Protein YA with P0/AP3 in the Cytoplasm and in Vitro: Implications for Developmental Regulation of YA's Subcellular Location

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The *Drosophila* nuclear lamina protein YA is essential for the transition from female meiosis to embryo mitosis. Its localization and, hence, function is under developmental and cell cycle controls. YA protein is hyperphosphorylated and cytoplasmic in ovaries. Upon egg activation, YA is partially dephosphorylated and acquires the ability to enter nuclei. Its function is first detected at this time. To investigate the cytoplasmic retention machinery that keeps YA from entering nuclei, we used affinity chromatography and blot overlay assays to identify cytoplasmic proteins that associate with YA. *Drosophila* P0/AP3, a ribosomal protein that is also an apurinic/apyrimidinic endonuclease, binds to YA in ovary and embryo cytoplasms. P0 and YA bind specifically and directly *in vitro* and are present in a 20S complex in the cytoplasmic extracts. YA protein can be phosphorylated by MAPK, but not by p34^{Cdc2} kinase, *in vitro*. This phosphorylation increases YA's binding to P0. We propose that the P0-containing 20S cytoplasmic complex retains hyperphosphorylated ovarian YA in the cytoplasm. In response to egg activation, YA is partially dephosphorylated and its binding to the 20S complex is reduced. Hence, some YA dissociates from the complex and enters nuclei. Consistent with this model, decreasing P0 levels partially suppress a hypomorphic *Ya* mutant allele.   © 2002 Elsevier Science (USA)

Key Words: Drosophila; P0/AP3; YA; MAPK; egg activation; nuclear entry; phosphorylation.

INTRODUCTION

Oocyte growth, maturation, and activation are critical processes for successful production of an embryo. During oogenesis, oocytes stockpile mRNAs and proteins that will be used subsequently for embryo development. During oocyte maturation, oocytes acquire the capability to be activated and fertilized. Mature oocytes in all vertebrates and many invertebrates, including *Drosophila*, arrest during meiotic metaphase awaiting egg-activation signals. Egg activation triggers completion of meiosis, allowing the formation of a haploid female pronucleus that can participate in further development. In several organisms, the phosphorylation states of some maternal proteins undergo changes that are important for proper progression through

oocyte maturation, metaphase arrest, and egg activation. For example, inhibition of phosphorylation of maternal proteins during meiotic metaphase arrest can activate *Xenopus* eggs (Zhang and Masui, 1992), suggesting that phosphorylation of maternal proteins is essential to maintain the meiotic metaphase arrest.

Maturation promoting factor (MPF; p34^{Cdc2} kinase and cyclin B complex) and mitogen-activated protein kinase (MAPK) are two key kinases during these processes. In most invertebrates and vertebrates (e.g., the bivalve *Ruditape*, ascidians, *Xenopus*, mouse), MPF and MAPK activities increase during oocyte maturation, remain high during oocyte metaphase arrest, and decrease after initiation of egg activation (Colas and Guerrier, 1995; Russo *et al.,* 1998). MPF activity is required for oocyte maturation and the maintenance of meiotic arrest, and its inactivation is essential for egg activation (Colas and Guerrier, 1995; Palmer and Nebreda, 2000). MPF is responsible for chromatin condensation and nuclear envelope disassembly during *Xenopus* metaphase arrest (Lohka, 1998). In most organisms, MAPK

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is not required for germinal vesicle breakdown (GVBD) or MPF activation during oocyte maturation, but is required for the maintenance of oocyte metaphase arrest (Fisher *et al.,* 1999; Gross *et al.,* 2000; Kosako *et al.,* 1994a,b). In *Xenopus*, a transient early high level of MAPK is required for the correct timing and efficiency of GVBD, and MAPK increases MPF activity (Fisher *et al.,* 1999; Gross *et al.,* 2000). MAPK is required to suppress entry into S phase between meiosis I and meiosis II (Gross *et al.,* 2000) and may trigger nuclear migration independent of MPF activity during oocyte maturation (Gavin *et al.,* 1999). MAPK is also sufficient for MPF activation at the onset of oocyte maturation (Gotoh *et al.,* 1995; Haccard *et al.,* 1995; Huang *et al.,* 1995; Yew *et al.,* 1992). The situation in some other organisms, such as *Chaetopterus*, is a bit different: MAPK is neither required nor sufficient for oocyte maturation or meiotic arrest (Eckberg, 1997). In mouse, high MAPK activity during oocyte maturation and metaphase arrest is important for the maintenance of chromosome condensation and the metaphase block, formation of the meiotic spindles, and prevention of pronuclear envelope formation (Choi *et al.,* 1996; Moos *et al.,* 1995; Verlhac *et al.,* 1994). The phosphorylation targets of MAPK that execute these functions are largely unknown, with the exception of p90[Rsk], which is phosphorylated by MAPK during *Xenopus* oocyte maturation (Bhatt and Ferrell, 1999; Gavin *et al.,* 1999; Gross *et al.,* 1999; Grove *et al.,* 1993; Sturgill *et al.,* 1988).

The role or modulation of MPF and MAPK in *Drosophila* oocytes is as yet unknown. *Drosophila* oocytes arrest at meiotic prophase I during most of oogenesis. Toward the end of oogenesis, they resume meiosis, only to arrest again at metaphase I (Page and Orr-Weaver, 1997). Egg activation occurs independent of and prior to fertilization (Doane, 1960; Heifetz *et al.,* 2001).

The YA (Young Arrest) phosphoprotein is essential for nuclear events during the developmental transition from female meiosis to embryo mitosis (Lin and Wolfner, 1991; Liu *et al.,* 1995; Yu *et al.,* 1999). Embryos from females lacking YA function arrest just after completing meiosis. Zygotic mitosis never occurs in these embryos (Lin and Wolfner, 1991; Liu *et al.,* 1995; Lopez, 1996; Berman, 2000). In normal embryos and activated oocytes, YA is found in interphase through metaphase nuclei, in the nuclear lamina layer of the nuclear envelope, where it associates with lamin (Goldberg *et al.,* 1998), and in the nucleoplasm, where it associates with chromatin (Lopez and Wolfner, 1997) through its direct interaction with DNA and with histone H2B (Yu and Wolfner, 2002). There is also a cytoplasmic pool of YA throughout the cell cycle. In ovaries, YA is in a more highly phosphorylated form and is excluded from oocyte nuclei, and away from chromatin (Yu *et al.,* 1999). Thus, only after egg activation does YA acquire its ability to enter nuclei, coincident with its partial dephosphorylation. Based on this correlation, we proposed that phosphorylation regulates YA's potential for nuclear entry during development (Yu *et al.,* 1999).

YA contains five predicted MAPK target sites and four predicted p34[Cdc2] kinase target sites (Liu and Wolfner, 1998). [443]Thr, which is both a p34[Cdc2] kinase and MAPK predicted target site, is one of at least two sites in YA that are differentially phosphorylated during this developmental transition (Yu *et al.,* 1999). This suggests that phosphorylation of YA by one or both of these kinases may regulate YA's subcellular location.

Phosphorylation can regulate a protein's nuclear entry by modulating the protein's interaction with cytoplasmic retention machinery (Jans and Hubner, 1996). In one phosphorylation state, the target protein binds the cytoplasmic retention machinery and stays in the cytoplasm. In another phosphorylation state, the protein loses affinity for the cytoplasmic retention machinery and can enter nuclei via its nuclear localization signals (NLS). This type of regulation is typified by the nuclear import of the *Drosophila* Dorsal protein (Drier *et al.,* 1999; Liu *et al.,* 1997; Whalen and Steward, 1993). Dorsal is retained in the cytoplasm by binding to Cactus protein. In response to dorsoventral signaling, Dorsal and Cactus are both phosphorylated, causing Dorsal to dissociate from Cactus and leading to Dorsal's nuclear entry.

