Regulated Unmasking of in Vivo Synthesized Maternal mRNA at Oocyte Maturation

A ROLE FOR THE CHAPERONE NUCLEOPLASMIN*

Funda Meric, Ken Matsumoto, and Alan P. Wolfe‡

From the Laboratory of Molecular Embryology, NICHD, National Institutes of Health, Bethesda, Maryland 20892-5421

We examine the translational regulation of histone H4 mRNA when Xenopus laevis oocytes are induced to mature with progesterone. Histone H4 mRNA synthesized from plasmid templates microinjected into oocyte nuclei is translationally silenced (masked). This masked mRNA becomes translationally active only after oocyte maturation. In contrast, histone H4 mRNA injected into the oocyte cytoplasm is translationally active both before and after oocyte maturation. Thus, transcription in vivo is required to mask histone H4 mRNA and to allow subsequent translational regulation. Protein association with histone H4 mRNA synthesized in vivo was determined before and after oocyte maturation. UV cross-linking of radiolabeled RNA to protein and immunoprecipitation of cross-linked proteins reveals an increased association of the chaperone nucleoplasmin with ribonucleoprotein particles dependent on the oocyte maturation process. The Y-box protein FRGY2 inhibits translation of histone H4 mRNA in vitro. Nucleoplasmin is able to partially relieve this repression. We discuss the potential role of nucleoplasmin in the remodeling of repressive ribonucleoprotein particles containing maternal mRNA to facilitate translational activation.

The translational control of maternal mRNA is a major regulatory mechanism for gene expression during early Xenopus development (1–3). Maternal mRNA is packaged into ribonucleoprotein particles (mRNPs, informosomes) (4, 5). RNA-binding proteins, including those of the Y-box family of proteins, maintain mRNA in a translationally repressed or masked form (6–9). Removal of the Y-box proteins from mRNA facilitates translational activation (10, 11). Thus one potential translational regulatory mechanism might involve the removal of repressive Y-box proteins from maternal mRNA. In addition, true activation mechanisms exist that further facilitate translation of maternal mRNA. Most masked mRNAs that are in the oocyte have short poly(A) tails that are lengthened during oocyte maturation concomitant with translational activation (unmasking) (12–14). The poly(A) tail directly stimulates the initiation of translation (15). The exact molecular events that might regulate the translational activation of any masked maternal mRNA are not yet known. Multiple molecular mechanisms are likely to contribute to the translational regulation of any specific mRNA.

In this work we investigate the translational regulation of histone H4 mRNA because the endogenous message is abundant in Xenopus oocytes and the vast majority of H4 mRNA is masked (16). The rate of histone H4 synthesis increases 50-fold on oocyte maturation without an increase in histone H4 mRNA polyadenylation (17, 18). In fact histone H4 mRNA is polyadenylated in oocytes (16, 18), and the poly(A) tail is removed during the maturation process (18). Thus the unmasking of histone H4 mRNA most probably involves the mobilization of mRNA from stores present as masked ribonucleoprotein particles by removal of a repressor protein rather than activation via polyadenylation dependent mechanisms (18, 19).

We first reconstitute the regulated unmasking of histone H4 during oocyte maturation using mRNA synthesized from plasmid DNA templates microinjected into the oocyte nucleus. We next examine the proteins associated with H4 mRNA before and after oocyte maturation by UV cross-linking to radiolabeled mRNA. We purify the major protein whose association with H4 mRNA increases during oocyte maturation. This protein is nucleoplasmin, a well known chaperone stored in the Xenopus oocyte nucleus in large amounts (290 ng/oocyte) until oocytes mature and the nuclear envelope breaks down (20). Nucleoplasmin has the role of removing specialized arginine-rich protamines from Xenopus sperm chromatin (21). We propose that it has a comparable role in facilitating the removal of the arginine-rich Y-box proteins from maternal mRNA, thereby potentiating translation.

MATERIALS AND METHODS

Plasmid Construction—The SP6H4F construct has been described elsewhere (22). The NH2-terminal FLAGged (IBI) H4 sequence was amplified from SP6H4F using the primers 5’-ACCTTCCATGGCTTTA-ACCTCCGGATCCTGACAGTGGCG-3’ and 5’-ATTGAATTCT-3’ to give the H4 fragment. This fragment was subcloned into the SP6 vector (Promega). The cytomegalovirus promoter (23) was subcloned into the HindIII/XbaI sites of the pCAT-Basic vector (Promega), and the cytomegalovirus promoter (23) was subcloned into the HindIII/XbaI sites to give CMV.H4F. All constructions were confirmed by sequencing.

