* function is first detected.

Unfertilized eggs (0–15 min) were collected from virgin females homozygous for the apparent null Ya^2 mutation. As controls, unfertilized eggs from females heterozygous for Ya^2 were also collected (see MATERIALS AND METHODS). In control unfertilized eggs, completion of meiosis I and II results in four equal-sized haploid meiotic products (DOANE 1960 and this work). Their chromosomes first become uncondensed (Figure 4A). Then the four nuclei condense their chromosomes and two or three of them start to fuse with one another (DOANE 1960 and this work). Eventually they fuse and form a star-shaped polar body. There are two features noticeable about this process. First, the nuclei are always synchronous, either all with their chromatin condensed or all with it uncondensed. Second, whenever a fusion is seen, all the nuclei have condensed chromatin.

Ya^2 unfertilized eggs do not follow the normal course. Instead, at the stage when Ya^2 eggs contain nuclei with uncondensed chromatin, they usually contain only two, rather than four nuclei (Figure 4, C and E). These two nuclei are of different sizes and, sometimes, their chromatin is of different condensation states, with that of the larger nucleus being uncondensed in most of those cases. Unfertilized eggs from Ya^2/Df females looked no different from those from Ya^2/Ya^2 females (data not shown), suggesting that the abnormal phenotype of Ya^2/Ya^2 unfertilized eggs is indeed due to the *Ya* mutation. As mentioned in the DISCUSSION, results from *in situ* hybridization using probes to specific chromosomes indicated that the bigger nucleus in Ya^2 unfertilized eggs has the DNA content of three haploid nuclei and the smaller nucleus, of one (J. M. LOPEZ, A. DERNBERG and M. F. WOLFNER, unpublished results). This is inconsistent with a meiotic block, suggesting that meiotic segregation is normal in Ya^2 unfertilized eggs, but that postmeiotic coordination between chromatin condensation and nuclear fusion is abnormal. The meiotic products in longer collections (2 hr) of Ya^2 unfertilized eggs can still fuse into one nucleus eventually, but its chromatin looks abnormally condensed (data not shown).

Localization of lamin and the mutant YA proteins in *Ya* mutant embryos and unfertilized eggs: As the first step toward understanding how YA functions in the nuclear envelope to coordinate chromatin condensation and nuclear fusion, we asked whether YA is required as a structural component for the formation of the nuclear envelope. To test this, we stained 0–15 min Ya^2 unfertilized eggs for lamin localization.

In wild-type unfertilized eggs, lamin is localized to the nuclear envelopes of the four meiotic products when their chromatin is uncondensed (Figure 4B). As described above, most Ya^2 unfertilized eggs contained only two nuclei of different sizes and chromatin condensation states (Figure 4, C and E). When they were stained for lamin, the nucleus with uncondensed chromatin, but not the one with condensed chromatin, also had lamin in the nuclear envelope (Figure 4, D and F). Thus, without functional YA protein, lamin can be localized to the nuclear envelope, at least to the nucleus with uncondensed chromatin, in *Ya* unfertilized eggs. These results suggest that YA is not required for the formation of the lamin-containing nuclear envelope.

When we stained *Ya* mutant embryos, however, we found that neither YA nor lamin was detected around the periphery of any nuclei in Ya^2, Ya^{76}, Ya^{70}, and Ya^{77} embryos (Figure 5, A and B). Western blots indicated that YA protein was present in normal amounts in Ya^{76}, Ya^{70}, and Ya^{77} embryos (Figure 3; we could not determine this for Ya^2, see above) and lamin was also present in normal amounts in all these embryos (Figure 5C).