Here, we present evidence that the changes in YA's subcellular localization may utilize an analogous mechanism, but one regulated during egg activation. We report that YA binds to P0/AP3, a *Drosophila* ribosomal protein which also has apurinic/apyrimidinic endonuclease activity (Yacoub *et al.,* 1996). *In vitro*, this interaction is influenced by YA's phosphorylation state: YA is a target of MAPK (but not p34[Cdc2] kinase) *in vitro*, and MAPK-phosphorylated YA binds more strongly to P0 than YA that was not phosphorylated by MAPK. We propose that MAPK phosphorylation of YA in ovaries results in YA's retention in the cytoplasm within a 20S complex that also contains P0. Upon activation, dephosphorylation of YA releases it from the complex, allowing it to enter nuclei. Consistent with this model, lowering the levels of P0 partially suppresses a hypomorphic *Ya* mutant allele. YA provides a molecular probe with which to assess the cause and consequences of phosphorylation changes during *Drosophila* egg activation.

MATERIALS AND METHODS

Production, Purification, and Coupling of Recombinant Proteins

To make a protein fusion of YA to a tag [*E. coli* maltose-binding protein (MBP)] and a site to allow labeling *in vitro* [a heart-muscle kinase (HMK) target site (Blanar and Rutter, 1992)], full-length *Ya* cDNA (Lopez *et al.,* 1994) was cloned into a modified pMALTM-c2 vector (NEB) called pMALC2-HMK(R) (Z. Li and M. L. Goldberg, personal communication). The fusion protein is called MBP-HMK-YA. A full-length P0 cDNA (Yacoub *et al.,* 1996) was cloned into pET21a(+) vector (Novagen) to express P0-His$_6$ fusion protein.

Protein induction and expression were done according to NEB's protein fusion and purification (pMALTM) instruction manual (for MBP-HMK-YA) or the pET system manual (Novagen) (for P0-His$_6$) with the following modifications. The construct was transformed

into *Escherichia coli* BL21(DE3)pLysS cells (for MBP-HMK-YA) or BL21(DE3) (for P0-His$_6$). An overnight culture was diluted 1:20 into fresh medium. IPTG was added to a final concentration of 1 mM (for MBP-HMK-YA) or 0.4 mM (for P0-His$_6$). All the following steps after harvest of cells were performed at 4°C. The cell pellet was immediately resuspended and sonicated. Triton X-100 was added to the cell lysate to a final concentration of 1%. The lysate was incubated for 1 h with continuous mixing, and centrifuged at 39,000g for 30 min. MBP-HMK-YA was purified from the supernatant with amylose beads, and P0-His$_6$ was purified with His-bind beads (Novagen). The peak fractions were pooled and analyzed by 7.5% SDS–polyacrylamide gels with Coomassie blue staining and Western blotting with affinity-purified anti-YA or anti-P0 antibodies.

Like endogenous YA (Liu and Wolfner, 1998), purified MBP-HMK-YA protein can interact with embryonic YA (data not shown). MBP-HMK-YA can also be incorporated into the nuclear envelope of *in vitro* assembled nuclei in *Xenopus* egg extracts (M.F.W., unpublished observations), consistent with its being capable of at least some of YA's normal behaviors.

A total of 4 mg of MBP-HMK or MBP-HMK-YA was coupled to 1 ml CNBr-activated Sepharose-4B beads (Sigma) following the procedure from the manufacturer. The coupling efficiency was above 90%, judging by SDS–PAGE.

Ovaries, Embryos, and Extracts for Column Purification

Ovaries, eggs, and embryos from females of the Oregon R P2 strain (Allis *et al.,* 1977) were used for all experiments. Ovaries were dissected from 2-day-old virgin females into 1× EBR buffer (Verheyen and Cooley, 1994) and ground in the indicated buffers. Ovaries were in 1× EBR buffer for less than 15 min before grinding to avoid activation of mature oocytes. Embryos at 0–2 h were collected as described in Allis *et al.* (1977) and homogenized with a Dounce homogenizer (tight pestle) in 1 vol of 1× Hyb(75) buffer (20 mM Hepes–KOH, pH 7.7, 75 mM KCl, 0.1 mM EDTA, 2.5 mM MgCl$_2$, 1 mM DTT) with 0.5% NP-40. Protease inhibitors including 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, 1 μg/ml pepstatin A, and 1 μg/ml antipain were included in all solutions. All steps after tissue/embryo collection were done at 4°C. After incubation on ice for 10 min, the homogenate was centrifuged at 20,000g for 10 min. The supernatant was diluted with 9 vol of 1× Hyb(75) buffer and incubated for 10 min. After another centrifugation, the supernatant was ready for MBP pull-down assay or for column purification.

For column purification, embryo extracts were loaded through an MBP-HMK-coupled affinity column. The flow-through was loaded onto an MBP-HMK-YA-coupled affinity column. After washing with 1× Hyb(75) buffer, proteins bound to the column were eluted with 1× Hyb(75) buffer with increasing concentrations of NaCl from 0.1 to 1 M.

Blot Overlay Assay

To make probes, 2 μg of MBP-HMK-YA or MBP-HMK was incubated in a 100-μl labeling reaction and incubated at room temperature for 3 h (Blanar and Rutter, 1992). Labeling of proteins was verified by SDS–PAGE followed by autoradiography.

Proteins to be analyzed by blot overlay assay were separated by SDS–PAGE and transferred to nitrocellulose membranes as de-

scribed in Lin and Wolfner (1991). The blot overlay assay was performed as Blanar and Rutter (1992) with minor modifications.

Protein Sequencing

Proteins for sequencing were separated on SDS–PAGE and transferred to PVDF membranes according to Lin and Wolfner (1991). The membrane was stained with Coomassie blue and destained with milliQ water. The band of interest was cut from the membrane and sequenced from the protein's N terminus with a PE/Applied Biosystems Precise 492, by the Cornell University BioResource Center. The unambiguous amino acids in the peptide sequence thus obtained were used to search the nr database to identify the protein.

Genomic PCR

The genomic region spanning the N-terminal coding sequence of P0 from the Oregon R P2 strain was amplified with primers: 5′-TTCTTAAATTCACCCGACGA-3′ and 5′-TGGTACGGATG-TTCTGCATC-3′ as described by Yu and Wolfner (2002). The sequence of the PCR product was determined and compared with the *D. melanogaster* genome sequence in Adams *et al.* (2000).

The Yeast Two-Hybrid Assay

Full-length P0 cDNA (a kind gift from M. Kelley; Yacoub *et al.,* 1996) was cloned into the pGAD424 vector. Full-length *Ya* cDNAs that happened to carry mutations in the five potential MAPK phosphorylation sites to glutamic acid (All-E) or to alanine (all-A) (S. Mani and M.F.W., unpublished results) were cloned into pGBT9 vector as in Goldberg *et al.* (1998). The pGBT9-YA constructs were cotransformed with pGAD424 (negative control), pGAD424-P0, or pGAD424-lamin Dm0 (positive control; Goldberg *et al.,* 1998) into yeast strains HF7c and SFY526 β-gal activity was assayed qualitatively (by color of the colonies; HF7c strains) and quantitatively (for β-Gal activity units; SFY526 strains) as described in Goldberg *et al.* (1998). Each combination was tested in duplicate. β-Gal units are the average of three determinations per experiment; data from one experiment per combination is shown.

