Assembly of the Yin Yang 1 Transcription Factor into Messenger Ribonucleoprotein Particles Requires Direct RNA Binding Activity*

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The early stages of vertebrate development depend heavily on control of maternally transcribed mRNAs that are stored for long periods in complexes termed messenger ribonucleoprotein particles (mRNPs) and utilized selectively following maturation and fertilization. The transcription factor Yin Yang 1 (YY1) is associated with cytoplasmic mRNPs in vertebrate oocytes; however, the mechanism by which any of the mRNP proteins associate with mRNA in the oocyte is unknown. Here we demonstrate the mechanism by which YY1 associates with mRNPs depends on its direct RNA binding activity. High affinity binding for U-rich single-stranded RNA and A:U RNA duplexes was observed in the nanomolar range, similar to the affinity for the cognate double-stranded DNA-binding element. Similar RNA binding affinity was observed with endogenous YY1 isolated from native mRNP complexes. In vivo expression experiments reveal epitope-tagged YY1 assembled into high molecular mass mRNPs, and assembly was blocked by microinjection of high affinity RNA substrate competitor. These findings present the first clues to how mRNPs assemble during early development.

YY1 clearly plays an important role in early embryonic development; however, there is compelling evidence that it functions through mechanisms other than transcriptional regulation. YY1 is localized entirely to the cytoplasm of mouse oocytes and has a mosaic pattern of nucleocytoplasmic distribution in cells of early embryos (20). Homozygous deletion of YY1 in mice causes peri-implantation lethality, and heterozygotes display severe neurulation defects (20). Studies in Xenopus show YY1 misexpression affects survival, neurulation, and patterning (7, 21, 22). Biochemical analysis has shown that YY1 is entirely restricted to the cytoplasm during early development, and an absence of YY1 transcriptional regulatory activity has been observed with promoter constructs containing YY1 consensus elements (19, 23). In oocytes and early embryos, YY1 is a component of cytoplasmic messenger ribonucleoprotein particles (mRNPs) (19, 23). Several groups have reported cytoplasmic localization of YY1 in mammalian cultured cells and differentiated cells in vivo (24–26).

The control of early vertebrate development depends heavily on mRNAs stored in the oocyte cytoplasm in ribonucleoprotein complexes termed mRNPs (27). The Xenopus oocyte has been the primary model system for studies of mRNPs. During oogenesis, mRNPs become highly concentrated in the cytoplasm and function in the storage and stabilization of maternally transcribed mRNAs required for subsequent embryonic development (27–30). The heterogeneous population of mRNAs associated with cytoplasmic mRNPs originate from a subset of maternally transcribed, intronless genes (31). Despite a relatively extensive body of experimental work from decades past, the structure and function of oocyte mRNPs are rather poorly understood. Common sequence features among these mRNAs that could possibly serve as sites of recruitment of mRNP proteins have not emerged (31). Thus, mRNPs constitute a highly heterogeneous population of complexes, which have thus far...
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resisted traditional biochemical and genetic characterization (27, 30, 32).

Oocyte mRNP components identified thus far include the RNA helicase Xp54 (33); the cold-shock domain-containing transcription factors FRGY2a and FRGY2b (32); CIRP2 (cold-inducible RNA-binding protein 2) (34); RAP55 (RNA-associated protein 55) (35–37); CAT-box transcription factor (38); and YY1 (19). There is evidence that Xp54 and FRGY2a/b may function in translational repression of associated mRNAs (29, 37, 39–43), and Xp54 and FRGY2a/b have been shown to associate with RNA in vitro (32, 39). However, the RNA binding activities of mRNP proteins have not been extensively examined nor has the role of these activities in the mechanism of mRNP assembly in vivo been elucidated.