In Vitro Transcription—In vitro transcription reactions were performed using SP6 RNA polymerase as described (24). Templates were linearized with EcoRI downstream from the poly(A) tract to give polyadenylated RNA. RNA synthesis was carried out in the presence of a 10:1 cap analog to rGTP ratio to obtain 5’ capped transcripts (8). Transcribed RNA was phenol/chloroform extracted and ethanol-precipitated.

Xenopus Oocyte Microinjection—Xenopus oocytes were prepared as described previously (25). Stage VI oocytes were sorted and used within 24 h of oocyte preparation. Oocytes were maintained at 18 °C in modified Barth’s saline (26). To ensure accuracy in the nuclear versus cytoplasmic injection experiments, the injection volume was kept to 9.2 nl and cytoplasmic injections were performed into the vegetal pole. After injection, only oocytes with homogenous pigmentation and normal turgor were collected. Collected oocytes were homogenized in 0.25 M Tris-HCl (pH 7.5) (10 µl/oocyte) and aliquoted for protein and RNA
Unmasking Maternal mRNA at Oocyte Maturation

In vitro oocyte maturation was carried out in oocyte culture medium (26) using 1 μg/ml progesterone (Sigma). Oocyte maturation was determined by the appearance of a white spot at the animal pole indicative of germinal vesicle breakdown.

**UV Cross-linking**—Labeled in vitro transcribed RNA was obtained by introducing 50 μCi of [3H]UTP or [32P]UTP into the reaction. In vitro labeling was obtained by injecting [3H]UTP or [32P]UTP (1 μCi/oocyte) in the oocyte nucleus. After the indicated time, oocytes (whole, nucleus, or cytoplasm) were homogenized in 10 mM Tris-HCl (pH 7.7), 100 mM KC1, 1 mM MgCl2, 0.1 mM CaCl2, 50 mM NaCl, 5 mM sucrose (3 μl/oocyte). After centrifugation for 10 min at 4 °C, protein extracts were exposed to UV light for 10 min on ice; then RNase A was added (0.5 μg/ml) and digestion was allowed for 1 h at 37 °C. Immunoprecipitation of the cross-linked proteins used antibodies against nucleoplasmin purified from serum using protein-A agarose (27). Using the fact that nucleoplasmin is soluble at 80 °C (28), proteins extracted from oocytes were heated for 10 min at 80 °C, then placed on ice for 10 min. The soluble proteins cross-linked to radiolabeled RNA were then incubated with 15 μg of purified IgG containing nucleoplasmin antibody and 50 μl of IgG Sorb (The Enzyme Center, Malden, Massachusetts) in RIPA buffer (50 mM HEPES [pH 7.5], 2 mM NaCl, 0.1% SDS, 1% Triton, 5 mM EDTA, 0.1% bovine serum albumin) for 2 h at room temperature. Complexes were washed three times with RIPA buffer, then three times with 50 mM HEPES (pH 7.5), 0.15 M NaCl, 5 mM EDTA, and then treated with RNase A as above. Proteins were released by adding 25 μl of a solution of 1% SDS, 0.2 M β-mercaptoethanol and heating 10 min at 100 °C.

**RNA Analysis**—RNA was extracted from the homogenate by the RNazol method (Cinna Scientific). RNA was analyzed by primer extension as described (29). Primer extension was performed using the primer 5'-GGCTTGGTGATGCCCTGGATGTTATCC-3' (primer 5'-end labeled with [32P]dATP). Primer 5'-end labeled with [32P]dATP was heated for 10 min at 80 °C, then placed on ice for 10 min. The soluble protein was released by adding 25 μl of a solution of 1% SDS, 0.2 M β-mercaptoethanol and heating 10 min at 100 °C. The pellet was resuspended in 10% cyanogen bromide in 70% formic acid and the pellet washed with acetone, 0.2% HCl, then acetone alone.

**Protein Sequence Determination**—The protein was sequenced using a protein sequencer (Applied Biosystems). The positional accuracy of the sequence was evaluated by comparison with the published sequence of the corresponding protein.

