mRNP4, a Major mRNA-binding Protein from Xenopus Oocytes Is Identical to Transcription Factor FRG Y2*

(Received for publication, March 26, 1992)

Stéphane Deschamps, Alain Vié, Manuel Garrigó, Herman Denis, and Marc le Maire

From the Centre de Genétique moléculaire, Laboratoire propre du Centre National de la RechercheScientifique associé à l'Université P. et M. Curie (Paris VI), F-91198 Gif-sur-Yvette Cedex, France, and the Section de Biophysique des Protéines et des Membranes, Département de Biologie Cellulaire et Moléculaire, Centre d'Etudes Nucléaires de Saclay, F-91191, Gif-sur-Yvette Cedex, France

Previous work has revealed a striking similarity between the sequence of mRNP4, a major mRNA-binding protein of Xenopus laevis oocytes and FRG Y2, a transcription factor specific for Y-box-containing promoters. However, the apparent molecular mass of mRNP4 exceeds by 50–60% the cDNA-deduced molecular mass of FRG Y2. To resolve this discrepancy we have measured the real molecular mass of mRNP4 by sedimentation equilibrium. The molecular mass of mRNP4 closely agrees with that of FRG Y2. We conclude that mRNP4 is identical to FRG Y2 and has a dual function in oocytes. As a DNA-binding protein, mRNP4 stimulates transcription from a specific set of promoters. As a mRNA-binding protein, mRNP4 favors accumulation of the transcripts it contributes to produce.

The oocytes of Xenopus laevis accumulate large amounts of ribosomes, tRNA, and mRNA (1). Most of mRNA occurs in oocytes as complexes with several proteins (2,3). The mRNA-containing particles, called mRNPs, are heterogeneous in size (2). They apparently serve two functions. First, they protect mRNA against degradation (2). Second, they prevent premature translation of mRNA (3). The mRNPs presumably dissociate in early embryos, thereby making mRNA available to the translation machinery. Much has still to be learned concerning the molecular mechanism by which mRNA is masked during oogenesis and progressively unmasked during embryonic development.

Four major proteins (mRNP 1–4)2 associate with mRNA in immature oocytes (2). These proteins share several properties, such as highly basic character and coordinate accumulation (2, 3). Much information has been obtained concerning mRNP3 (also called p54 or p56; Refs. 4–6) and mRNP4 (also called p56 or p60; Refs. 4–6). These proteins accumulate to a high concentration in early oocytes (Dumont stages I and II) and decline in later oocytes (7, 8). mRNP3 and mRNP4 differ from other mRNP proteins by two remarkable properties. First, they do not precipitate when the cell extracts are heated at 80 °C (9). Second, they can be phosphorylated by kinases associated with the mRNPs (4–6, 9). Purification and partial sequencing of mRNP3 and mRNP4 have shown that these proteins are homologous (9, 10). Recently, two cDNAs encoding mRNP3 and the C-terminal part of mRNP4 have been isolated and sequenced (10).

The amino acid sequence of mRNP4 is very similar to the sequence encoded by FRG Y2 cDNA (11). FRG Y2 is a germ cell-specific factor stimulating transcription from Y-box promoter (12, 13). However, the identity between mRNP4 and FRG Y2 has not been formally proved, because the apparent molecular mass of mRNP4 (56–59 kDa; Refs. 4–6 and 9) exceeds by 50–60% the deduced molecular mass of FRG Y2 (37 kDa; Ref. 11), as does that of bacterially expressed FRG Y2 (14). Since the 5′-terminal part of mRNP4 cDNA is not yet sequenced, we do not know if mRNP4 and FRG Y2 are really identical or differ in their N-terminal portion. In this report we demonstrate that mRNP4 has a molecular mass of 38 kDa and an extended conformation in SDS which can explain its unusual size deduced from SDS-gel electrophoresis. We conclude that FRGY2 is identical to mRNP4. Like TFI11A (15, 16), FRG Y2/mRNP4 has a dual function. As a transcription factor, it stimulates mRNA synthesis in oocytes (11). As a mRNP component, it binds and protects mRNA against degradation (2).

