The Yin Yang 1 Transcription Factor Associates with Ribonucleoprotein (mRNP) Complexes in the Cytoplasm of Xenopus Oocytes

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Yin Yang 1 (YY1) is a multifunctional transcription factor that activates, represses, or initiates transcription of a diverse assortment of genes. Previous studies suggest a role for YY1 in cellular growth and differentiation, but its biological function during development of the vertebrate oocyte or embryo remains to be determined. We recently showed that YY1 is abundantly expressed throughout oogenesis and early embryonic stages of Xenopus, but it is sequestered in the cytoplasm and does not function directly in transcriptional regulation. In the present study we used a series of biochemical analyses to explore the potential function of YY1 in the oocyte cytoplasm. YY1 was isolated from oocyte lysates by oligo(dT)-cellulose chromatography, suggesting that it associates with maternally expressed mRNA in vivo. RNA mobility shift assays demonstrate that endogenous YY1 binds to labeled histone mRNA. Size exclusion chromatography of oocyte lysates revealed that YY1 exists in high molecular mass complexes in the range of 480 kDa. Destruction of endogenous RNA by RNase treatment of lysates, abolished the binding of YY1 to oligo(dT)-cellulose and resulted in redistribution from 480-kDa complexes to the monomeric form. Microinjection of RNase directly into the cytoplasm released YY1 from 480-kDa complexes and unmasked its DNA-binding activity, but did not promote translocation to the nucleus. These results provide evidence that YY1 is a component of ribonucleoprotein (mRNP) complexes in the Xenopus oocyte, indicating a novel function for YY1 in the storage or metabolism of maternal transcripts.

Yin Yang 1 (YY1) is a highly conserved GLI-Kruppel transcription factor that functions as a repressor, activator, or initiator of transcription of a large number of cellular and viral genes (reviewed in Refs. 1 and 2). The multiple functions of YY1 are influenced by the promoter context of its binding site(s), the cellular environment, and by virtue of its interactions with other proteins. Indeed, numerous protein interactions have been identified, and there is evidence to suggest that these facilitate various adaptations of YY1 activity in a diversity of contexts. Proteins such as c-Myc (3), Sp1 (4, 5), polycomb group protein EED (6), cAMP-response element-binding protein (7), E1A (8), and retinoblastoma protein (9) act as transcriptional adaptors, coactivators, or corepressors to modulate activation/repression domains, its ability to bind to promoters, or its association with the basal transcriptional machinery. YY1 also exerts its transcriptional effects by modification of chromatin structure through interactions with histone deacetylases (10) or histone acetyltransferases p300/CBP (11), and these proteins may carry out direct covalent modification of YY1 (12). Other interacting proteins, such as poly(ADP-ribose) polymerase (PARP-1), are also known to modify YY1 directly (13, 14). Clearly, YY1 is subject to very complex regulation, and not surprisingly, the mechanisms underlying coordination and specification of its repressor or activator functions in various contexts remain to be elucidated.

Recognition sites for YY1 have been identified in the promoters of a large number of genes that are expressed in a variety of tissues including muscle, blood, testes, and bone (1). Evidence from numerous tissue culture studies have suggested YY1 controls expression of developmentally regulated genes, and therefore it has been hypothesized to play an essential role in cell growth, development, and differentiation. However there have been few direct examinations of YY1 in vertebrate developmental models, and its biological role in embryonic development remains poorly understood. In mouse YY1 is abundantly expressed in oocytes and early embryos, displays a dynamic pattern of subcellular localization in early development, and is selectively expressed in certain tissues later in development (15). Heterozygous mutation in the mouse resulted in growth and neurulation defects, and full knockout caused embryonic lethality around implantation, suggesting YY1 functions at multiple stages of mouse development (15).