Immunofluorescence staining

Embryos at 0–2 h were collected and processed as described in Lin and Wolfner (1991). Embryos were incubated with affinity-purified anti-P0 antibodies (1:50). Anti-P0 antibodies were purified from crude antiserum kindly provided by M. R. Kelley (Yacoub *et al.,* 1996) by using a GST-P0 protein-coupled Sepharose 4B column. The embryos were then stained with FITC-conjugated anti-rabbit IgG antibodies (1:100) and counterstained with DAPI. Images were collected by using laser scanning confocal microscopy (Bio-Rad MRC 600 system on a Zeiss inverted microscope).

Coimmunoprecipitation Assay and Antibodies

Cytoplasmic extracts were made from 10 μl of packed 0- to 1-h embryos or from 80 ovaries dissected from 2-day-old virgin females. To half the extracts, affinity-purified anti-P0 antibodies were added; the other half received preimmune serum. After 2 h of incubation, 10 μl protein A-Sepharose CL-4B beads (Sigma) were added and the mixture was rotated end-to-end for 6 h. The beads

were then harvested and washed with 1× Hyb(75) with 0.05% NP-40 eight times. Proteins were eluted from the beads by boiling in SDS–PAGE sample buffer. One-twentieth of the supernatant after binding and half the eluate from the beads were analyzed by Western blotting with affinity-purified anti-P0 antibodies and anti-YA antibodies.

For coimmunoprecipitation with anti-YA antibodies, 2 μl of affinity-purified anti-YA antibodies (Lin and Wolfner, 1991) were added to cytoplasmic extracts from 5 μl of packed 0- to 2-h embryo from wild-type or Ya^2 mutant females. Presence of YA and P0 in samples corresponding to 1 μl of embryos was assessed by Western blotting; supernatant after binding and the eluate from the beads were examined.

Sucrose Density Gradient Sedimentation Assay

Embryos at 0–2 h were homogenized in 4 vol of buffer D (50 mM Tris–HCl, pH 7.5, 50 μl MgCl$_2$, 145 mM sucrose) with 100 mM NaCl and 0.25% Triton X-100. The supernatant was loaded onto a 5–35% sucrose density gradient in the same buffer. Centrifugation conditions were as described in Swanson and Vacquier (1997). The collected fractions were precipitated with 2 vol of acetone. The pellet was dissolved in 1× SDS–PAGE sample buffer, boiled for 5 min, and analyzed by Western blotting. All steps were performed at 4°C. Molecular weight markers (MW-GF-1000; Sigma) were run in the sucrose density gradient at the same time and analyzed by SDS–PAGE with silver staining. Purified polyclonal anti-DS3 was provided by M. R. Kelley (Wilson *et al.,* 1994).

MBP Pull-Down Assay

To test the specificity of the cytoplasmic proteins' binding to YA, extracts from 10 μl of packed 0- to 2-h embryos were incubated overnight with 20 μl of either MBP-HMK-YA beads or MBP-HMK beads in the absence or presence of 2 μg MBP-HMK or MBP-HMK-YA. The beads were then washed with 1× Hyb(75) + 0.05% NP-40 eight times. Proteins bound to the beads were eluted with 1× Hyb(75) + 0.05% NP-40 + 1 M NaCl.

To test purified P0's direct binding to YA, 2 μg of purified MBP-HMK-YA or 2 μg of purified MBP-HMK were incubated with 4 μg of P0-His$_6$ in 1× Hyb(75) + 0.05%NP-40 with protease inhibitors (1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin) for 1 h. Amylose beads (30 μl) were then added to the solution, which was then rotated end-to-end for 5 h. After centrifugation, the beads were harvested and washed four times with 1 ml of 1× Hyb(75) + 0.05% NP-40 + 200 mM NaCl with protease inhibitors. The beads were eluted by boiling in 4× SDS–PAGE sample buffer for 5 min. The supernatant after the binding was precipitated with 2 vol of acetone at −20°C overnight. The pellet was dissolved in 1× SDS–PAGE sample buffer and boiled for 5 min. The supernatant after binding and half of the eluate from the beads were analyzed by SDS–PAGE followed by Western blotting with affinity-purified anti-P0 antibodies. All steps were performed at 4°C.

To test the effect of phosphorylation of YA on YA's interaction with P0, different dilutions of *in vitro* phosphorylated YA and mock phosphorylated YA (0.7 or 0.3 μg) were mixed with 3 μg P0-His$_6$ in a final volume of 250 μl containing 1× Hyb(75), 0.05% NP-40, and protease inhibitors. The binding reaction was performed as above.

Immunoprecipitation of p34^{Cdc2} Kinase from Hela Cells

Cell culture, synchronization, and lysis of Hela S3 cells were performed according to Resnick *et al.* (1997). All buffers following cell lysis were supplemented with protease inhibitors and phosphatase inhibitors (25 mM NaF, 1 mM Na vanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin). Anti-p34^{Cdc2} kinase antibodies (10 μg) (Gibco Life Technologies) were added to cell lysates containing about 500 μg protein and incubated for 2 h at 4°C. Protein A-Sepharose CL-4B beads (20 μl; Sigma) were then added and the mixture was rotated for 1 h at 4°C. The beads were harvested and washed once with KLB (Resnick *et al.,* 1997) three times and with 1× kinase buffer (NEB). The beads were then resuspended in 50 μl 1× kinase buffer and stored at 4°C.

In Vitro Phosphorylation Reactions

MBP-HMK-YA fusion protein (0.5 μg) or 1 μg histone H1 in 1× kinase buffer was incubated with 2 μl immunoprecipitated p34^{Cdc2} kinase or 0.5 μl MAPK (ERK2, NEB) in a final volume of 25 μl containing 1× kinase buffer and 100 μM ATP and 20 Ci/mmol [γ-^{32}P]ATP (final concentration). MBP-HMK-YA (4 μg) was incubated in a final volume of 100 μl containing 1× kinase buffer and 200 μM ATP with or without (mock phosphorylation) 14 μl immunoprecipitated p34^{Cdc2} kinase and/or 4 μl MAPK. [γ-^{32}P]ATP was added to a 10-μl aliquot of the above reaction mix to a final concentration of 20 mCi/μmol. All buffers were supplemented with protease inhibitors and phosphatase inhibitors (25 mM NaF, 1 mM Na vanadate, 10 μg/ml leupeptin, 10 μg/ml aprotinin). All the reactions were incubated at room temperature for 4 h with rotation. The reactions with radioactive ATP were analyzed by SDS–PAGE. Reactions with nonradioactive ATP were used for MBP pull-down assays.

Genetic Suppression Analysis

P0^{306}/MKRS stock was kindly provided by M. V. Frolov and J. A. Birchler (Frolov and Birchler, 1998). X^X, *y Ya^77 cv v f*/Y, *Ya^+ y^+ w^+ B^s* (Liu *et al.,* 1995) virgin females were mated with P0^{306}/MKRS males. Then, 0- to 2-h embryos were collected from X^X, *y Ya^77 cv v f*/Y; *P0^{306}*/+, and X^X, *y Ya^77 cv v f*/Y; +/*MKRS* females, respectively. All embryos were stained with DNA dye DAPI and examined with an Olympus BX50 equipped with a Pentamax camera (Princeton Instruments). Three independent repeats of the experiment gave comparable results. The results of one of those experiments is shown.