The association of YY1 and other mRNP proteins with maternal transcripts could potentially be mediated by protein-protein interactions, through direct interaction with mRNA molecules, or through a combination of protein-nucleic acid interactions. Given that YY1 possesses a potential RNA-binding zinc finger motif (19), we examined the ability of YY1 to bind directly to RNA. Here we report that both native mRNP-associated YY1 isolated from oocytes and recombinant bacterially expressed YY1 possess high affinity RNA binding activity. In vitro binding assays conducted with RNA probes exhibiting a range of sequence and structural features reveal that YY1 binds with highest affinity to U-rich single-stranded RNA and to A:U RNA duplexes. Nascent YY1 expressed in vitro assembled into high molecular mass mRNPs recoverable by oligo(dT) chromatography. Microinjection of high affinity RNA substrates effectively blocked the assembly of YY1 into mRNPs in vivo, demonstrating that association with mRNPs is dependent on RNA binding activity. We conclude the mechanism of YY1 assembly into mRNPs requires direct association with maternal mRNA.

**EXPERIMENTAL PROCEDURES**

Preparation of Recombinant Protein—The coding sequence of *X. laevis* YY1 (GenBank™ accession number NM_001087615) was inserted in-frame, downstream of the His6 tag of pRsetB (Invitrogen), to produce pRsetBYY1. For bacterial expression, pRsetBYY1 was transformed into BL21(DE3) pLysS (Invitrogen), to produce pRsetBYY1. For expression, rrYY1 (supplemental sequences) was radiolabeled using [α-32P]dCTP (PerkinElmer Life Sciences) and MLuV reverse transcriptase. Single-stranded DNA and RNA probes were prepared by labeling reciprocal strands and indicated. Size exclusion chromatography was performed as described previously (19).

Preparation of Radiolabeled Nucleotide Probes—Oligonucleotide probes (supplemental sequences) were radiolabeled and purified as follows. Double-stranded DNA probes were end-ﬁll labeled using [α-32P]dCTP (PerkinElmer Life Sciences) and MLuV reverse transcriptase. Single-stranded DNA and RNA probes were end-labeled using [γ-32P]ATP (PerkinElmer Life Sciences) and T4 polynucleotide kinase. Double-stranded RNA probes were prepared by labeling reciprocal strands and then puriﬁed and annealed to the complementary unlabeled strand. Double-stranded RNA probe integrity was further veriﬁed by insensitivity to single-stranded ribonucleases (data not shown). Labeled probes were puriﬁed over Sephadex-G-50 (Sigma), extracted with phenol/chloroform (1:1) and chloroform/isooamyl alcohol (19:1), ethanol-precipitated, resuspended in TEN buffer (100 mM NaCl, 20 mM Tris-HCl (pH 7.6), 2 mM EDTA), and spin-dialed with TEN buffer ﬁve times using Microsep 3K Omega spin tubes (Pall Corp., Waltham, Ontario, Canada). The state of purity was monitored by electrophoresis on 20% polyacrylamide, 0.5× TBE gels containing 8.3 M urea. Once no trace of unincorporated label could be detected by 12 h of radiography using Kodak XB-1 ﬁlm, oligonucleotides were quantiﬁed by UV spectroscopy on a Bio-Rad SmartSpec® using millimolar extinction coefﬁcients speciﬁc to the oligonucleo-
tide being measured. Only samples with an $A_{260}/A_{280}$ ratio greater than 1.8 were used.

**Electrophoretic Mobility Shift Assays (EMSA), Western Blotting, and Antibodies**—Binding reactions for EMSA contained 0.1 pmol (10 nM) of labeled probe, 50 mM NaCl, 50 mM Tris-HCl (pH 7.5), 2 mM MgCl$_2$, 1 mM DTT, 10 units of RNasin (Fermentas). Reactions with recombinant protein contained a final volume of 10 μl. Reactions containing oocyte lysate or purified mRNPs contained a final volume of 20 μl. For competition experiments, oligonucleotides (1 pmol) or antibodies (200 ng) were added directly to binding reactions. Reactions were incubated at room temperature for 20 min, mixed with 2 μl of loading dye (0.1% (w/v) bromphenol blue, 0.1% (w/v) xylene cyanol FF, 50% (v/v) glycerol), and immediately loaded onto 5% polyacrylamide 0.5× TBE gels and electrophoresed at 150 volts for 2.5 h at 4 °C. Gels were dried and autoradiographed at −80 °C for 16 h with an intensifying screen.