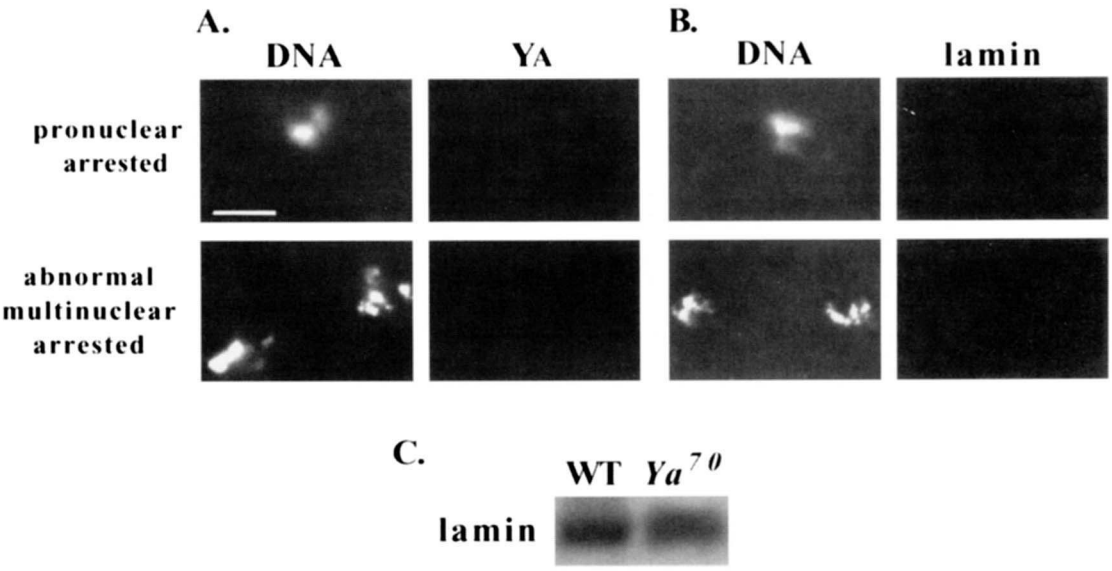

FIGURE 5.—Localization of lamin and the mutant YA proteins in embryos from the *Ya* mutant alleles. Embryos (0–2 hr) from homozygous *Ya* mutant females were stained with DAPI and anti-YA antibodies (A), or with DAPI and anti-lamin antibodies (B) under identical conditions. The top panels show the staining pattern of pronuclear-arrested embryos from Ya^{76}, and the bottom panels are the staining patterns of the embryos arrested with abnormal multiple nuclei from Ya^{70}. Neither YA nor lamin localization is observed in these embryos. The pronuclear-arrested embryos from Ya^{2}, Ya^{70} and Ya^{77} and abnormal multiple-nuclear-arrested embryos from Ya^{2}, Ya^{76} and Ya^{77} show the same staining pattern (data not shown). Bar, 10 μm and applies to all panels. (C) A Western blot of 0–2 hr embryo extracts from wild-type and Ya^{70} mutant probed with anti-lamin antibodies. Equal amount of protein was loaded in each lane according to Bradford assays. Normal amounts of lamin are present in Ya^{70} mutant embryos. Results for Ya^{2}, Ya^{76}, and Ya^{77} embryos are the same (data not shown).

Therefore, the lack of YA and lamin nuclear envelope staining in *Ya* mutant embryos suggests that the nuclear envelope in *Ya* mutant embryos is abnormal. Since lamin is localized to the nuclear envelope in *Ya* unfertilized eggs, the abnormal nuclear envelope staining in *Ya* mutant embryos is likely a result of an earlier defect due to lack of YA function.

Consistent with this, when we stained 0–2 hr embryos from Ya^{70}/Ya^{77} females for lamin and YA, we found that the 15% of embryos that developed to late cleavage stages because of the interallelic complementation had both lamin and YA localized to the nuclear envelope (Figure 6). However, there was no lamin or YA staining in the nuclear envelope of arrested Ya^{70}/Ya^{77} embryos, similar to that of the arrested *Ya* mutant embryos (data not shown, see Figure 5, A and B).

Taken together, the above results suggest that a normal nuclear envelope is not present in arrested *Ya* mutant embryos. However, this abnormality is likely to result from an earlier defect caused by the lack of YA function. The results also suggest that YA's function in the nuclear envelope is not for the formation of a lamin-containing nuclear envelope.