We recently showed that YY1 is an abundantly expressed maternally derived protein synthesized in early oocytes and stable throughout subsequent embryonic development of Xenopus (16). Its DNA-binding activity is high during early oogenesis then is repressed until after the mid-blastula stage, raising the intriguing possibility that it could function to activate or repress a battery of target genes at specific points in development. However, we found that YY1 is sequestered in the cytoplasm in oocytes and in cells of the early embryo, and expression analyses showed it does not play a direct role in oocyte or embryonic transcription (16). This led to the hypothesis that cytoplasmic retention may provide a means to inhibit or delay gene regulatory activity of YY1 until later in development, but it was also possible that YY1 could carry out an essential, as yet unidentified, function(s) in the cytoplasm. Also, it has been shown that overexpression of YY1 in embryos induces an ec-

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**EXPERIMENTAL PROCEDURES**

**Oocytes, Cell Culture, and Cell Lysates—Portions of Xenopus laevis oocytes were obtained surgically from adult female frogs (Xenopus I, Ann Arbor ML) and follicle cells were removed by gentle agitation in Ca²⁺/Mg²⁺-free OR2 (82.5 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1 mM NaH₂PO₄, 5 mM Hepes, 100 mM streptomycin sulfate, 10 mM benzyl penicillin) containing 0.2% type II collagenase (Sigma) for 4 h. Stage VI oocytes were selected, washed extensively in OR2 and maintained at 18°C. For in vitro RNase experiments, oocytes were microinjected with 200 ng of RNase A (Sigma) in a volume of 20 nl and incubated at 8 h at 18°C. Nuclear and cytoplasmic extracts were prepared by manual dissection as described previously (20). For RNA and DNA-mobility shift assays, lysates were prepared by homogenization of oocytes in buffer C (50 mM Tris-HCl, pH 7.8, 20% glycerol, 50 mM KCl, 0.1 mM EDTA, 2 mM dithiothreitol, 10 μg/ml aprotinin, 10 μg/ml leupeptin) as previously described (18). Lysates for oligo(dT)-cellulose chromatography were prepared essentially as described previously (21). Oocytes were homogenized at 4°C in 4 volumes of buffer (8% glycerol, 50 mM NaCl, 2 mM MgCl₂, 10 mM Tris-HCl, pH 7.5, 100 units/ml RNAGuard (Promega)). Homogenates were centrifuged at 10,000 × g for 12 min at 2°C, and supernatants were centrifuged a second time to remove residual cell debris. Yolk proteins were removed by extraction with an equal volume of 1,1,2-trichlorofluoroethane, and supernatants were adjusted to 100 mM NaCl. For size exclusion chromatography (SEC), lysates were prepared by homogenization in buffer C containing 100 units/ml RNAGuard (Promega). Homogenates were centrifuged at 10,000 × g for 10 min at 4°C, and supernatants were filtered through 0.2-μm nylon membranes (Gelman Laboratory) and stored at −80°C. Hepes cells were maintained from American Type Culture Collection (ATCC) and grown in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum. Cells were washed, and lysates were prepared by three freeze-thaw cycles in 5 volumes of lysis buffer (50 mM Tris-HCl, 50 mM KCl, 20% v/v glycerol, 100 μM EDTA, 10 μg/ml aprotinin, 10 μg/ml leupeptin). Oligo(dT)-cellulose chromatography—Oligo(dT)-cellulose chromatography was carried out using previously described protocols (22). Lysates (300 μl) were applied to 100 μl of oligo(dT)-cellulose (New England Biolabs) pre-equilibrated with 10 volumes of binding buffer (100 mM NaCl, 2 mM MgCl₂, 10 mM Tris-HCl, pH 7.5) and incubated 2 h at 4°C with rotation. Matrix was washed in 6 volumes of binding buffer, and bound material was eluted in 400 μl of binding buffer containing 25% (v/v) formamide. Columns were run using a BioLogic HR (Bio-Rad) high pressure chromatography control unit at a flow rate of 0.1 ml/min. Lysates (300 μl) were applied to 1-ml columns, and the flow-through was re-applied to columns four times. Poly(A)⁺ bound proteins were eluted in 200-μl fractions with binding buffer containing a range of NaCl from 100 mM to 2 M. Proteins were precipitated in 5 volumes of acetone at −80°C.