**RESULTS**

**Translational Masking of Histone H4 mRNA Requires Transcription in Vivo**—In earlier work we have described the translational masking of histone H1 mRNA by quantitating protein synthesis per mass of mRNA (8). Histone H1 mRNA synthesized in vivo from a plasmid DNA molecule injected into the oocyte nucleus was translated 50–100-fold less efficiently than the H1 mRNA microinjected into the nucleus (8). Histone H1 mRNA is a bona fide translationally masked mRNA in the oocyte (30, 33). We have also examined whether the requirement for transcription in vivo to establish efficient masking of mRNA was a general phenomenon (14). Heterologous mRNAs such as those encoding chloramphenicol acetyltransferase or luciferase are translationally active if they are injected into the oocyte cytoplasm but are translationally masked if they are synthesized in vivo (14). Endogenous H4 mRNA (Fig. 1A, lanes 1–3) is not translationally active (Fig. 1A, lanes 1–3) as expected from earlier results (19). To discriminate between endogenous and exogenous experimental H4 mRNA we modified the coding sequence to contain a FLAG (IBI) epitope tag at the amino terminus (see “Materials and Methods”). This additional nucleotide sequence enabled distinction between endogenous and exogenous H4 mRNA using primer extension assays (Fig. 1A,

**Fig. 1.** In vivo transcribed histone H4 mRNA is masked. Stage VI oocytes were injected with either 3 ng of H4 mRNA (lane 2) or 3 ng of H4 DNA (lane 3), this mRNA and DNA encode an epitope-tagged histone H4 (see "Materials and Methods"). These oocytes and non-injected control oocytes (lane 1) were then injected with [3H]arginine and [3H]lysine and incubated another 6 h. A, primer extension analysis demonstrates levels of H4 mRNA. The primer extension products from the nuclear transcript synthesized from injected DNA (in vitro synthesized exogenous H4 mRNA), the injected transcript (in vitro synthesized exogenous H4 mRNA), and the endogenous message (endogenous H4 mRNA) are indicated by arrows. B, radiolabeled proteins synthesized in these oocytes. The arrow indicates the epitope-tagged H4 protein. The markers (M) include proteins of 98, 64, 46, and 36 kDa together with core histones purified from Xenopus erythrocytes. The position of wild type histone H4 is marked with an asterisk.

EcoRI-linearized pSP H4F (22). RNA was heated at 65 °C for 10 min and quickly chilled on ice before use. In vitro translation was performed with the nuclelease-treated rabbit reticulocyte lysate system (Promega). Histone H4 mRNA (0.13 μg) was incubated at 30 °C for 60 min in a 25-μl reaction mixture consisting of 15 μl of rabbit reticulocyte lysate, 25 μM each of amino acids including 87.5 μCi of [3H]lysine and 17.5 μCi of [3H]arginine, 20 units of RNasin (Promega), and Tail domain protein (FRG2D, see Ref. 31). The mixture was then digested with 5 μg of RNase A at 30 °C for 5 min. Acid-soluble protein was prepared as described (8). The aliquots were subjected to electrophoresis in a 15% polyacrylamide gel containing 7 M urea and 5% acetic acid (32). The gel was fixed, treated with Amplify (Amer sham Corp.), and dried. The translation products were detected by fluorography.

**RESULTS**

**Translational Masking of Histone H4 mRNA Requires Transcription in Vivo**—In earlier work we have described the translational masking of histone H1 mRNA by quantitating protein synthesis per mass of mRNA (8). Histone H1 mRNA synthesized in vivo from a plasmid DNA molecule injected into the oocyte nucleus was translated 50–100-fold less efficiently than the H1 mRNA microinjected into the nucleus (8). Histone H1 mRNA is a bona fide translationally masked mRNA in the oocyte (30, 33). We have also examined whether the requirement for transcription in vivo to establish efficient masking of mRNA was a general phenomenon (14). Heterologous mRNAs such as those encoding chloramphenicol acetyltransferase or luciferase are translationally active if they are injected into the oocyte cytoplasm but are translationally masked if they are synthesized in vivo (14). Endogenous H4 mRNA (Fig. 1A, lanes 1–3) is not translationally active (Fig. 1A, lanes 1–3) as expected from earlier results (19). To discriminate between endogenous and exogenous experimental H4 mRNA we modified the coding sequence to contain a FLAG (IBI) epitope tag at the amino terminus (see “Materials and Methods”). This additional nucleotide sequence enabled distinction between endogenous and exogenous H4 mRNA using primer extension assays (Fig. 1A,
“Materials and Methods”). Note that the histone H4 mRNA synthesized from the CMV.H4F expression vector following injection into the Xenopus oocyte nucleus (Fig. 1A, lane 3, in vivo synthesized exogenous H4 mRNA) is longer at the 5′ end than the histone H4 mRNA synthesized in vitro using SP6 RNA polymerase and the SP6 H4F expression vector (Fig. 1A, lane 2, in vitro synthesized exogenous H4 mRNA). This additional sequence in the 5′-untranslated region has no influence on the translational activity of the H4 mRNA, or the translational activity of the H4 mRNA, or mRNA stability (see Bouvet and Wolffe (8)).