DISCUSSION

We have reported here further genetic and molecular characterization of YA. Our detailed analyses of the lesions and effects of four *Ya* alleles have extended our understanding of the function of YA in the nuclear envelope in several ways. Phenotypic analyses of *Ya* mutant eggs and embryos suggest a role of YA during and after egg maturation. Our data suggest that it functions in the nuclear envelope to enable proper chromatin condensation of the nuclei. In addition, determination of

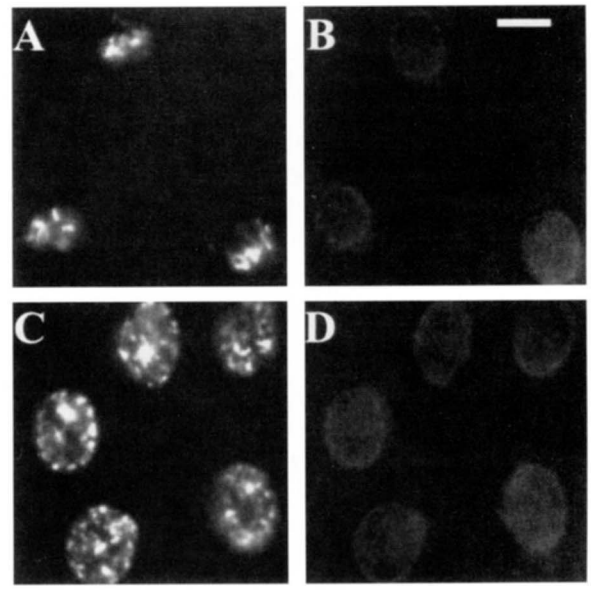

FIGURE 6.—Localization of YA and lamin in Ya^{70}/Ya^{77} embryos. Embryos (0–2 hr) that are at late cleavage stage from Ya^{70}/Ya^{77} females were fixed and stained with DAPI (A and C) and Rhodamine-conjugated anti-YA (B) or anti-lamin (D) antibodies. In these embryos, both lamin and YA are localized to the nuclear envelope. Bar, 20 μm.

the mutant lesions has begun to shed light on the nature of this essential nuclear envelope protein.

Egg maturation in *Drosophila melanogaster* occurs independent of fertilization (reviewed in FOE *et al.* 1993). Without fertilization, completion of meiosis I and II produces four meiotic products, whose chromatin first becomes decondensed. These nuclei then condense their chromatin and fuse (DOANE 1960 and this work). Unlike control eggs, unfertilized *Ya* eggs from 0–15 min collections contain nuclei of different sizes and chromosome condensation states (Figure 4). The size difference could result from either abnormal segregation during meiosis or from abnormal fusion of the meiotic products. Using probes to specific chromosomes, *in situ* hybridizations indicated that the bigger nucleus in Ya^2 unfertilized eggs has the DNA content of three haploid nuclei and the smaller nucleus, of one (J. M. LOPEZ, A. DERNBERG and M. F. WOLFNER, unpublished results). This suggests that meiotic segregation is normal in Ya^2 unfertilized eggs, but that fusion of the meiotic products is abnormal, occurring too early, before coordination of the chromatin condensation states of the nuclei. Therefore, YA function is required after meiosis but during egg maturation.

The arrested phenotype of *Ya* mutant embryos is consistent with YA's primary function being during egg maturation. Without functional YA, the female pronucleus might already be abnormal before the first mitotic division (gonomeric division), resulting in abnormalities in that, and subsequent, divisions. Or, since YA is acquired by the nuclear envelope of the male pronucleus in the fertilized egg, lack of YA function might not only affect the coordination of the female meiotic products, but also the coordination of the two pronuclei, resulting in an arrest of *Ya* mutant embryos in the gonomeric division. This arrest would eventually lead to degeneration of the arrested embryos. Similarly, incomplete YA function during egg maturation could result in the abnormal subsequent divisions as seen with the leaky alleles. The hypothesis that *Ya* functions during egg maturation is also consistent with our observation that YA is epistatic to *gnu* in unfertilized eggs as well as in fertilized eggs from *Ya; gnu* mothers (LIU *et al.* unpublished observations). The maternal effect *gnu* mutation causes uncontrolled DNA replication in unfertilized eggs as well as embryos, resulting in formation of giant nuclei (FREEMAN *et al.* 1986; FREEMAN and GLOVER 1987). This phenotype suggests that gnu^+ functions to suppress DNA replication prior to fertilization and to couple DNA replication and nuclear division in zygotes. The epistasis of *Ya* to *gnu* in unfertilized eggs is consistent with YA playing a role prior to fertilization.