**Size Exclusion Chromatography (SEC)—Lysates (300 μl) were loaded onto size exclusion columns using either Bio-Sil SEC 250–5 (Bio-Rad; separation range 10–300 kDa) or Superdex 200 HR 10/30 (Amersham Biosciences; separation range, 10–11,000 kDa) using a Bio-Logic HR HPLC (Bio-Rad) at a flow rate of 0.5 ml/min with column buffer (50 mM Tris-HCl, 50 mM NaCl, 0.1 mM EDTA). Fractions (200 μl) were collected and precipitated in 5 volumes of acetone at −80°C. Samples were resuspended in 60 μl of Laemmli buffer. Columns were calibrated using Gel Filtration Standard Bio-Rad catalog number 151–1901 containing the following markers: thyroglobulin (670 kDa), γ globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), vitamin B-12 (1.35 kDa).

**Antibodies and Immunoblotting—** Proteins were electrophoresed on 10% SDS-polyacrylamide gels, and immunoblotting was performed as previously described (20). Antibodies used for immunoblotting were: rabbit anti-human YY1 polyclonal (SC-281, Santa Cruz), anti-human PCNA monoclonal (SC-56, Santa Cruz), rabbit anti-Xenopus nucleolin (gift of Dr. Patrick DeMario Louisiana State University), rabbit anti-YY1 mAb (gift of Dr. K. Matsumoto, Riken, Wako, Saitama, Japan). Antibody-protein interactions were detected by chemiluminescence (Renasssance System; PerkinElmer Life Sciences) and exposure to x-ray film.

**Gel Mobility Shift Assays—** Binding reactions and DNA gel mobility shift assays were performed as described previously (16). RNA gel mobility shift assays were performed essentially using the methods as described previously (25) with minor modifications. Binding reactions were carried out for 20 min at 4°C in RNA binding buffer (20 mM Tris-HCl, pH 7.4), 4 mM MgCl₂, 100 mM KC₃, 30% v/v glycerol, 1 mM dithiothreitol, 20 μg/ml bovine serum albumin) with 0.5 ng of labeled RNA probe and 20 μg of soluble protein in a total reaction volume of 20 μl. Antibodies against YY1 and PCNA (see above) were added directly to the binding reactions in a range of concentrations and then immediately loaded onto non-denaturing 5% polyacrylamide gels containing 6.7 mM Tris-HCl, pH 7.5, 1 mM EDTA, 3.3 mM sodium acetate, 5% glycerol. Gels were maintained at 4°C during electrophoresis for 2.5 h at 160 volts. Gels were dried and exposed to x-ray film overnight at −80°C. The mRNA probe encoding Xenopus histone H2A was synthesized from a plasmid (pBluescript) containing a 393-nucleotide H2A PCR product inserted into the Smal site. Plasmid was linearized at the BamHI site at the 3’-end of the insert (a gift of Dr. A. Johnson, Florida State Univ, Tallahassee, FL), and 3²P-labeled RNA was synthesized in vitro with modifications to the method as described previously (26).

**RESULTS**

**RNA-binding Activity of YY1 in the Oocyte Cytoplasm—** Previous domain analysis of YY1 shows that it contains four C₂H₂ zinc finger domains that function in DNA binding (1, 2). This structure of the YY1 molecule provided an important first clue to its potential role in the cytoplasm. Certain RNA-binding proteins, including, TFIIIA which is known to bind oocyte 5S RNA (27), display sequence similarity to zinc finger DNA-binding transcription factors, particularly in the spacing of RNA-binding motifs (28) in the amino acid sequence of TFIIIA (PDB accession number JC2426) and TFIIIA (PDB accession number 19). The protein sequence alignment with TFIIIA precludes YY1 (29). The protein sequence alignment with TFIIIA suggests that YY1 has been highlighted (boxed) as a DNA-binding transcription factor and mRNP suggests that YY1 might bind RNA in the oocyte. YY1, in addition to having a C₂H₂ zinc finger domain, also contains a 160-amino acid region, the leucine-rich YY1 box, which is conserved in YY1. The YY1 box has been shown to interact with RNA in vitro (28) and to bind RNA in a specific manner (29).