Microinjection of exogenous histone H4 mRNA synthesized in vitro into the oocyte cytoplasm (Fig. 1A, lane 2), or microinjection of a plasmid DNA molecule (H4 DNA CMV.H4F) into the oocyte nucleus directing the synthesis of exogenous H4 mRNA (Fig. 1A, lane 3) was followed by radiolabeling of newly synthesized protein in a 6-h period (Fig. 1B). Comparable levels of in vitro or in vivo synthesized exogenous H4 mRNA (Fig. 1A, compare lanes 2 and 3, Table I) show very different efficiencies for synthesis of epitope-tagged histone H4 protein (Fig. 1B, compare lanes 2 and 3; Table I). The in vitro synthesized exogenous H4 mRNA is translated into epitope-tagged H4 protein (Fig. 1B, lane 2, arrowhead) much more efficiently than the in vivo synthesized mRNA (Fig. 1B, lane 3). Note that the epitope-tagged histone H4 (indicated as H4 epitope-tagged, arrowhead) migrates as a larger protein than endogenous histone H4 (indicated by the asterisk). Thus like histone H1, chloramphenicol acetyltransferase, and luciferase mRNAs, the exogenous histone H4 mRNA is translationally active if injected into the oocyte cytoplasm, but is translationally re-

**TABLE I**

Translational efficiency of in vitro and in vivo transcribed RNA after injection into oocytes

The experiment shown in Fig. 1 were quantified. H4 mRNA level was quantified by use of the PhosphorImager (Molecular Dynamics). Quantification of 3H-labeled H4 protein was performed by scanning the autoradiogram using a densitometer. Translational efficiency was standardized by the level of mRNA.

| mRNA (A.U.) | Protein (A.U.) | Ratio |
|-------------|---------------|-------|
| H4 RNA      | 652,754       | 250   | 100  |
| H4 DNA      | 1,706,080     | 25    | 3.8  |

**Fig. 2.** In vivo transcribed histone H4 mRNA is translationally activated after oocyte maturation. A, Scheme. Stage VI oocytes were injected with 3 ng of histone H4 mRNA or 3 ng of H4 DNA. After an 8-h incubation, the oocytes from both groups were placed into oocyte culture medium with or without progesterone. After an additional 16 h of incubation, matured oocytes and their not matured counterparts were collected and analyzed. B, the various forms of histone H4 mRNA as indicated (see Fig. 1A, legend) were assayed by primer extension (“Materials and Methods”) at the end of the experiment. Histone H4 mRNA abundance is compared when the oocytes are matured with progesterone (+) or incubated in control buffer (−). C, epitope-tagged exogenous H4 protein synthesized was detected through immunoblotting with anti-FLAG antibody (“Materials and Methods”). Lane 1 corresponds to non-injected control (c) oocytes. Lanes 2 and 3 shows oocytes injected with histone H4 mRNA into the cytoplasm, without or with oocyte maturation. Lanes 4 and 5 shows oocytes injected with histone H4 DNA into the nucleus, without or with oocyte maturation. The position of epitope-tagged histone H4 protein is indicated. D, distribution of H4 mRNA in RNPs pre- and post-oocyte maturation. RNPs from homogenates before and after oocyte maturation were fractionated on 20–60% nycodenz gradients. RNA from these fractions were deproteinized, and resolved on a denaturing agarose gel before Northern blotting and hybridization.
Unmasking Maternal mRNA at Oocyte Maturation

Unmasking of histone H4 mRNA synthesized in vivo from plasmid DNA templates comes under the appropriate developmental control at oocyte maturation, whereas histone H4 mRNA microinjected into oocyte cytoplasm does not. In earlier work (30), we made use of density gradient centrifugation to show that masked maternal mRNA is assembled exclusively into mRNPs, but that on release to the translational machinery mRNA is distributed between mRNPs and ribosomes. Histone H4 mRNA exhibits similar behavior. Histone H4 mRNA is exclusively in mRNPs prior to oocyte maturation (Fig. 2D, Pre) and redistributes between mRNPs and ribosomes post-maturation (Fig. 2D, Post). Note that these Northern blots of gradient fractions are only to compare the distribution of H4 mRNA between various RNP fractions. Since there is no transcription in Xenopus from oocyte maturation until the mid-blastula transition of Xenopus embryogenesis (34, 35) there is no increase in H4 mRNA abundance from levels in the oocyte until after that time. We next examined the molecular mechanisms responsible for the release of H4 mRNA from storage ribonucleoprotein particles to the ribosomes.