RESULTS

Drosophila P0 Interacts with YA

To identify cytoplasmic proteins that interact with YA and could serve as YA's cytoplasmic retention machinery, we employed a blot overlay assay to detect proteins that interact with YA directly, and affinity chromatography to purify those proteins. The same bands from ovary and syncytial-stage embryo cytoplasmic samples were detected by a ^{32}P-labeled MBP-HMK-YA probe (data not shown), suggesting that the same group of proteins from ovary and embryo cytoplasm interact with YA. As it is easier to get

large amounts of embryos for protein purification, embryos rather than ovaries were used as starting material to purify YA-interacting proteins from cytoplasm.

We then tested whether the proteins that bound to the ^{32}P-labeled MBP-HMK-YA probe in the blot overlay assay could bind to MBP-HMK-YA specifically and in a nondenatured condition suitable for affinity column purification. Cytoplasmic extracts from 0- to 2-h embryos were incubated with MBP-HMK-YA-coupled beads or MBP-HMK-coupled beads in the presence or absence of excess amounts of uncoupled MBP-HMK-YA or MBP-HMK protein (see Material and Methods). Proteins bound to the beads were eluted and analyzed by blot overlay assay. As shown in Fig. 1 of the blot probed with ^{32}P-labeled MBP-HMK-YA probe, in the presence of excess unlabelled MBP-HMK, six proteins that bind to MBP-HMK-YA-coupled beads were detected by the probe (Fig. 1, lane 2). Of these, three proteins, P35, P12 and P10, did not bind to MBP-HMK-coupled beads (Fig. 1, lane 1), suggesting that they only bound to the YA moiety. P25 bound to MBP-HMK beads very weakly but to MBP-HMK-YA beads strongly, suggesting that it has weak nonspecific binding to MBP-HMK and/or the beads, but it has strong affinity for YA. Confirming this, the binding of P35, P25, P12, and P10 to MBP-HMK-YA-coupled beads was competed by MBP-HMK-YA in solution (Fig. 1, lane 3), but not by MBP-HMK in solution (Fig. 1, lane 4). The other two proteins, P42 and P30, bound to both MBP-HMK-YA-coupled beads and MBP-HMK-coupled beads (Fig. 1, lanes 1 and 2), suggesting that they could bind to the MBP-HMK, YA moiety, and/or the beads. Their binding to MBP-HMK-YA beads was competed by MBP-HMK-YA, but not MBP-HMK, in solution (Fig. 1, lanes 3 and 4), suggesting that they bound to both the YA moiety of MBP-HMK-YA and the beads. Since P42 and P30 do not bind to YA specifically, they were not studied further. The blot probed with ^{32}P-labeled MBP-HMK probe in the presence of excess unlabeled MBP-HMK (Fig. 1) did not have any signal, suggesting that the blockage by the nonlabeled MBP-HMK of any potential ^{32}P-labeled MBP-HMK probe's binding to the proteins on the blot is complete; it also confirms that the signals from the blot probed with ^{32}P-labeled MBP-HMK-YA came from YA's binding to the proteins on the blot.

We used affinity chromatography to enrich the four proteins that interact with YA specifically (P35, P25, P12, and P10) (see Materials and Methods). We obtained enough P35 for N-terminal sequencing; we did not pursue the other three proteins further in this study. The N-terminal sequence of P35 was determined (Fig. 2A). Of the 20 amino acids that the Cornell sequencing facility was able to identify, 14 were unambiguous; there was uncertainty in 6 due to the limited amount of sample. The unambiguous sequence was used to search the *Drosophila* genome (Adams *et al.,* 2000). There was only one match—*Drosophila* P0/AP3 (Fig. 2A), an apyrinic/apurimidinic endonuclease that is also a ribosomal protein (Yacoub *et al.,* 1996); we will abbreviate this protein as P0. Four amino acids do not
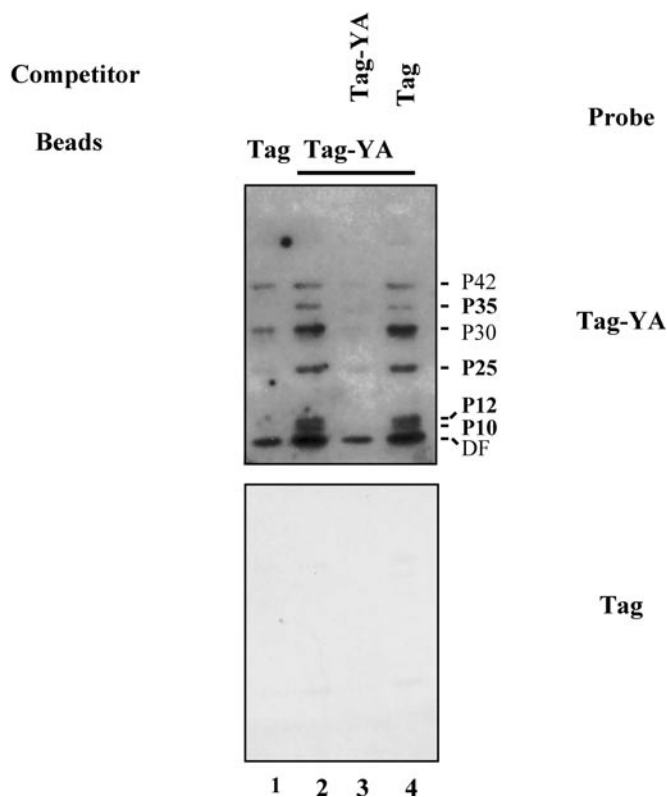

FIG. 1. Four cytoplasmic protein bands bind specifically to YA. Cytoplasmic extracts of 0- to 2-h embryos were incubated with either MBP-HMK-YA-coupled beads (Tag-YA) (lanes 2– 4) or MBP-HMK-coupled beads (Tag) (lane 1) in the presence or absence of competitor MBP-HMK-YA or MBP-HMK. The proteins eluted from the beads were analyzed by blot overlay assays. Probes were either ^{32}P-labeled MBP-HMK-YA (Tag-YA) or ^{32}P-labeled MBP-HMK (Tag) with a 4000-fold excess of unlabeled MBP-HMK. Shown in this autoradiograph, when probed with ^{32}P-labeled MBP-HMK-YA (top), three proteins, P35, P12 and P10, bound to MBP-HMK-YA beads only (lane 2). P25 bound to MBP-HMK-YA beads strongly and to MBP-HMK beads very weakly (lanes 1 and 2). Two proteins, P42 and P30, bound to both MBP-HMK beads and MBP-HMK-YA beads (lanes 1 and 2). The binding of all six proteins to MBP-HMK-YA beads was competed by MBP-HMK-YA (lane 3); the remaining residual binding is probably due to the incomplete competition by MBP-HMK-YA competitor; their binding was not competed by MBP-HMK (lane 4). The proteins were not labeled in a similar blot probed with ^{32}P -MBP-HMK (bottom), suggesting that the signals on the ^{32}P -MBP-HMK-YA probed blot are from the proteins' binding to YA. DF, dye front. The proteins' molecular weights are 42, 35, 30, 25, 12 and 10 kDa.