**YY1 Associates with mRNPs in the Oocyte Cytoplasm—** YY1 interacts with mRNA in the oocyte cytoplasm. YY1 binds RNA in the oocyte cytoplasm, and its interaction with RNA is necessary for the binding of YY1 to mRNPs. YY1 binds to mRNPs in the oocyte cytoplasm, and its interaction with RNA is necessary for the binding of YY1 to mRNPs. YY1 binds to mRNPs in the oocyte cytoplasm, and its interaction with RNA is necessary for the binding of YY1 to mRNPs. YY1 binds to mRNPs in the oocyte cytoplasm, and its interaction with RNA is necessary for the binding of YY1 to mRNPs.
the cellulose matrix, the bound material was eluted in a buffer containing 25% formamide. The eluants were then analyzed for the presence of proteins by Coomassie staining and Western blotting. A small subset of proteins were retained on the oligo(dT) matrix (Fig. 2A), similar to what has been previously reported (22). Western blots show that YY1 was among the proteins present in the bound fraction, as was nucleolin, a known component of oocyte mRNPs (31)(Fig. 2B). In control experiments, other proteins such as nuclear PCNA, a nuclear marker protein in *Xenopus* oocytes, did not bind to oligo(dT) and were not isolated by this procedure (Fig. 2B).

We also applied oocyte lysates to oligo(dT)-cellulose columns, washed extensively, and eluted bound proteins using a salt gradient. YY1 eluted from the column at 600–750 mM NaCl (Fig. 2C). The binding of YY1 to oligo(dT)-cellulose indicates that YY1 binds to maternal poly(A)+ mRNA in the oocyte cytoplasm. To rule out the possibility that YY1 has high affinity for oligo(dT), lysates were treated with RNase prior to chromatography. Destruction of RNA abolished the retention of YY1 (Fig. 2C), demonstrating that its affinity for oligo(dT) was dependent on its interaction with intact RNA in oocyte lysates. These results provide initial evidence that YY1 associates with maternal mRNA in the cytoplasm, which strongly suggests that it is a component of cytoplasmic mRNPs.

We next used in vitro RNA mobility shift assays to test more directly for the ability of YY1 to associate with RNA. Oocyte lysates were incubated with a 393-nucleotide radiolabeled histone mRNA probe, and samples were electrophoresed on nondenaturing 5% polyacrylamide gels containing 5% glycerol. The rationale for the use of histone mRNA as a probe in these experiments was that it is a small message amenable to the mobility shift assay and it is an abundant mRNA species in the oocyte (32) providing a biologically relevant binding target.

Also, previous studies have used histone mRNA probes to demonstrate *in vitro* binding activity of mRNPs (19). At least two prominent protein-mRNA complexes were detected as retarded bands (Fig. 3). Formation of the more slowly migrating complex was disrupted in a concentration-dependent manner by addition of YY1-antibodies directly into the binding reactions (Fig. 3A). In controls, neither complex was affected by addition of antibodies against PCNA (Fig. 3B), suggesting that disruption of the upper band was due to specific YY1-antibody recognition of a YY1-containing mRNA-complex. This data confirms that native YY1 in oocyte lysates is capable of associating with mRNA, but whether YY1 binds directly or as part of a larger multi-protein complex, or if there is sequence-specificity, remains to be determined.