Western blotting was performed as described previously (23). Anti YY1 (catalog number SC-281, Santa Cruz Biotechnology) was used at a dilution of 1:1000; anti-PCNA (catalog number SC-56, Santa Cruz Biotechnology), anti-HA (catalog number SC-7392, Santa Cruz Biotechnology), and anti-His$_6$ (catalog number SC-803, Santa Cruz Biotechnology) were used at 1:5000. Secondary antibodies used were goat anti-rabbit horseradish peroxidase conjugate (catalog number 170-6515, Bio-Rad) at 1:5000 and goat anti-mouse horseradish peroxidase conjugate (catalog number 170-6516, Bio-Rad) at a dilution of 1:5000.

**Nitrocellulose Filter Binding Assays**—Nitrocellulose filter binding assays were performed according to Romanik (45) with slight modifications. Binding reactions were carried out with 0.5 pmol probe at a concentration of 10 nM. After 20 min of incubation at room temperature, reactions were diluted with 50 μl of Filter Binding Buffer (50 mM NaCl, 50 mM Tris-HCl (pH 7.5), 2 mM MgCl$_2$) and applied immediately to pre-equilibrated 0.45-μm nitrocellulose membranes (Bio-Rad). Filters were washed twice with 100 μl of Filter Binding Buffer and then dried for 90 min at 80 °C in vacuo. Filters were dissolved in 500 μl of 0.5 M benzenethionium hydroxide in methanol and mixed with 15 ml of scintillation fluid, and radioactivity was measured in a Tri-Carb 3100TR scintillation counter (Packard Biosciences, Meriden, CT). Presented data are the average of three replicate experiments.

**Determination of Minimal Binding Site**—RNA fragments were generated by digestion of 5 pmol of end-labeled U$_{280}$ probe with 1 ng of RNase A in 200 μl of TMN (100 mM NaCl, 20 mM Tris-HCl (pH 7.6), 2 mM MgCl$_2$) for 20 min at room temperature and purified as described above for RNA probes with the exclusion of spin dialysis. Binding reactions were assembled by resuspending probe in 200 μl of binding buffer (50 mM NaCl, 50 mM Na$_2$HPO$_4$/Na$_2$HPO$_4$ (pH 7.5), 2 mM MgCl$_2$, 0.5 mM 2-mercaptoethanol, 1 unit/ml RNasin), and addition of 50 μl of nick-translation NTA-agarose beads and 0.2 nmol of YY1. Reactions were incubated 30 min at room temperature, and beads were then recovered by centrifugation (1000 × g, 1 min) and washed (2 × 1 ml) with binding buffer. Bound probe was eluted with 50 μl of 80% (v/v) formamide and electrophoresed on 20% DNA sequencing gels followed by autoradiography.

**RESULTS**

**Analysis of Recombinant YY1**—Following bacterial expression and purification of His$_6$-tagged Xenopus YY1 (Fig. 1A), activity of the recombinant protein was verified by DNA-binding assays using the consensus promoter element. YY1 required the presence of both zinc and magnesium ions for DNA binding (Fig. 1B). Formation of YY1-DNA complexes was competed with both oligonucleotide and antibody competitors (Fig. 1B), in a manner similar to that of endogenous YY1 (23). Recombi-
Yy1 bound with high affinity to consensus DNA (Fig. 1C) as observed with previous studies using mammalian Yy1 (46, 47). No binding was observed with single-stranded forward and reverse strands of either consensus or mutant DNA probes (data not shown). Therefore, Yy1 did not possess affinity for single-stranded DNA.