The proposed timing of YA function during egg maturation is consistent with the timing of YA's nuclear envelope localization. YA protein in the ovary is not localized to the nuclear envelope (SONG 1994). It first becomes detectable in nuclear envelopes of unfertilized eggs, at the stage when four meiotic products are visible and their chromatin is uncondensed. This is also the first stage at which we observe an abnormal phenotype in *Ya* mutants. Therefore, we hypothesize that YA function is first needed in nuclear envelopes of maturing eggs. Since YA is detectable in nuclear envelopes after this time as well (LIN and WOLFNER 1991), it is possible that YA may also function during cleavage divisions, though this has not been demonstrated.

How does YA function in the nuclear envelope? One function of the nuclear lamina is to provide a structural framework for the nuclear envelope (reviewed in GERACE 1986). However, our data do not support a role for YA in nuclear envelope assembly. The presence of lamin at the periphery of the meiotic products in *Ya* mutant eggs suggests that YA is not essential for the formation of a lamin-containing nuclear envelope (Figures 4–6). Consistent with this, nuclei formed *in vitro* in extracts of Ya^2 oocytes are surrounded by normal-looking nuclear envelopes (J. M. LOPEZ and M. F. WOLFNER, unpublished data).

Instead, the mutant phenotypes of *Ya* eggs and embryos suggest a role for YA related to chromatin condensation in the nuclei. This would fit with another function suggested for the nuclear lamina, which is to provide attachment sites for chromosomes in organizing the nucleus. In support of this hypothesis, unfertilized *Ya* eggs from short collections contain nuclei of different chromatin condensation states (Figure 4), which is never observed in unfertilized eggs from wild type. Also consistent with a role of YA in chromatin organization, chromatin appears abnormally condensed in nuclei of pronuclear-arrested *Ya* mutant embryos and in *Ya* unfertilized eggs from long collections. It is possible that YA participates in chromatin condensation by direct interactions with the chromosomes, thus associating them with the nuclear envelope. YA protein has two potential Cys2-His2-type zinc fingers and an SPKK motif (LIN and WOLFNER 1991 and this work), which are known DNA binding motifs (CHURCHILL and SUZUKI 1989; SUZUKI 1989; BERG 1990; COLEMAN 1992). Moreover, YA associates with decondensed chromatin *in vitro* and with interbands of salivary gland polytene chromosomes upon ectopic expression (J. M. LOPEZ and M. F. WOLFNER, unpublished observations). Therefore, YA might associate with decondensed chromatin in eggs and embryos to assist in facilitating proper chromatin condensation or in other processes that require association of chromosomes with the nuclear envelope.

In addition to extending our understanding of YA function in embryos and eggs, our studies of *Ya* mutants have shed some light on the molecular nature of the YA protein itself. Sequences of the four *Ya* mutant alleles indicate that both the N- and the C-termini of the YA protein are functionally important, and that the C-terminal one third of the YA protein, including the potential

nuclear localization signals, the FSPKK motif and the polar C-terminal end, is essential for YA function. Since the C-terminal lesion in YA^{70} and the N-terminal lesion in YA^{77} complement each other, YA may function by interacting with itself either directly or indirectly.

Therefore, as a developmentally regulated nuclear envelope protein, YA might function by mediating interactions of chromosomes and the nuclear envelope in eggs and embryos to allow proper chromatin condensation to occur. We have shown that this function is required immediately after meiosis of the oocyte and that YA might exert its function by interacting with itself either directly or indirectly. Further studies on the biochemical basis of how YA exerts its function in the nuclear envelope and which part of the protein is carrying out the specific functions will help understand the nuclear envelope and mitotic divisions in early embryos.

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