**Fig. 3.** Nucleoplasmin is UV cross-linked to histone H4 mRNA dependent on oocyte maturation. A, oocytes were injected with radiolabeled H4 mRNA either into the nucleus (N) or the cytoplasm (C) and incubated for 8 h before a further 12-h incubation with or without progesterone treatment as indicated. Oocyte homogenates were UV cross-linked, treated with RNase A, and resolved by 10% SDS-PAGE (“Materials and Methods”). The UV cross-linked proteins were visualized by autoradiography (lanes 1–4). The positions of FRGY2 (asterisk) and the 34-kDa protein (arrow) whose cross-linking increases on oocyte maturation are indicated. In lane 5 extracts of 100 injected oocytes treated as in lane 4 were heat-treated at 80°C for 10 min (“Materials and Methods”) before resolution of soluble proteins by 10% SDS-PAGE, B, cleavage of the 34-kDa protein with cyanogen bromide. As the NH2 terminus of the 34-kDa protein was blocked upon sequencing attempts, it was electroeluted and cleaved with cyanogen bromide (“Materials and Methods”). The cleavage products were resolved by 4–20% gradient SDS-PAGE, transferred to a PVDF membrane, and stained with Coomassie Blue. The arrows indicate the two cleavage products detected. C, alignment of the protein sequence of the cleavage product 2 with the nucleoplasmin sequence (39).

pressed if synthesized from a plasmid template injected into the oocyte nucleus. Quantitation of relative translational efficiencies indicate a ~25-fold repression of in vivo synthesized versus in vitro synthesized H4 mRNA (Table 1).

**Regulated Unmasking of Histone H4 mRNA on Oocyte Maturation**—We have established conditions for the translational masking of histone H4 mRNA following synthesis of the mRNA in vivo (Fig. 1). We next examined whether this masked mRNA would be released for translation on oocyte maturation and hence come under the appropriate developmental control. We repeated the experiment of injecting histone H4 mRNA into the cytoplasm or synthesizing H4 mRNA in vivo, however, we added the additional step of inducing oocyte maturation with progesterone (Fig. 2A). As a control we made use of our primer extension assay to determine the level of the various histone H4 mRNAs at the end of our experiments (Fig. 2B). Incubation in the presence of progesterone leads to a small decline in the abundance of all the histone H4 mRNAs (Fig. 2B, compare lanes 1 and 2). This result was confirmed by Northern blotting (data not shown). We next examined the translation of the exogenous H4 mRNA. In the absence of progesterone treatment, immunoblotting with antibodies specific for the FLAG epitope indicates that exogenous histone H4 mRNA synthesized in vitro and injected into the cytoplasm is translated (Fig. 2C, lane 2), whereas that synthesized in vivo is masked (Fig. 2C, lane 4). Maturation of oocytes with progesterone has no effect on the translation of histone H4 mRNA injected into the cytoplasm (Fig. 2C, compare lanes 2 and 3), but leads to the unmasking of histone H4 mRNA synthesized in vivo (Fig. 2C, compare lanes 4 and 5). Thus histone H4 mRNA synthesized in vivo from plasmid DNA templates comes under the appropriate developmental control at oocyte maturation, whereas histone H4 mRNA microinjected into oocyte cytoplasm does not. In earlier work (30), we made use of density gradient centrifugation to show that masked maternal mRNA is assembled exclusively into mRNPs, but that on release to the translational machinery mRNA is distributed between mRNPs and ribosomes. Histone H4 mRNA exhibits similar behavior. Histone H4 mRNA is exclusively in mRNPs prior to oocyte maturation (Fig. 2D, Pre) and redistributes between mRNPs and ribosomes post-maturation (Fig. 2D, Post). Note that these Northern blots of gradient fractions are only to compare the distribution of H4 mRNA between various RNP fractions. Since there is no transcription in Xenopus from oocyte maturation until the mid-blastula transition of Xenopus embryogenesis (34, 35) there is no increase in H4 mRNA abundance from levels in the oocyte until after that time. We next examined the molecular mechanisms responsible for the release of H4 mRNA from storage ribonucleoprotein particles to the ribosomes.