match the P0 genomic sequence reported by Adams *et al.* (2000); these mismatches are not due to strain differences based on genomic PCR from the strain we used for protein purification (data not shown); we therefore believe they are due to machine error given to the limited amount of protein available for sequencing. The first amino acid, Met, is in the
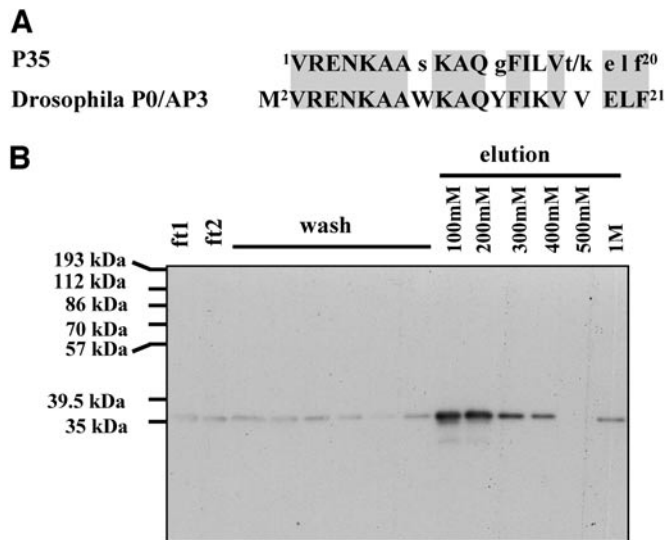
A

P35

Drosophila P0/AP3

[1]VRENKAA s KAQ gFILVt/k e l f[20]

M[2]VRENKAAWKAQYFIKV V ELF[21]

B

FIG. 2. P35 is *Drosophila* P0. (A) The N-terminal sequence of P35 purified from MBP-HMK-YA column aligned with the N-terminal sequence of *Drosophila* P0/AP3, a ribosomal protein that also has apurinic/apyrimidinic endonuclease activity (Yacoub *et al.,* 1996). The residues in lowercase are those for which the sequencing facility reported uncertainty. (B) Western blot of embryo cytoplasmic proteins that passed through an MBP-HMK coupled column and were loaded onto a MBP-HMK-YA column (ft1), the flow through from the MBP-HMK-YA column (ft2), the wash (wash), and the eluate from the MBP-HMK-YA column with increasing concentrations of NaCl (elution). The fractions separated by SDS–PAGE were probed with anti-P0 antibodies. These antibodies recognize P35, the 35-kDa band that bound to MBP-HMK-YA. The ft1 and ft2 lanes each contain 0.5 U of extracts (Fisher *et al.,* 1982); the other lanes each contain 20 U of extracts.

FIG. 3. P0-His$_6$ binds specifically to YA *in vitro*. A total of 2 μg of MBP-HMK (Tag-YA) or MBP-HMK (Tag) was incubated with 4 μg of P0-His$_6$ and pulled down with amylose beads. All of the supernatant (Sup.) and half of the proteins bound to beads (Beads) were analyzed by Western blotting with anti-P0 antibodies. P0-His$_6$ was pulled down with MBP-HMK-YA (lane 3) but not with MBP-HMK (lane 4).

predicted P0 sequence but not in the sequence of P35. Since the P0 sequence is derived by conceptual translation from the cDNA, this suggests that this Met is cleaved from the protein after translation *in vivo* (Bradshaw *et al.,* 1998). Four lines of evidence support the conclusion that P35 is *Drosophila* P0. First, the predicted molecular weight of P0 (34 kDa) and the size of its *in vitro* translation product (Kelley *et al.,* 1989) are the same as the size of P35 (35 kDa). Second, affinity-purified anti-P0 antibodies (Yacoub *et al.,* 1996) cross-react with a band that migrates at the same molecular weight as P35 and on Western blots parallels the distribution of P35 during purification (Fig. 2B). Third, purified recombinant P0 protein binds to YA directly *in vitro* (Fig. 3). His$_6$-tagged P0 was made and purified from *E. coli* and incubated with MBP-HMK-YA or MBP-HMK. Amylose beads were added to pull down the MBP-HMK fusion proteins (see Materials and Methods). P0-His$_6$ was pulled down by amylose beads with MBP-HMK-YA, but not with MBP-HMK, indicating that P0 binds specifically to the YA moiety of MBP-HMK-YA. Finally, YA interacts with *Drosophila* P0 in the yeast two-hybrid system as seen with
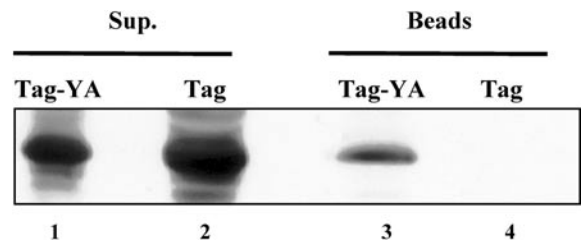
both qualitative and quantitative assays (Table 1). These data, taken together, indicate that the YA-interacting protein P35 is *Drosophila* P0.

P0 and YA Are Members of a Complex in Both Embryo and Ovary Cytoplasmic Extracts

P0 is an abundant multifunctional protein that is expressed throughout *Drosophila* development, and is reported to be found in both nuclear and cytoplasmic subcellular fractions (Yacoub *et al.,* 1996). We confirmed that it is abundant in the cytoplasm of 0- to 2-h embryos (Fig. 4).

To further examine the interaction of YA and P0 in embryos and ovaries, we performed coimmunoprecipitation assays with embryo and ovary cytoplasmic extracts (Fig. 5A). In the presence of affinity-purified anti-P0 antibodies, YA was precipitated with P0 from both ovarian and embryo cytoplasm (Fig. 5A, lanes 2 and 4). Immunoprecipitation

TABLE 1

Interaction of YA and P0 As Measured by the Yeast Two-Hybrid Assay

Genes fused to pGBT9/ Genes fused to pGAD424		β-Gal color	β-Gal activity (U)
YA/none	All-A	White	0.00638
YA/P0	All-A	Light blue	0.184
YA/lamin	All-A	Blue	2.693
YA/none	All-E	White	0.141
YA/P0	All-E	Light blue	0.671
YA/lamin	All-E	Blue	3.538

Note. Full-length *Ya* cDNA with mutations in all potential MAPK phosphorylation sites to alanine (All-A) or to glutamic acid (All-E) were cloned into pGBT9 vector. The resulting construct was cotransfected with pGAD424 (the negative control), pGAD424-P0, or pGAD424-lamin (the positive control). β-Gal color and liquid assays were performed with each cotransfection.
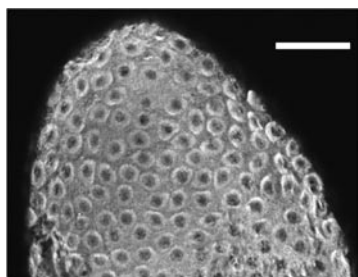
P0

FIG. 4. P0 is abundant in embryo cytoplasm. Embryos at 0–2 h were stained with affinity-purified anti-P0 antibodies. P0 levels are high in the cytoplasm. Parallel staining of embryos with different primary antibodies (anti-YA; data not shown) confirmed that the cytoplasmic staining is not due to staining conditions or to background from the secondary antibodies used. The bar denotes 100 μm.

with preimmune serum did not precipitate either P0 or YA (Fig. 5A, lanes 3 and 5), indicating that the precipitation of YA and P0 is specific to anti-P0 antibodies. The YA and P0 bands were also not from anti-P0 antibodies or the protein A beads used for immunoprecipitation, since immunoprecipitation of buffer alone with anti-P0 antibodies showed neither of the bands (Fig. 5A, lane 1). This indicates that YA and P0 are in the same protein complex in both ovarian and embryo cytoplasmic extracts. The ratio of YA to P0 is similar in immunoprecipitates from ovary and embryo cytoplasmic extracts; however, in the supernatants, the ratio of YA to P0 is much higher in embryo samples than in ovary samples, suggesting that there is more YA unassociated with P0 in embryos than in ovaries. Since P0 is more abundant than YA, we interpret the higher level of P0-free YA in embryos to mean that embryo YA has a lower affinity for P0 than ovary YA, rather than that there is only limited availability of P0 in embryos.