Nitrocellulose filter binding reactions were used for comparative determination of dissociation constants of various probes and to quantitatively verify results of the EMSA analysis. The filter-binding assay measures the proportion of the total radioactivity bound, which is converted to a molar quantity of bound probe as a function of the specific activity. High affinity of Yy1 for consensus DNA probe was verified by filter binding assay (Kd = 3.2 ± 0.1 nM, see Table 1). Moderate to low affinity binding to mutant probe (Kd = 54.9 ± 1.2 nM) and no detectable binding to heat shock element were observed (Fig. 1C and Table 1). Yy1 did not exhibit free nucleotide binding activity to adenosine, uridine, or deoxycytidine triphosphates (data not shown).

**Examination of Yy1 RNA Binding Activity**—We examined the possibility that assembly of Yy1 into mRNPs may involve direct RNA binding activity. Initially we tested RNA sequences complementary to the consensus promoter element, both single-stranded (forward and reverse) and duplex consensus as well as mutant probes. Yy1 binding to single-stranded consensus as well as mutant RNA probes was barely detectable, and Yy1 bound very weakly to both consensus and mutant duplex RNA probes (Fig. 2 and Table 1).

The next experiments measured Yy1 affinity for short (20 nucleotides) synthetic oligoribonucleotides to examine sequence and structural specificity. EMSA and filter-binding assays revealed high affinity for U20 (Kd = 1.2 ± 0.2 nM) and weak affinity for A20 (Kd = 24.8 ± 1.3 nM) and G20 (Kd = 114 ± 4 nM), and no detectable binding was observed with C20 (Fig. 2 and Table 1). Analysis of binding to G20 by EMSA was not in accordance with filter binding data. We surmise that gel shift assays were complicated by the presence of the excessive secondary structure present in this probe (data not shown), and we

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**TABLE 1**

**Dissociation constants of Yy1-probe complexes**

| Oligonucleotide | Kd (nM) | Oligonucleotide | Kd (nM) |
|-----------------|---------|-----------------|---------|
| C DNA (consensus dsDNA) | 3.2 ± 0.1 | C20 (poly(C) ssRNA) | NA |
| M DN (mutant dsDNA) | 54.9 ± 1.2 | A20 (poly(A) ssRNA) | 24.8 ± 1.3 |
| HSE (heat shock element dsDNA) | NA | (AC) (GU) | NA |
| CF RNA (consensus forward ssRNA) | NA | (CU) (GU) | NA |
| CR RNA (consensus reverse ssRNA) | NA | (AG) (GU) | NA |
| MF RNA (mutant forward ssRNA) | NA | A:U (dsRNA) | 3.4 ± 0.1 |
| MR RNA (mutant reverse ssRNA) | NA | C:G (dsRNA) | NA |
| ds C RNA (consensus dsRNA) | NA | A:U (dsRNA) | 73.4 ± 1.5 |
| ds M RNA (mutant dsRNA) | NA | (CU) (GU) | NA |
| A20 (poly(A) ssRNA) | 24.8 ± 1.3 | (AC) (GU) | NA |
| U20 (poly(U) ssRNA) | 1.2 ± 0.2 | (CU) (AG) | NA |
| G20 (poly(G) ssRNA) | 114 ± 4 | (AU):(AU) | NA |
| ds C RNA (consensus dsRNA) | NA | (AU):(AU) | NA |
| ds M RNA (mutant dsRNA) | NA | (GC):(GC) | NA |
| A20 (poly(A) ssRNA) | 24.8 ± 1.3 | (AC):(GU) | NA |
| U20 (poly(U) ssRNA) | 1.2 ± 0.2 | (CU):(AG) | NA |
| G20 (poly(G) ssRNA) | 114 ± 4 | (AU):(AU) | NA |

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**FIGURE 2. Effect of sequence and secondary structure on Yy1 RNA binding.** Binding reactions containing a gradient of Yy1 concentrations (indicated above panel) and 10 nM RNA probe (probes indicated at left) were subjected to EMSA. Supplemental sequences RNA probe sequences are provided in the Supplemental Material. CF, consensus forward; CR, consensus reverse; MF, mutant forward; MR, mutant reverse. All probes were single-stranded unless designated as double-stranded (ds).
note that this was the only case in any of the RNA-binding experiments in which apparent complex formation was not concentration-dependent.