**Nucleoplasmin Associates with Ribonucleoprotein Particles Containing Histone H4 mRNA following Oocyte Maturation**—A major event associated with oocyte maturation is the breakdown of the nuclear envelope. This leads to a mixing of nuclear and cytoplasmic components with important consequences for transcription (35) and translation (36). We wished to explore whether oocyte maturation would alter the distribution of proteins that could be cross-linked to radiolabeled histone H4 mRNA. In earlier work we had documented the association of proteins with radiolabeled mRNA synthesized in vitro using this strategy (8, 37). The Y-box protein FRGY2 interacts with mRNA microinjected into the oocyte nucleus or cytoplasm (8, 30, 31). The capacity for FRGY2 to bind to histone H4 mRNA is unchanged in this assay following oocyte maturation (Fig. 3A, compare lanes 1 with 2 and 3 with 4). FRGY2 is indicated with an asterisk. The identity of FRGY2 was demonstrated by immunoprecipitation experiments (Ref. 8; data not shown). This result is consistent with the continued presence of histone H4 mRNA in mRNPs after oocyte maturation (Fig. 2D). Several other radiolabeled proteins appear, two have masses of ~250 and 30 kDa, respectively (Fig. 3A). The identity of the ~250-kDa protein is unknown. The properties of the 30-kDa protein suggest that it is phosphovit; this is an egg yolk protein of ~30 kDa, that contains 56% of the amino acids in the protein as potentially phosphorylated serines (38). This protein is readily radiolabeled following injection of any [32P]nucleotide triphosphate into Xenopus oocytes, it is heat-stable (see Fig. 4), and is not extracted by phenol/chloroform treatment. It is a highly radioactive contaminant of our experiments. Our attention focused on a protein of apparent molecular mass of 34 kDa that associates with histone H4 mRNA after oocyte maturation, but not before (Fig. 3A, arrowhead). We substantially scaled up this analysis and resolved total protein after heat purification (Fig. 3A, lane 5, see below) on SDS-PAGE before transfer to PVDF membranes. Note that the radioactive 30-kDa protein (Fig. 3A, lanes 1–4) is not visualized by Coomassie Blue staining, suggesting that it is a minor contaminant of our preparations in terms of protein mass. NH2-terminal sequencing of the 34-kDa protein was attempted, but the NH2 terminus was found to be blocked. After repeat electrophoresis of the extract, the gel was stained with Coomassie Blue and the appropriate protein band was cut out and electroeluted. The protein was cleaved with cyanogen bromide before resolution by SDS-PAGE and transfer to a PVDF membrane (Fig. 3B). The cleavage products were analyzed by NH2-terminal sequencing, Product 1 (Fig. 3B) was blocked; however, Product 2 gave the sequence indicated (Fig. 3B).
Fig. 4. Detection of nucleoplasmin association with histone H4 mRNA synthesized in vivo following oocyte maturation by immunoprecipitation. Oocytes were either injected with in vitro transcribed radiolabeled H4 mRNA or coinjected with H4 DNA and [32P]UTP. After an 8-h incubation, oocytes were treated with or without progesterone as indicated for 12 h. A, oocytes were UV-treated to cross-link proteins to mRNA, the nucleoprotein complex was digested with RNase A, and then radiolabeled proteins resolved by 10% SDS-PAGE. In lanes 1 and 2 oocytes were injected with H4 mRNA into the cytoplasm and in lanes 3 and 4, oocytes were injected with H4 DNA into the nucleus. The arrowhead indicates the 34-kDa protein. B, oocytes were injected with H4 DNA and [32P]UTP as above. The nuclei and cytoplasm of 20 oocytes that had not been matured were manually fractionated. The nuclear (N) (lane 1) and cytoplasmic (lane 2) fractions as well as total extract (T) from oocytes that had not been matured (lane 3) or that had been matured (lane 4) were UV cross-linked, digested with RNase A, and heat-fractionated. The arrowhead indicates the 34-kDa protein. C, oocytes injected with H4 DNA and [32P]UTP were incubated for 8 h before a further 12 h of incubation without or with progesterone. Matured and control oocytes were UV cross-linked, digested with RNase A, and then heat-purified. The labeled proteins were directly resolved by gel electrophoresis (lanes 1 and 2) or immunoprecipitated (lanes 3–6). Immunoprecipitation was carried out with anti-nucleoplasmin antibodies (lanes 3 and 4) or with preimmune sera (lanes 5 and 6). Nucleoplasmin is indicated by the arrowhead.
the possibility that nucleoplasmin might interact with the Y-box proteins and thereby facilitate translation of the mRNAs with which they are associated.

In earlier work we established in vitro conditions where the binding of FRGY2 or the Tail domain of FRGY2 to mRNA led to translational repression (31). We repeated these experiments with histone H4 mRNA, adding sufficient excess of the Tail domain containing the arginine-rich sequences to repress translation (Fig. 5A, lanes 1–3). We then titrated increasing amounts of nucleoplasmin into the translation reaction (Fig. 5A, lanes 3–6). Under these conditions nucleoplasmin provides a modest stimulation of translation, quantitation indicates a 5% relief of repression. The addition of nucleoplasmin alone to the translation reaction in the presence of naked H4 mRNA was without effect on translation efficiency (Fig. 5A, lanes 7–9).