EXPERIMENTAL PROCEDURES

Purification of mRNP4—Postmitochondrial extracts from immature ovaries were heated at 80 °C for 10 min (9). mRNP4 was purified from the extracts using preparative electrophoresis (17). The gels were stained with 0.5% Coomassie blue for 5–10 min (18). The protein band containing mRNP4 was cut off from the gels and washed first with 250 mM Tris-HCl, pH 9, 250 mM EDTA (3 × 10 min) and second with 20 mM Tris-HCl, pH 6.8, 150 mM glycine, 0.01% SDS (10 min). mRNP4 was electroeluted from the gel as described (17) and concentrated in Centricron 10 (Amicon).

Determination of Molecular Mass—The apparent molecular mass of mRNP4 was measured by SDS-gel electrophoresis under three different conditions (19–21). The pH 7 gel system (21) could be expected to give more correct estimates of the true molecular mass than the other systems (19, 20), as proved to be the case of calsequestrin (22).

The true molecular mass of mRNP4 was determined by sedimentation equilibrium using a Beckman TL-100 ultracentrifuge and a TLA-100 rotor (23). Purified mRNP4 (final concentration, 0.1 mg/ml) was dissolved in 50 mM Tris-HCl, pH 8.3, 100 mM NaCl, 0.1% SDS, containing 0.5% Dextran T40 in order to stabilize the protein

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed.

‡ The abbreviations used are: mRNP, messenger ribonucleoprotein particle; SDS, sodium dodecyl sulfate; HPLC, high pressure liquid chromatography.

1 Present address: Dept. of Cellular and Molecular Biology, The Biological Laboratories, Harvard University, 16 Divinity Ave., Cambridge, MA 02138.

2 In this report we adopt the nomenclature originally proposed by Darnbrough and Ford (2) to designate the mRNP proteins. A nomenclature based on molecular masses (p54, p56, p60 etc.) is confusing because a given protein is often given different names (4, 5). Furthermore, the apparent molecular mass of the mRNP proteins (4, 5) is very different from their real molecular mass (this paper).
concentration gradient formed at completion of the sedimentation run. Each tube was loaded with 120 μl of protein solution and centrifuged for 48–60 h at 20 °C at different velocities ranging from 23,000 to 35,000 rpm. At the end of the centrifugation run, the content of the tubes was fractionated from the meniscus (10–15 fractions), and its volume was calculated from the solution density. The protein concentration was determined by analyzing fraction samples by electrophoresis followed by microdensitometry with a Vernon Phi 5 spectrophotometer and integrator (24). The linearity of the response was checked by using known amounts of mRNP4. The radial distance of the midvolume of each fraction was determined as described (23). The slopes of the equilibrium plots yield a quantity that is formally equal to \( M_p(1 - \phi \rho) \), where \( M_p \) is the molecular mass of the protein component of the sedimenting particle (excluding bound water and detergent), \( \phi \) is the effective partial specific volume per g of protein, and \( \rho \) is the solvent density. As previously described (25), the contribution of bound detergent (\( \delta_0 \) in g/g of protein) can be allowed for by the relation,

\[
M_p(1 - \phi \rho) = M_p[(1 - \delta_0 \rho) + \delta_0(1 - \delta_0 \rho)]
\]

where \( \delta_0 \) is the partial specific volume of the protein and \( \delta_0 \) is the partial specific volume of the bound detergent. A value of \( \delta_0 = 0.707 \) cm3/g was calculated on the basis of the amino acid composition of FRG Y2 (11) by the method of Cohn and Edsall (26). A value of \( \delta_0 \) for SDS was taken as 0.864 cm3/g (27). Equation 1 can be solved if \( \delta_0 \) is measured independently (see below) or if one can adjust the solvent density to the point where \( 1 - \delta_0 \rho = 0 \), i.e. for \( \rho = 1/\delta_0 \) (28, 32). In that case, the contribution of the detergent to Equation 1 vanishes regardless of the amount of detergent bound to the protein. In order to alter the solvent density, we used various concentrations of water isotopes (H2O, D216O, and D218O). Appropriate corrections were made for the small increase in protein molecular mass due to the replacement of exchangeable H atoms in the protein by D atoms (present in buffers containing D416O and D418O) and the corresponding decrease in protein partial specific volume (32). Buffer densities were determined by weighing measured volumes relative to the same volume of distilled water.