We next analyzed YY1 affinity for the single-stranded dinucleotide combinations (AC)10, (AG)10, (CU)10, and (GU)10. The dinucleotides (AU)10 and (GO)10 form self-complementary duplexes and are analyzed below. YY1 bound to (GU)10 with moderate affinity (K_d = 38.0 ± 1.5 mM) and (CU)10 and (AG)10 with low affinity, and no binding to (AC)10 was detected (Fig. 2 and Table 1). These data reveal a preference for U-rich single-stranded probes.

The effect of RNA secondary structure on YY1 binding was examined in greater detail using duplex-forming RNA oligonucleotides. Six possible duplexes can be made from the 10 consecutive nucleotides and larger (Fig. 3). YY1 bound to (GC):(GC), (AG):(CU), or (AC):(GU) RNA duplexes with some degree of sequence specificity.

To determine unequivocally the identity of the complexes described above, YY1 was combined with radiolabeled RNA in the presence or absence of zinc and magnesium as well as with specific and nonspecific competitors (Fig. 3A). Complex formation depended on the presence of both magnesium and zinc ions. Furthermore, addition of cold specific DNA or RNA competitor, or anti YY1 antibody, reduced or abolished complex formation. Nonspecific competitors and antibodies had no effect.

To determine the minimal binding site of YY1, single-stranded U_20 RNA was partially digested with RNase A, generating a population of probe fragments ranging in size from 1 to 20 nucleotides. Binding reactions were assembled with digested probe mixture and recombinant YY1 in the presence of nickel-charged NTA-agarose beads. YY1-RNA complexes formed in solution were recovered by binding of YY1 to the affinity matrix via the N-terminal His tag. The smallest fragment retained corresponds to the minimal binding site of YY1. Sequencing gel analysis of bound probe revealed YY1 retained fragments of 11 nucleotides and larger (Fig. 3B). Probe was not retained in control reactions without YY1 (data not shown). A minimum of 11 consecutive nucleotides are therefore required for binding of YY1 to RNA, and the YY1-RNA complexes observed with uncapped probes (data not shown), suggesting YY1 does not bind to the 5'-terminal region of RNA.

Because numerous cellular factors are known to associate with the 5'-cap structure of both polysomal and quiescent mRNAs, we also tested the potential effect of the 7-methylguanosine cap structure on YY1 RNA binding by determining the affinity of YY1 for capped versus uncapped synthetic RNA probes. No significant difference in affinity was detected between capped and uncapped probes (data not shown), suggesting YY1 does not bind to the 5'-terminal region of RNA.