These experiments (Fig. 5A) use 130 ng of H4 mRNA associated with 2,500 ng of Y-box protein. The addition of nucleoplasmin (3,000 ng) under these conditions provides a very limited relief of translational repression. The in vitro translation assay conditions constrain the amount of histone H4 mRNA required to obtain a strong translation signal; moreover, we can only concentrate nucleoplasmin to a limited extent before precipitation. However, in vivo in the oocyte there is 290 ng of nucleoplasmin and 21 ng of total mRNA associated with approximately 40 ng of Y-box proteins (30, 49); thus, the ratio of nucleoplasmin to Y-box protein is greater in the oocyte than in our in vitro reaction. If we reduce the excess of Y-box proteins in the in vitro translation reaction by 50%, we find that nucleoplasmin (3,000 ng) more effectively relieves translational repression (25% relief of repression, Fig. 5B, compare lanes 2 and 3). Our experimental system is limited by the concentration of purified nucleoplasmin; nevertheless, our results suggest that the ratio of Y-box protein to nucleoplasmin can influence the relief of translational repression. We suggest that nucleoplasmin can contribute to the partial relief of translational repression directed by the Tail domain of FRGY2.

**DISCUSSION**

Our results demonstrate that histone H4 mRNA synthesized from a plasmid DNA template in vivo comes under the appropriate developmental control of translation on oocyte maturation. The H4 mRNA synthesized in vivo is translationally silent prior to maturation and is released to the translational machinery after maturation (Fig. 2). Histone H4 mRNA microinjected into the oocyte cytoplasm does not come under this translational control. These results further emphasize the importance of in vivo transcription to establish translational silencing in the oocyte (5, 8). Although it is possible to activate translation on oocyte maturation in the oocyte using particular synthetic mRNAs injected into the oocyte cytoplasm (12, 13), the range of translational regulation that might be achieved is potentially greater if the same mRNA is synthesized in vivo (Fig. 2; Ref. 14). This amplification of responsiveness is due to the reduction in translational efficiency dependent on association of the mRNA with repressive proteins such as FRGY2. Earlier work has comprehensively excluded regulated increases in polyadenylation as exerting an activating function on histone H4 mRNA translation (18, 19). Thus other regulatory mechanisms must contribute to the unmasking of H4 mRNA.

In the immature oocyte nucleoplasmin stores histones H2A and H2B in the oocyte nucleus (20, 50). However, 290 ng of nucleoplasmin (molecular mass 22 kDa) is available to store 70 ng of histone H2A and H2B (which exist as a heterodimer of molecular mass 25 kDa) (20, 51). Thus it is likely that additional functions exist for nucleoplasmin. One of these functions is the unpackaging of the paternal genome within sperm chromatin following fertilization (21). This unpackaging is greatly facilitated by the hyperphosphorylation of nucleoplasmin that occurs on oocyte maturation (21, 42). Phosphorylated nucleoplasmin sequesters the arginine-rich sperm protamines allowing the paternal genome to be assembled into nucleosomes (43, 52). Our results suggest that a comparable function might exist for nucleoplasmin in facilitating the unpackaging of maternal mRNA.

Maternal mRNA is packaged by the Y-box protein FRGY2 (30, 41, 46). The COOH-terminal Tail domain of FRGY2 has marked sequence similarities with sperm-specific protamines (46, 47). Nucleoplasmin does not interact with naked histone H4 mRNA in vitro (data not shown), or efficiently with histone H4 mRNA in the nucleus (Fig. 4B), but it does interact with H4 mRNA following oocyte maturation as assayed by UV cross-linking (Figs. 3 and 4). These results imply that nucleoplasmin
association is a regulated process dependent on protein-protein interactions with the mRNP. Earlier immunofluorescence studies had suggested that nucleoplasmin might associate with ribonucleoprotein particles on the lambrush chromosomes of the oocyte (53). Thus some association of nucleoplasmin with nuclear ribonucleoprotein particles is likely to occur. We suggest that the affinity of nucleoplasmin for ribonucleoproteins will be increased following the hyperphosphorylation of nucleoplasmin during oocyte maturation (21, 42). The increased association of nucleoplasmin with masked maternal mRNPs following oocyte maturation may depend on interactions of the Tail domain with FRGY2. However, our experiments have so far failed to show a stable interaction between nucleoplasmin and FRGY2 in the absence of mRNA (data not shown). It is the interaction of hyperphosphorylated nucleoplasmin with sperm-specific protamines that leads nucleoplasmin to associate with sperm chromatin (21). A tethering of nucleoplasmin by the Tail domain might facilitate cross-linking to mRNA. It is also probable that nucleoplasmin association will destabilize the interaction of the translationally repressive Tail domain with mRNA (31). Nucleoplasmin can partially relieve the translational repression of H4 mRNA caused by the FRGY2 Tail domain in vitro (Fig. 5). Clearly other factors must contribute to translational regulation; however, nucleoplasmin or comparable chaperone activities are likely to have a role in unpackaging masked maternal mRNA following oocyte maturation.