**Molecular Size and RNase Sensitivity of Native YY1 Complexes**—The specific retention of YY1 on oligo(dT)-cellulose (Fig. 2) and its presence in protein-mRNA complexes (Fig. 3) provided initial evidence that YY1 in its native state associates with or is a component of mRNPs in the oocyte cytoplasm. To confirm this, we fractionated oocyte lysates by SEC in order to examine the size distribution of native YY1. In the initial experiments, lysates were applied to BIO-SILECT 250 (Bio-Rad) columns, fractions were separated by SDS-gel electrophoresis, and fractions were analyzed by Western blotting. All of the YY1 was detected in the column flow-through (Fig. 4). Its presence in the void material suggested that in its native state YY1 exists in complexes larger than 300 kDa in the cytoplasm. To more accurately determine the size of YY1 complexes, we next applied lysates to SEC using Superdex 200, a matrix with the capacity to separate larger protein complexes. YY1 was detected in fractions around 480 kDa (Fig. 5). Monomers were not detected in untreated native lysates using either matrix, suggesting that the majority of endogenous YY1 is bound in large complexes. FRG2, the major mRNP component in *Xenopus* oocytes (7, 33–36) was isolated in a higher size range,
in comparison to YY1, over 500 kDa (Fig. 5). The elution profiles of YY1 and FRGY2 were partially overlapping, but not identical.

To confirm the identity of the 480-kDa YY1 complexes as mRNPs, lysates were treated with RNase prior to fractionation to destroy endogenous mRNA. This resulted in a complete redistribution of YY1 to fractions around 60 kDa, close to its predicted molecular size as a monomer (Figs. 4 and 5). Thus, targeted destruction of RNA in vitro destabilized 480-kDa YY1 complexes present in oocyte lysates and resulted in the release of monomers. This demonstrates that the integrity of native complexes is dependent on the presence of intact maternal mRNA. In control experiments, we treated aliquots of the same lysates with DNase prior to SEC, which did not significantly alter the size distribution of YY1 (Fig. 5). Therefore, RNase-mediated destabilization of 480-kDa YY1 complexes was likely a direct result of RNA destruction rather than an artifact of the experimental protocol. The effects of RNase seen in these experiments provide strong evidence that YY1 associates with mRNPs particles in the cytoplasm of oocytes. The isolation of YY1 in large RNase-sensitive complexes is entirely consistent with its binding to oligo(dT)-cellulose (Fig. 2) and mRNA-binding activity (Fig. 3). YY1 is clearly a component of mRNP particles in the *Xenopus* oocyte.

We also carried out a direct comparison between the relative size distribution of cytoplasmic and nuclear YY1 by SEC. HepG2 cell lysates were used as a source of nuclear YY1 since we have shown that it is entirely a nuclear protein in adult liver cells (16). Most if not all of YY1 from liver cell lysates was detected in fractions around its predicted size as a monomer or dimer (Fig. 5).

**RNA-dependent Association of YY1 with mRNPs in Vivo**—To analyze the association of YY1 with mRNPs *in vivo*, we tested the effects of degrading maternal mRNA *in vivo* after microinjection of a high concentration of RNase directly into the oocyte cytoplasm. Similar techniques have been used to examine the behavior of the CBTF in *Xenopus* embryos (18). First, we compared the relative size of YY1 in native and RNase-treated extracts by SEC. Microinjected RNase resulted in a redistribution of YY1 from high to low molecular weight fractions (Fig. 6A), confirming that association of YY1 in mRNP complexes is dependent on intact maternal mRNA.

In a previous paper, we demonstrated that YY1 is localized to the cytoplasm through early development and displays high levels of DNA-binding activity in early (stage I-II) oocytes, which is masked through subsequent oogenesis and reactivated at the mid-blastula stage after 12 h of embryonic development (16). Therefore, we postulated that the DNA-binding activity of YY1 may be physically blocked by virtue of direct mRNA-binding and/or association with mRNPs. Since SEC analysis shows targeted RNA degradation appears to release YY1 monomers from mRNP complexes (Figs. 4–6), we next tested whether this might also unmask its DNA-binding activity. Oocytes were microinjected with RNase, incubated for several hours, then manually dissected into nuclear and cytoplasmic.
mic fractions. These were then analyzed by gel mobility shift assays using a double-stranded DNA probe containing a consensus YY1 binding site. The YY1-DNA complex typically migrates at a position below a ubiquitous nonspecific DNA-binding activity attributable to protein(s) in the nucleus (16, 37). Specific YY1-DNA complexes were not detected in untreated lysates (Fig. 6B), consistent with our previous finding that its DNA-binding activity is masked in later oocyte development (16). In contrast, high levels of the specific YY1-DNA complex were detected in whole cell lysates and the cytoplasmic fractions from RNase-injected oocytes but not in nuclear fractions. This suggests that the DNA-binding activity of cytoplasmic YY1 is unmasked upon release of YY1 monomers from mRNP complexes.