In the converse immunoprecipitation experiment, incubation of cytoplasmic extracts from embryo with affinity-purified anti-YA antibodies also precipitated both YA and P0. As shown in Fig. 5B, anti-YA antibodies precipitated both P0 and YA from wild-type extracts, but it precipitated neither YA nor P0 from cytoplasmic extracts of embryos whose YA was a truncated mutant form that is not recognized by the anti-YA antibodies we used (Lin and Wolfner, 1991; Liu *et al.,* 1995).

We used sucrose density gradient analysis to further confirm that YA and P0 are in the same protein complex in cytoplasmic extracts. Though P0 is distributed in all fractions, its abundance peaks in the same gradient fractions as YA's in the gradient (highest amount in fraction 8 and slightly less in fraction 9), consistent with the conclusion that YA and P0 are members of a complex. This complex migrates at 20S in the sucrose gradient (Fig. 6). Although P0

is predicted based on its sequence to be a component of the large ribosomal subunit (Grabowski *et al.,* 1991), the 20S complex that contains P0 and YA is not a ribosomal subunit; it is smaller than even the small ribosomal subunit (40S). Moreover, another *Drosophila* ribosomal component, DS3 (Wilson *et al.,* 1994), did not comigrate with this 20S complex (data not shown). Together with the coimmunoprecipitation experiments, these results strongly suggest that YA and P0 are present in the same protein complex *in vivo*, in both embryos and ovaries, and that ovarian YA is
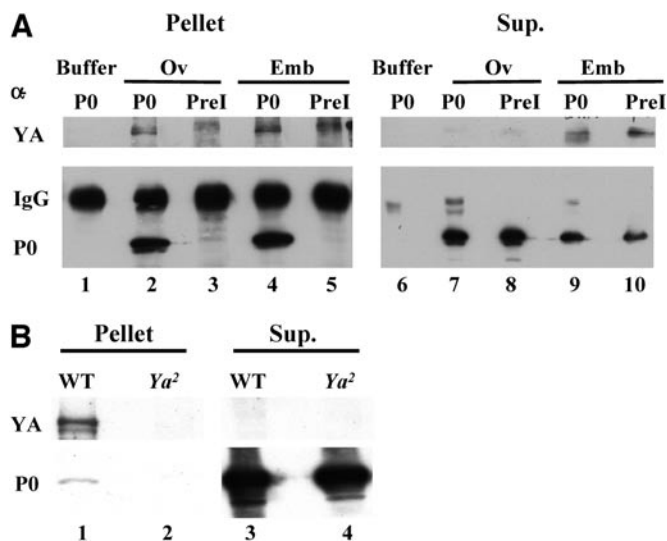

FIG. 5. YA and P0 coimmunoprecipitate from cytoplasmic extracts. (A) YA coimmunoprecipitates with P0 from cytoplasmic extracts of both ovaries and 0- to 2-h embryos. Affinity-purified anti-P0 antibodies, or preimmune serum (PreI), were added to ovarian (Ov) (40 ovaries) or embryo (Emb) (5 μl) cytoplasmic extracts. The antibodies were then pelleted with protein A-Sepharose 4B beads. Half of the proteins bound to the beads (Pellet) and 1/20 of the supernatant after binding (Sup.) were analyzed by Western blotting with anti-YA antibodies (YA) or anti-P0 antibodies (P0). The YA doublet was precipitated with P0 by anti-P0 antibodies (lanes 2 and 4) but not by preimmune serum (lanes 3 and 5). The signals in the preimmune serum lanes are not YA, as they are also present in preimmune serum not incubated with extracts (data not shown). The ratio of YA to P0 in the pellet is similar between ovary and embryo samples, but higher in embryo supernatant samples than in ovary supernatant samples. (B) P0 coimmunoprecipitates with YA from embryo cytoplasmic extracts. Cytoplasmic extracts from 5 μl of packed 0- to 2-h wild-type (WT) or Ya^2 mutant (Ya^2) embryos were incubated with anti-YA antibodies and pelleted with protein A-Sepharose 4B beads. The eluate from the beads (Pellet) and the supernatant after binding (Sup.) equivalent to that from 1 μl of embryos were analyzed with Western blotting with either anti-YA antibodies (YA) or anti-P0 antibodies (P0). P0 was pulled down together with YA from the wild-type embryo cytoplasmic extracts (lane 1), but not from the Ya^2 mutant extracts whose truncated YA is not recognized and pulled down by the anti-YA antibodies used (lane 2).
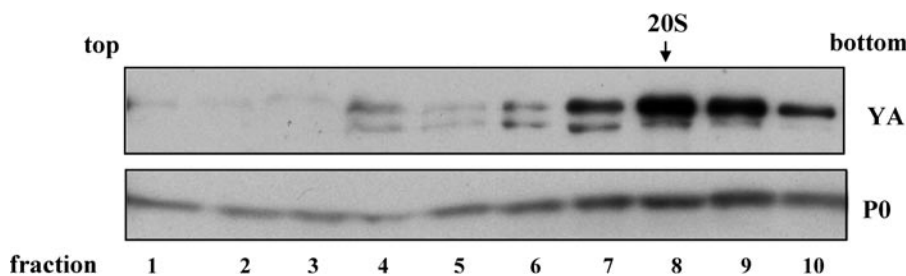
FIG. 6. YA and P0 are present in a 20S complex in cytoplasm. Cytoplasmic extracts from 0- to 2-h embryos were loaded onto a 5–35% sucrose density gradient. Fractions were analyzed by Western blotting with anti-YA antibodies (YA; top panel) and anti-P0 antibodies (P0; bottom panel). Both YA and P0 peaked at fraction 8 in the density gradient. This fraction is 20S, based on the size markers run at the same time (data not shown). In addition to their comigration in a 20S complex, YA and P0 appear to also be found (separately) in other complexes.

likely to associate with this complex more strongly than does embryo YA.

MAPK Phosphorylates YA in Vitro and This Phosphorylation Increases Binding of YA to P0

The changes in YA's phosphorylation state at a potential MAPK and $p34^{Cdc2}$ kinase site during egg activation suggested that YA may be phosphorylated by MAPK or $p34^{Cdc2}$ kinase in oocytes and dephosphorylated after the initiation of egg activation (Yu *et al.,* 1999). Therefore, we tested whether YA can be phosphorylated by one or both of these kinases.

Purified MBP-HMK-YA was incubated *in vitro* under phosphorylation-permissive conditions with $p34^{Cdc2}$ kinase immunoprecipitated from mitotic Hela S3 cells, with MAPK, or with both $p34^{Cdc2}$ kinase and MAPK. As shown in Fig. 7, MBP-HMK-YA was not phosphorylated in the presence of active $p34^{Cdc2}$ kinase alone (Fig. 7, lane 1), but was phosphorylated in the presence of MAPK alone and of both kinases (Fig. 7, lanes 2 and 3). These results indicate that, *in vitro*, YA is a substrate for MAPK but not for $p34^{Cdc2}$ kinase.