**RNA Binding of Endogenous YY1 from Native mRNPs**—The results obtained with recombinant protein were confirmed using native preparations of the endogenous protein isolated from oocyte mRNPs. Complexes isolated by oligo(dT)-cellulose chromatography were eluted and renatured, yielding an active preparation of isolated mRNP proteins, including YY1. The 10 nM consensus YY1 DNA probe was added to binding reactions with YY1. The position of the YY1-DNA complex is indicated at left. RNA-EMSA (lower panel) was performed with renatured mRNP samples and 10 nM A:U duplex RNA probe. The position of YY1-RNA complexes is indicated at left. The RNA (1 pmol), DNA (1 pmol), and antibody (200 ng) competitors are indicated at the top of the panel. The results obtained with recombinant YY1 were confirmed using native preparations of the endogenous protein isolated from oocyte mRNPs. Complexes isolated by oligo(dT)-cellulose chromatography were eluted and renatured, yielding an active preparation of isolated mRNP proteins, including YY1. Complex formation depended on the presence of both magnesium and zinc ions. Furthermore, addition of cold specific DNA or RNA competitor, or anti YY1 antibody, reduced or abolished complex formation. Nonspecific competitors and antibodies had no effect.
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**FIGURE 5. Binding of YY1 to mRNA in vitro and in vivo.** A, binding reactions containing 10 pmol of YY1 and 0.5 μg of poly(A⁺) mRNA isolated from oocytes were subjected to oligo(dT)-cellulose chromatography. Bound (B) and unbound (U) fractions were analyzed by Western blotting for YY1. Some reactions were treated with 1 μg of RNase A or 20 pmol of rU20 or rC20 competitors as indicated. Con, control. B, Western blot of size exclusion chromatography analysis of lysates from HA-YY1 expressing oocytes (upper panel) and control, un.injected oocytes (lower panel). Positions of fractions and molecular mass standard (MMS) are indicated above the panel. HA-YY1 expressing oocytes were sham-injected (Con) or microinjected with 5 pmol of U20 (+rU) or C20 of (+rC) 4 h prior to preparation of extracts. C, competition reactions with RNase abolished recovery of YY1 in bound fractions. Some reactions were able to inhibit YY1-mRNP assembly in vitro but not by C20, results that are consistent with the direct RNA-binding experiments shown in Fig. 2 and Table 1. Addition of U20 to reactions at the concentrations utilized did not significantly reduce total recovery of mRNA/mRNPs in bound fractions (data not shown). These in vitro data demonstrate that YY1 is able to bind directly to native maternal mRNA in the absence of other mRNP constituents.

The process of YY1-mRNP assembly was investigated in vivo by expression of HA-tagged YY1 from a plasmid construct (HA-YY1) microinjected into oocytes, and subsequent examination of cytoplasmic extracts by size exclusion and oligo(dT) chromatography (Fig. 5, B and C). The results show that nascent HA-YY1 assembled into poly(A⁺) mRNPs with a mean molecular mass of 480 kDa, identical in size to native YY1-containing mRNPs (Fig. 5B). Additionally, HA-YY1 was retained on oligo(dT)-cellulose via interaction with poly(A⁺) mRNAs. RNase treatment of samples abolished retention on oligo(dT). Time course experiments revealed HA-YY1 could be detected in mRNPs as early as 2 h after microinjection with peak levels of mRNP-associated HA-YY1 appearing after 12 h (data not shown). This indicates that the molecular machinery responsible for the assembly of mRNPs is completely functional in the mature oocyte and that epitope-tagged YY1 is competent to assemble into mRNPs. In some experiments, oocytes were nucleated after 8 h of expression, and nuclear and cytoplasmic extracts were analyzed by Western blot. HA-YY1 was found to be completely localized to the cytoplasm (data not shown), identical to what has been observed for native YY1 (19, 23). The assembly of HA-YY1 into mRNPs was significantly and specifically reduced by cytoplasmic microinjection of competitor RNA (U20) (Fig. 5C). Microinjection of RNAs for which YY1 displayed low affinity in vitro did not affect assembly of HA-YY1-mRNPs. The observation that high affinity RNA substrates are able to inhibit YY1-mRNP assembly in vivo clearly demonstrates that the RNA binding activity of YY1 is not only biologically relevant but is an absolute requirement for its assembly into mRNP complexes. These observations also show that protein-protein interactions between YY1 and other mRNP proteins are not sufficient for association of YY1 with mRNPs.