**Determination of Bound Detergent**—The amount of [35S]SDS bound to protein was measured on a size exclusion HPLC column (29). The column (TSK 3000 SW; 0.75 × 30 cm, Toyopearl Soda Japan) was equilibrated in 50 mM Tris-HCl, pH 8, 100 mM NaCl, 0.1% [35S] SDS (80,000 cpn/mg; Amersham Corp.). The protein samples (mRNP4 or bovine serum albumin and carbonic anhydrase used as control proteins) were dissolved in the SDS buffer, loaded as 0.2–1-mg aliquots, and eluted at a flow rate of 1 ml/min. Protein was detected in the eluate by its absorbance at 280 nm and determined by microdensitometry (24) or by colorimetry, using bovine serum albumin as a standard (30). The amount of [35S]SDS present in the protein solutions was measured by scintillation counting.

**Determination of the Stokes Radius**—The Stokes radius was measured by HPLC size exclusion chromatography. The HPLC column was calibrated as described (31). The void volume (\( V_d \)), the total volume (\( V_t \)), and the elution volume of a given protein (\( V_e \)) allow the partition coefficient (\( K_v \)) of this protein to be calculated as follows.

\[
K_v = (V_t - V_d)/(V_e - V_d)
\]

The viscosity Stokes radius (\( R_v \)) was calculated from the experimental calibration curve relating the \( K_v \) to the \( R_v \) of several proteins with known \( R_v \) in SDS (31).

**RESULTS**

All the sequence data concerning mRNP4 and FRG Y2 are summarized in Fig. 1. There is an almost perfect agreement between both sequences. A single point has still to be elucidated. The apparent molecular mass of mRNP4 determined by three different gel electrophoresis systems (54–60 kDa; Fig. 2) considerably exceeds the deduced molecular mass of FRG Y2 (37 kDa; Ref. 11). To definitively resolve this discrepancy, we measured the true molecular mass of mRNP4 by analytical ultracentrifugation. We performed a number of sedimentation equilibrium experiments, and the plots lnC versus \( r^2 \) (C is the protein concentration and \( r \) the radial distance) were linear through-out the centrifugation cell (data not shown). This indicated that the protein solutions were homogenous and that there was no change in weight-average molecular mass with concentration and therefore no protein self-aggregation.

Since mRNP4 used for the \( M_0 \) determination was purified in the presence of SDS, the amount of detergent bound to the protein had to be taken into account. The problem was solved in two different ways. In the first method the sedimentation equilibrium data were combined with independent measurement of the amount of bound SDS, obtained by HPLC chromatography of mRNP4. A value of 1.36 g of SDS/g of mRNP4 was obtained, and a corresponding molecular mass (\( M_p \)) of 39.5 kDa was calculated from Equation 1. In the second method the amount of bound detergent was not directly measured, but the sedimentation experiments were done in...
aqueous buffers of various densities, obtained by addition of water isotopes (Fig. 3). At the density corresponding to the inverse partial specific volume of SDS (1/\(\phi_c\); arrow in Fig. 3), the contribution of the detergent to the molecular mass of the complex is zero. The molecular mass of mRNP4 calculated by this method is 38 kDa, which is in excellent agreement with the deduced molecular mass of FRG Y2 (11).

The slow migration of mRNP4 in SDS gels (Fig. 2) cannot be ascribed to an anomalously low SDS content of the protein-detergent complex, since mRNP4 binds no less SDS than standard proteins (1.4 g/g; Ref. 33). It is rather due to a highly extended conformation of the complex. We confirmed this deduction by measuring the Stokes radius of the mRNP4-SDS complex. A value of 7.3 nm was obtained (Fig. 4A), which is significantly greater than the value expected for a standard 37-kDa protein in SDS (5.5 nm). A viscosity Stokes radius (Rs) of 7.3 nm would normally correspond to a 33-kDa protein (Fig. 4B).

**DISCUSSION**

mRNP4 has an \(M_r\) of approximately 38,000, which is significantly lower than the measurements obtained by SDS-gel electrophoresis. Those measurements are in error because mRNP4 has an extended conformation in SDS and hence an anomalously low electrophoretic mobility. A similar observation has been reported for calasequin (22). Our sedimentation equilibrium, detergent binding, and chromatographic size exclusion results are also likely to be relevant for mRNP3 which is very similar to mRNP4 (9, 19); bacterially expressed mRNP3 has an apparent \(M_r\) that exceeds by far the cDNA-deduced \(M_r\) (10). The extended conformation of mRNP4 (and probably mRNP3) in SDS is probably due to its high content in proline and charged amino acids (Fig. 1). The explanation found here may apply to a whole group of RNA- or DNA-binding proteins with unusual electrophoretic behavior (see for example Refs. 35–37). Interestingly, this group includes several transcription factors (c-myc, c-fos, and GCN4; Refs. 38–40).