We then tested whether phosphorylation of MBP-HMK-YA by MAPK affects MBP-HMK-YA's binding of P0. Equal amounts of phosphorylated MBP-HMK-YA and unphosphorylated MBP-HMK-YA (from a "mock" phosphorylation reaction) were incubated with the same amount of purified P0-His$_6$. The complex was pulled down with amylose beads. At 0.3 μg, a concentration of MBP-HMK-YA below saturation for binding, more P0 was pulled down together with phosphorylated MBP-HMK-YA (Fig. 8, lane 3) than with unphosphorylated MBP-HMK-YA (Fig. 8, lane 4). This indicates that MAPK phosphorylated YA binds more strongly to P0 than unphosphorylated YA *in vitro*.

Decreased P0 Levels Partially Suppress a Hypomorphic YA Mutant Allele

For further confirmation of interaction between YA and P0, we examined the genetic interaction between YA and

P0. We examined the effect of maternal heterozygosity for a P0 null allele $P0^{306}$ (Frolov and Birchler, 1998) on embryos from females homozygous for the hypomorphic Ya^{77} mutation ("Ya^{77} embryos"). Ya^{77} embryos are classified into three categories based on their arrest phenotype (Liu *et al.,* 1995). Pronuclear arrest embryos are arrested at the pronuclear stage at the end of meiosis. This is the most severe
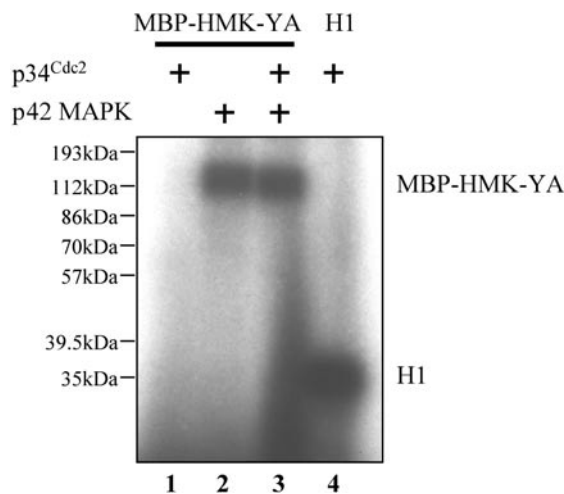

FIG. 7. YA is phosphorylated by MAPK but not by $p34^{Cdc2}$ kinase. Purified MBP-HMK-YA was incubated with $p34^{Cdc2}$ kinase immunoprecipitated from mitotic Hela cell lysate, MAPK, or both kinases in the presence of $[\gamma\text{-}^{32}P]ATP$. The kinase reactions were analyzed by SDS–PAGE and autoradiography. MBP-HMK-YA was not phosphorylated by $p34^{Cdc2}$ kinase (lane1). The lack of signal in this lane is not due to inactivity of the kinase, since the parallel positive control reaction showed histone H1 being phosphorylated by $p34^{Cdc2}$ kinase (lane 4). MBP-HMK-YA was phosphorylated by MAPK in the absence or presence of $p34^{Cdc2}$ kinase (lanes 2 and 3). Phosphorylation of MBP-HMK-YA by MAPK is due to phosphorylation of the YA moiety and not of the MBP-HMK moiety, since the 60-kDa MBP-HMK-containing degradation product in the MBP-HMK-YA preparation is not labeled.
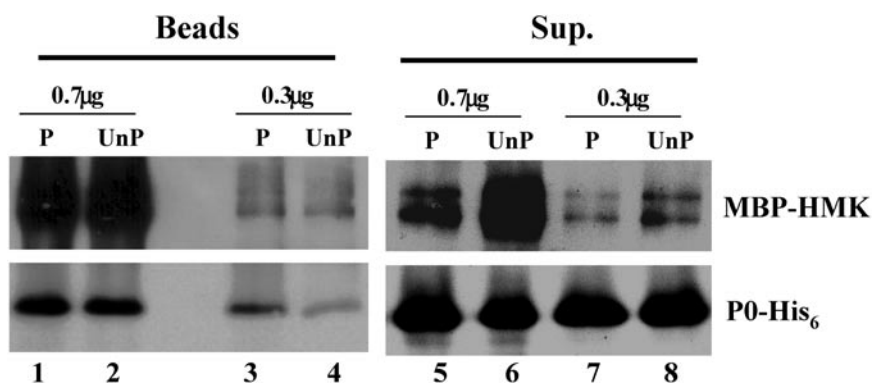
FIG. 8. MAPK phosphorylation increases YA's binding to P0. A total of 4 μg of MBP-HMK-YA was incubated with p34^{Cdc2} kinase and MAPK as in Fig. 7; an equal amount of MBP-HMK-YA was incubated in a parallel "mock" reaction from which the kinases were omitted. Then, 3 μg of P0-His$_6$ was incubated with 0.7 or 0.3 μg of phosphorylated YA (P) or unphosphorylated YA (from the mock reaction; UnP). Amylose beads were then added to pull down the complexes containing MBP-HMK-YA. 1/25 of the supernatant after binding (Sup.) and half of the proteins eluted from the beads (Beads) were analyzed by Western blotting probed with anti-YA antibodies or anti-P0 antibodies. A total of 0.7 μg of phosphorylated or unphosphorylated MBP-HMK-YA pulled down similar amount of P0 (compare lanes 1 and 2, P0 panel), indicating that this concentration is above saturation for binding. As YA becomes limiting, difference in affinity are detectable: 0.3 μg of phosphorylated MBP-HMK-YA pulled down more P0 than 0.3 μg of unphosphorylated MBP-HMK-YA (compare lanes 3 and 4, P0 panel).

phenotype. Type II embryos escape the pronuclear arrest and undergo a few of rounds of nuclear division before arresting. Type I embryos are thought to be degenerating from pronuclear or type II arrests. As shown in Table 2, lowering the P0 level by half ($P0^{306}$/+) partially but significantly ($P = 0.02$) suppressed the Ya^{77} phenotype. Fewer embryos from X^X, $y\ Ya^{77}\ cv\ v\ f$/Y; $P0^{306}$/+ mothers arrested at the pronuclear stages than those from control X^X, $y\ Ya^{77}\ cv\ v\ f$/Y; +/*MKRS* mothers, and more embryos escaped the pronuclear arrest and underwent a couple of rounds of division (Type II). This suggests that P0 normally functions to antagonize YA function, consistent with our proposal that P0 functions to keep YA out of nuclei, where YA

functions. Heterozygous P0 null females are fertile, suggesting that half the normal dose of P0 is still enough to retain hyperphosphorylated YA in the cytoplasm of oocytes.

DISCUSSION

The subcellular location of YA proteins is modulated during development; this correlates with changes in YA's phosphorylation state, including at one putative MAPK target site (Yu *et al.,* 1999). In this study, we showed that four proteins from cytoplasmic extracts bind to YA directly *in vitro* and identified one of them as *Drosophila* P0/AP3. Our observations here that the cytoplasmic protein P0/AP3 associates with YA in a 20S complex in the cytoplasm and that this association is stronger when YA has been phosphorylated by MAPK suggest the following model (Fig. 9): During oogenesis, YA is synthesized from maternal mRNA and phosphorylated by MAPK in the developing egg chamber. This phosphorylation causes more YA to be retained in a 20S cytoplasmic complex that serves to retain YA outside of nuclei. Upon egg activation, YA is partially dephosphorylated, including at one MAPK site. This dephosphorylation decreases YA's retention by the 20S complex. Those YA molecules released from the complex are freed to enter nuclei via their NLS. The future purification and identification of the other three cytoplasmic proteins that bind to YA will identify their roles in the retention of YA during development. Our data from *in vitro* assays (affinity chromatography, blot overlay, and pull-down) and from yeast two-hybrid analysis are consistent with this model. The coimmunoprecipitation and the sucrose density gradient experiments with cytoplasmic extracts strongly argue that