**DISCUSSION**

Here we report that a novel activity for YY1, the capacity to bind RNA directly with high affinity, is required for assembly of YY1-containing mRNPs. The results of in vitro analysis of RNA binding activity (Fig. 2) were confirmed using natural YY1 isolated from oocyte mRNPs (Fig. 4). YY1 bound to poly(A⁺) mRNA in vitro, and assembly of YY1 into mRNPs was specifically blocked in vivo by high affinity RNA substrates (Figs. 3 and 5). The major conclusions arising from these experiments are that YY1 has sequence-specific RNA binding activity for U-rich single-stranded and A-U duplex RNA and associates with maternal mRNA in vivo via direct RNA binding activity. The minimal length of single-stranded RNA substrate was found to be 11 nucleotides. Our findings reveal that the mechanism by which cytoplasmic YY1 associates with maternally derived mRNPs is through direct RNA binding activity.
YY1 displayed highest affinity for single-stranded poly(U) RNA (Table 1), on the same order as affinity for its DNA consensus site (46, 50, 51). YY1 displayed sequence specificity in its RNA-binding characteristics, with high affinity for A:U duplexes and single-stranded U-rich regions. We hypothesize that these sequences are likely targets of YY1 in mRNPs in vivo. Binding to several single-stranded substrates other than U20 was also observed; however, intramolecular A, G, or C residues significantly reduced or abolished binding for U-rich regions in both single- and double-stranded substrates (Fig. 2 and Table 1). Low affinity binding was observed for several substrates, with affinities substantially lower than for the DNA consensus site (46, 50, 51). YY1 displayed sequence specificity in its RNA binding. The general nonspecific RNA-binding characteristics of mRNP proteins Xp54 (33, 39), FRGY2a/b (32), and Rap55 (37) contrast with the apparent sequence specificity of YY1. The functional consequences of these differences remain to be determined.

We conclude that YY1 recognizes internal regions of mRNAs, perhaps at U-rich or A:U-rich duplex regions within mRNAs (Fig. 2). This is supported by data showing YY1 did not bind appreciably to the 7-mGpppG 5'-cap structure (not shown) or single-stranded poly(A) probes (Table 1), which would mimic the poly(A) tail of maternal mRNAs. In any event, it is clear that binding to mRNA by YY1 is the primary mechanism by which YY1 is assembled into mRNPs.

Of all known zinc finger RNA-binding proteins, the precise mode of RNA-interaction has only been elucidated for three, including the Xenopus C2H2 zinc finger proteins TFIIIA and ZFa and the human CCCH zinc finger protein TS11d (52–54). Whereas the mode of DNA recognition by zinc finger proteins invariably involves recognition helix-major groove interactions, the same general principles do not apply to RNA recognition. The RNA-binding zinc fingers of TFIIIA make specific contacts with RNA bases in highly structured helical and non-helical regions, and it binds a double-stranded region of the 5 S RNA with high sequence specificity (54). ZFa, in contrast, binds double-stranded RNA with little apparent sequence specificity, primarily through interaction with the RNA backbone (52). YY1 displays less apparent RNA sequence specificity than TFIIIA but more than ZFa (Fig. 2 and Table 1). YY1 displays high affinity for a single-stranded substrate, implying divergence in the mode of RNA recognition between C2H2 proteins. YY1 behavior more closely resembles the CCCH zinc finger protein TS11d. TS11d has high affinity for AU-rich duplex RNA, and the solution structure of this protein in complex with RNA has been determined (54). Sequence specificity of TS11d is apparently mediated entirely by peptide backbone contacts with Watson-Crick base pairs in helical RNA. Given the capacity of YY1 to bind single-stranded substrates (poly(U)), it would appear there is great variability in the mechanisms by which zinc finger proteins recognize RNA.

The present study constitutes the most comprehensive analysis of the RNA binding activity of any of the known Xenopus mRNP proteins and the first demonstration that RNA binding is an absolute requirement for assembly of mRNPs. Biochemical analysis of mRNPs has been difficult because they constitute a highly heterogeneous population of proteins and mRNAs. Moreover, they include a preformed, static population in the oocyte, and so genetic analyses and knockdown type experiments are not possible. Thus the major finding that YY1 assembles into mRNPs via direct RNA binding is an important first step in understanding the biochemical mechanisms of mRNP assembly and metabolism.

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