Recently, it was shown that a subunit of the CCAAT box transcription factor, CBTF122, is tethered to the cytoplasm through association with mRNPs, illuminating a novel mechanism by which the gene regulatory activity of a transcription factor may be regulated during development (16). Since YY1 is excluded from the nucleus in oocytes and during early embryonic development (16), it was possible that it might be subject to a similar RNA-dependent cytoplasmic anchoring mechanism. We tested if destruction of maternal mRNA in the oocyte cytoplasm, which releases YY1 monomers from 480-kDa complexes. These were then analyzed by gel mobility shift assays using a double-stranded DNA probe containing a consensus YY1 binding site. The YY1-DNA complex typically migrates at a position below a ubiquitous nonspecific DNA-binding activity attributable to protein(s) in the nucleus (16, 37). Specific YY1-DNA complexes were not detected in untreated lysates (Fig. 6B), consistent with our previous finding that its DNA-binding activity is masked in later oocyte development (16). In contrast, high levels of the specific YY1-DNA complex were detected in whole cell lysates and the cytoplasmic fractions from RNase-injected oocytes but not in nuclear fractions. This suggests that the DNA-binding activity of cytoplasmic YY1 is unmasked upon release of YY1 monomers from mRNP complexes.

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DISCUSSION

Taken together, the biochemical data presented in this paper establish that YY1 is a constituent of mRNP complexes in the Xenopus oocyte, a novel and distinct activity that has not been previously described for this transcription factor. YY1 in its native state in oocyte lysates is specifically bound by oligo(dT)-cellulose, suggesting it binds poly(A)\(^+\) mRNA in vitro. It has a conserved RNA-binding zinc finger/knuckle in the DNA-binding domain and displays RNA-binding activity in gel mobility shift assays. It is fractionated by SEC in high molecular weight complexes around 480 kDa. Importantly, both the ability to bind oligo(dT) and the integrity of the 480-kDa complexes are disrupted by targeted degradation of maternal RNA by both in vitro and in vivo RNase treatments. Hence, it appears that YY1 is an example of a multifunctional protein capable of binding both DNA and RNA. In this regard, YY1 is immediately comparable with the Y box-binding protein FRGY2 (mRNP3 + 4) (33–36, 38), a well characterized regulator of both transcription and translation in the oocyte. The cytoplasmic location and its association with mRNPs suggest the hypothesis that YY1 functions in the storage and translational control of maternal mRNA in the oocyte.

Xenopus oocytes accumulate a vast store of maternal mRNA, most of which is stabilized and translationally repressed in cytoplasmic mRNPs (17) until they are recruited onto polysomes for protein synthesis later in development (39). FRGY2, the major constituent of cytoplasmic mRNPs in the Xenopus oocyte, was originally identified as a transcription factor recognizing the Y box element of various promoters (24, 33, 40). The ability of FRGY2 to mask translation of maternal mRNAs has been well characterized (19, 31, 41). By comparison, therefore, it is possible that YY1 might function to repress transcription of maternal mRNA.

In addition to FRGY2, there are at least two notable examples of transcription factors with dual DNA and RNA binding capacity that function to regulate RNA in Xenopus oocytes and embryos. Indeed, the behavior of YY1 is comparable with TFIIIA, a zinc finger-containing factor that functions in both transcription and stabilization of SS rRNA (27). It is noteworthy that the spacing of key histidine and cysteine residues in the DNA-binding domain of YY1 is conserved with the general zinc finger/knuckle present in TFIIIA and other RNA-binding proteins. Although the data suggests that YY1 complexes bind to mRNA, the possibility that YY1 might associate with SS storage particles cannot yet be excluded.