TABLE 2

Lowered P0 Level Partially Suppresses a Hypomorphic *Ya* Mutant Allele

	Phenotype		
Genotype	Pronuclear arrest	Type II	Type I
X^X, Ya^{77}; $P0^{306}$/+ ($n = 158$)	112 (70.9%)	7 (4.4%)	39 (24.7%)
X^X, Ya^{77}; +/*MKRS* ($n = 105$)	90 (85.7%)	2 (1.9%)	13 (12.4%)

Note. Embryos at 0–2 h from X^X, $y\ Ya^{77}\ cv\ v\ f$/Y; $P0^{306}$/+ and X^X, $y\ Ya^{77}\ cv\ v\ f$/Y; +/*MKRS* females were collected and stained with DAPI. Embryos were scored for their arrest phenotype according to Liu *et al.* (1995). X^X, Ya^{77} stands for X^X, $y\ Ya^{77}\ cv\ v\ f$/Y. χ^2, 7.81; degrees of freedom, 2; $P = 0.02$.

- ▲ P0-containing complex
- ∽ Phosphorylated YA
- ʃ Partially dephosphorylated YA
- ○ Oocyte nucleus (4n) lacking YA
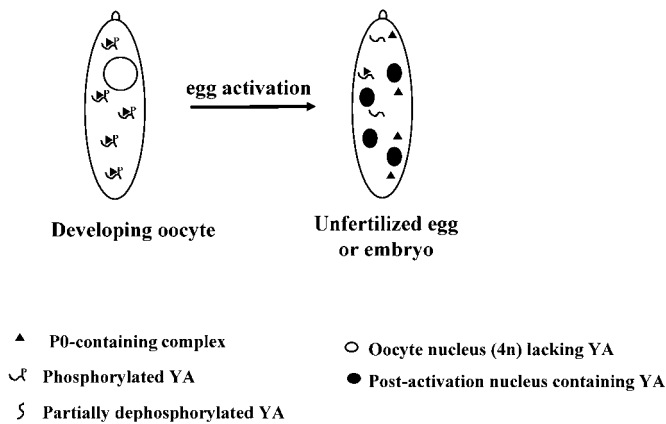- ● Post-activation nucleus containing YA

FIG. 9. A model for the regulation of YA's nuclear entry during development by the 20S cytoplasmic complex. Hyperphosphorylated YA in ovaries is more strongly retained in the 20S complex and is kept completely in the cytoplasm. Partially dephosphorylated YA in activated eggs and in embryos is less strongly retained in the cytoplasmic complex, so some YA dissociates from the complex and is free to enter nuclei using its NLS.

YA and P0 are together in a complex *in vivo* and also support this model. Our genetic analysis showing the suppression of a weak *Ya* allele by lowered P0 dosage is also consistent with the model.

This model has several implications for understanding macromolecular events before and during egg activation.

Our data suggest that MAPK is active in meiosis-arrested *Drosophila* oocytes. MAPK activity is known to increase during oocyte maturation and remain high in meiotic metaphase-arrested oocytes of, for example, *Xenopus* and mouse (Colas and Guerrier, 1995; Russo *et al.,* 1998), but to our knowledge this has not been demonstrated in *Drosophila*. Yu *et al.* (1999) had shown that ^{443}Thr, a potential MAPK and p34^{Cdc2} kinase phosphorylation site in YA, is phosphorylated in oocytes. Our results here show that MAPK but not p34^{Cdc2} kinase can phosphorylate YA *in vitro*. Taken together, these suggest that MAPK phosphorylates YA *in vivo* in oocytes, which suggests that MAPK activity will be detected in *Drosophila* oocytes, though such activity could potentially be restricted in time or place, as is the case for p34^{Cdc2} kinase in early *Drosophila* embryos (Su *et al.,* 1998). Because YA is not a target for p34^{Cdc2} kinase *in vitro*, our data cannot address whether MPF activity is high in meiotic metaphase-arrested oocytes as it is, for example, in *Xenopus* (Gerhart *et al.,* 1984; Masui and Markert, 1971; Smith and Ecker, 1971).

Our data suggest that modulation of MAPK phosphorylation occurs during egg activation in *Drosophila*. Yu *et al.* (1999) had shown that dephosphorylation of a potential MAPK and P34^{Cdc2} target site, ^{443}Thr, during egg activation correlated with changes in YA's subcellular location. That YA is, at least *in vitro*, a target of MAPK but not p34^{Cdc2} kinase suggests that MAPK activity is modulated. It is

possible that modulation of MAPK phosphorylation is a general regulatory mechanism during *Drosophila* egg activation, similar to other organisms. Future studies of other proteins active or activated at this time in *Drosophila* (Axton *et al.,* 1994; Chen *et al.,* 2000; Edgar *et al.,* 1986; Elfring *et al.,* 1997; Fenger *et al.,* 2000; Freeman and Glover, 1987; Freeman *et al.,* 1986; Lieberfarb *et al.,* 1996; Loppin *et al.,* 2000; Page and Orr-Weaver, 1996; Santamaria, 1983; Santamaria and Gans, 1980; Shamanski and Orr-Weaver, 1991) will be useful in testing this.

YA may provide a link between MAPK activity and regulation of chromosome condensation state. YA is likely to be involved in modulating chromatin condensation during the end of meiosis (Liu *et al.,* 1995). It has been shown in mice that chromosome condensation state follows MAPK, but not MPF, activity during oocyte metaphase arrest and egg activation (Verlhac *et al.,* 1994). If the same holds true for *Drosophila*, it would be tempting to postulate that MAPK does so via its regulation of proteins that modulate chromosome condensation, such as YA.

The exclusion of YA from nuclei prior to egg activation (Yu *et al.,* 1999), and its chromatin binding (Lopez and Wolfner, 1997) and nuclear organization (Liu *et al.,* 1995) functions, might suggest that exclusion of YA from nuclei might be essential for oogenesis. Perhaps it is important to exclude from meiotic nuclei a protein that is needed for postmeiotic chromatin remodeling, in order not to interfere with meiotic chromosome condensation. If this were the case, then disabling potential phosphorylation sites that control YA's subcellular location during development should result in meiotic arrest. Our finding here that YA is a target of MAPK makes it possible to test this hypothesis.

We identified a 20S cytoplasmic complex that contains YA and P0 and that we propose serves to retain YA in the cytoplasm in response to developmental signals. Modulation of a protein's interaction with a cytoplasmic protein is one of the modes in which phosphorylation regulates a protein's nuclear entry (Jans and Hubner, 1996). The nuclear entry of NF-κB and Dorsal have been shown to be regulated in this fashion (Drier *et al.,* 1999; Li and Sedivy, 1993; Whalen and Steward, 1993). It will be interesting to see whether the 20S cytoplasmic complex that includes YA, or other analogous complexes, regulates the subcellular location of other *Drosophila* maternal proteins before and during egg activation.

The molecular events leading to and during *Drosophila* oocyte metaphase arrest and egg activation are not yet well understood. Our identification of an interaction between YA and P0, and its capacity for regulation by MAPK, points to the involvement and regulation of MAPK activity in these critical developmental processes. This regulation of YA's subcellular localization, and hence its ability to access chromatin where it could potentially sense or modulate condensation, could suggest a mechanism for MAPK's role in the regulation of chromosome condensation state at this critical developmental time in *Drosophila*.

ACKNOWLEDGMENTS

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