Our molecular mass determination of mRNP4 leaves little doubt concerning the identity of this protein with FRG Y2. The mRNP4 sequence does not extend beyond the limit set by the first ATG codon found in frame within the FRG Y2 cDNA sequence (11). Furthermore, mRNP4 does not appear to undergo extensive posttranslation modifications that would significantly increase its molecular mass. Note, however, that the N-terminal residue of mRNP4 is blocked by the first ATG codon found in frame within the FRG Y2 cDNA sequence (11).

The identity between mRNP4 and FRG Y2 implies that mRNP4 has two functions in oocytes. As a DNA binding factor mRNP4 promotes transcription of a specific set of genes (11). As a mRNA-binding protein, mRNP4 prevents degradation of the transcripts it contributes to produce. mRNP4 is not the first bifunctional protein to be discovered in oocytes. TFIH is another DNA- and RNA-binding pro-

---

\(M_r\) is the molecular mass, \(\phi_c\) is the partial specific volume of the complex, and \(\mu\) is the frictional coefficient.

**Fig. 3.** Sedimentation equilibrium of the mRNP4-SDS complex at various solvent densities obtained by using water isotopes. The arrow indicates the value of \(p = 1/\phi_c\) for SDS. At this density the contribution of bound SDS to the molecular mass of the complex is zero. The molecular mass of mRNP4 can thus be calculated to be 38 kDa (Equation 1). The appropriate corrections due to H/D exchange were made by plotting \(M_r(1 - \phi'/\mu)\) from the \(D_2^{18}\)O and \(D_2^{18}\)O data.

**Fig. 4.** A, calibration curve for a HPLC TSK 3000 SW column equilibrated in SDS. The viscosity-based Stokes radius of various protein-SDS complexes used as standards is plotted as a function of the partition coefficient \(K_d\). Composition of effluent medium: 0, 250 mM sodium phosphate, pH 7.2, 3.4 mM SDS; \(\Phi\), 50 mM Tris HCl, pH 7.8, 100 mM NaCl, 3.47 mM SDS. Standards: \(\beta\) Gal, \(\beta\)-galactosidase; Phl, phosphorylase; Trf, transferrin; BSA, bovine serum albumin; Ova, ovalbumin; CA, carbonic anhydrase; STI, soybean trypsin inhibitor; \(\alpha\)-Lac, \(\alpha\)-lactalbumin. The \(K_d\) of the mRNP4-SDS complex (indicated by an arrow) corresponds to a Stokes radius of 7.3 nm. B, relation between the Stokes radius and the molecular mass of various proteins. The data are calculated from Table 1 of Ref. 34. \(\Delta\), viscosity-based Stokes radius; \(\Phi\), frictional coefficient-based Stokes radius. The apparent \(M_r\) of mRNP4 deduced from the calibration curve shown in A is 53,000.

---

2 S. Deschamps, A. Viel, M. Garrigos, H. Denis, and M. le Maire, unpublished observations.
tein, known to be involved in 5 S RNA transcription and storage (15, 16). mRNP4 and TFIIIA are highly concentrated in growing oocytes. In the case of TFIIIA, this may be a crucial factor for maintaining a high rate of transcription of the oocyte-type 5 S RNA genes (41). It is possible that a high concentration of mRNP4 is also required for selective expression of protein-encoding genes in oocytes.

Homologues of several housekeeping proteins are being used by amphibian oocytes as components of their storage particles. This is the case for both 42 S particle proteins (thesaurin a and thersain b; Refs. 42 and 43). Thesaurin a is a divergent form of eukaryotic elongation factor 1α (EF-1α; Refs. 44–47). Thesaurin b is a divergent form of TFIIIA (48). It is possible that mRNP4 is also a modified form of the ubiquitous Y-box transcription factors with a specialized mRNA storage role in oocytes.

Acknowledgments—We thank Dr. A. Wolfe for helpful discussions about identities between FRGY2 and mRNP4. We are also very grateful to Dr. M. T. Murray for sending us a preprint of her paper. We thank A. Gomez de Garcia for expert technical assistance.

REFERENCES