We are also compelled to compare the dual activity of YY1 in the oocyte to the 122-kDa subunit of the CBTF122 in the developing embryo. Brzostowski et al. (18) recently identified CBTF122 as a cytoplasmic mRNP-associating protein in fertilized eggs that translocates to the nucleus at gastrula stage to activate transcription of GATA-2. CBTF122 is tethered to the cytoplasm by virtue of its interaction with RNA, illustrating a novel regulatory mechanism to control a transcription factor during development. Though we could speculate that YY1 comes under similar regulation, there are certain limitations in the comparison of these proteins. First, their respective patterns of sub-cellular localization during development are quite different. Whereas YY1 remains cytoplasmic in early development (16), CBTF is nuclear in oocytes, becomes cytoplasmic and associates with mRNPs after fertilization, and then migrates to the nucleus at gastrulation (18). Second, YY1 does not translocate to the nucleus after microinjection of RNase (Fig. 6) suggesting that interaction with mRNA is not solely responsible for its cytoplasmic location. In contrast, mRNA binding is sufficient for cytoplasmic retention of CBTF122, and nuclear localization is linked to mRNA degradation at the gastrula stage (18). Notwithstanding these discrepancies, it is clear that the ability of transcription factors to bind RNA and associate with mRNPs has emerged as an important regulatory mechanism at work during development. Since YY1 expressed in the oocyte remains in the cytoplasm of the embryo, it is likely that it continues to interact with and possibly regulate mRNAs during embryonic development.

Several questions regarding the function and behavior of YY1 in the oocyte cytoplasm remain to be answered. For example, SEC of oocyte lysates showed that YY1 and FRGY2 have overlapping but not identical elution profiles (Fig. 5). Thus, certain subsets of mRNP complexes might contain both YY1 and FRGY2. We acknowledge that the constituents of YY1-containing mRNPs remain to be determined and the possibility that YY1 functionally associates with FRGY2 in translational masking of mRNAs awaits further study.

RNA mobility shift experiments using native oocyte lysates demonstrated that YY1 has the ability to bind labeled mRNA in vitro (see Fig. 3). Thus, YY1 may contact RNA directly perhaps through the zinc finger/knuckle present in the DNA-binding domain, in which case we predict that RNA binding would preclude DNA binding. Such a scenario is consistent with reactivation of DNA-binding activity by RNase degradation of endogenous mRNA in the cytoplasm (see Fig. 6). Further examination of YY1 will be required to establish the relation-
ship between RNA- and DNA-binding activities at the biochem-
ical and organismal levels.

Similar to FRGY2 and other mRNPs such as CIRP2 (22), YY1 is a very abundant protein expressed early during oocyte development (16). We suggest the abundance of YY1 facilitates packaging of maternal mRNA, most of which are synthesized early in oogenesis (42) into ~2 x 10^14 mRNPs that accumulate by stage II (17). It is interesting that a high level of YY1 DNA-binding activity is detectable in early stage oocytes and then declines at stage II coincident with the bulk of mRNA synthesis (16). This pattern of DNA binding might therefore reflect the availability of uncomplexed YY1 in early oocytes and hypothesize that it plays a role in early development (16). We suggest the abundance of YY1 facilitates the regulation of maternal mRNA. We note that immunocy-
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Finally, we have established here that YY1 is a constituent of mRNPs in Xenopus oocytes and hypothesize that it plays a role in the regulation of maternal mRNA. We note that immunocytochemical analyses reveal that YY1 is a cytoplasmic protein in mouse oocytes, as well as in cells of the trophctoderm and inner cell mass of E3.5 embryos (15). In the mouse embryo, knockout of YY1 was lethal around implantation, indicating an indispensable role for YY1 in early development (15). Given its cytoplasmic location in the two embryonic models studied thus far and its high degree of conservation (1), the association of YY1 with mRNPs revealed here is likely to be of significance to the development of other organisms.

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