REFERENCES
1. Standart, N. (1992) Semin. Dev. Biol. 3, 369–379
2. Wickens, M. (1992) Semin. Dev. Biol. 3, 399–412
3. Wormington, M. (1994) BioEssays 16, 533–535
4. Spirin, A. S. (1994) Mol. Rep. Dev. 3, 533–535
5. Standart, N., and Jackson, R. (1994) Exp. Cell Res. 213, 107–117
6. Adamson, E. D., and Woodland, H. R. (1977) J. Biol. Chem. 252, 773–780
7. Runyan, M., Tufari, S. R., and Wolffe, A. P. (1993) J. Biol. Chem. 268, 24255–24261
8. Bouvet, P., and Wolffe, A. P. (1991) J. Biol. Chem. 266, 3064–3072
9. Pollard, S. C., and Chalkley, R. (1986) Biochemistry 25, 3080–3087
10. Richter, J. D., and Smith, L. D. (1984) Nature 309, 378–380
11. Kiefer, D., Barnett, P., Cummings, A., and Sommerville, J. (1987) Nucleic Acids Res. 15, 4099–4109
12. McGrew, L. E., Dworkin-Rastl, E., Dworkin, M. B., and Richter, J. D. (1989) Mol. Biol. Cell. 1, 369–379
13. Sheets, M. D., Wu, M., and Wickens, M. (1995) Nature 374, 511–516
14. Meric, P. M., Starfoss, A. M., Wormington, M., and Wolffe, A. P. (1996) J. Biol. Chem. 271, 30804–30810
15. Hershey, J. W. B. (1991) Annu. Rev. Biochem. 60, 717–755
16. Ruderman, J. V., Woodland, H. R., and Sterges, E. A. (1979) Dev. Biol. 71, 71–82
17. Adamson, E. D., and Woodland, H. R. (1977) Dev. Biol. 57, 136–149
18. Ballantine, J. M., and Woodland, H. R. (1986) FEBS Lett. 180, 224–228
19. Woodland, H. R., and Wilt, F. H. (1980) Dev. Biol. 73, 214–221
20. Mills, A. D., Lasky, R. A., Black, P., and De Robertis, E. M. (1980) J. Mol. Biol. 139, 561–568
21. Leno, G. H., Mills, A. D., Philpott, A., and Lasky, R. A. (1996) J. Biol. Chem. 271, 7253–7256
22. Freeman, L., Kurumizaka, H., and Wolffe, A. P. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12780–12785
23. Bachar, M., Weber, F., Gerhard, J., Dorsch-Hasler, K., Fleckenstern, B., and Schafer, W. (1985) Cell 41, 521–530
24. Krieg, P. A., and Melton, D. A. (1984) Nucleic Acids Res. 12, 7057–7070
25. Almouzni, G., and Wolffe, A. P. (1993) Genes Dev. 7, 2033–2047
26. Heasman, J., Holwill, S., and Wylie, C. C. (1991) Methods Cell Biol. 36, 213–231
27. Dimitrov, S., and Wolffe, A. P. (1996) EMBO J. 15, 5897–5906
28. Lasky, R. A., Honda, B. M., Mills, A. D., and Finch, J. T. (1978) Nature 275, 416–420
29. Toyoda, T., and Wolffe, A. P. (1992) Dev. Biol. 153, 150–157
30. Tafuri, S. R., and Wolffe, A. P. (1993) J. Biol. Chem. 268, 24255–24261
31. Matsumoto, K., Merie, P., and Wolffe, A. P. (1996) J. Biol. Chem. 271, 22706–22712
32. Panini, S., and Chalkley, R. (1969) Biochemistry 8, 3972–3979
33. Woodland, H. R., Flynn, J. M., and Whiffle, A. J. (1979) Cell 18, 165–171
34. Newport, J. W., and Kirschner, M. W. (1982) Cell 30, 657–666
35. Marca, M. J., Fidler, M. C. F., Smith, L. D., and Krem, K. (1975) Dev. Biol. 47, 384–393
36. Varnum, M. M., Burney, C. A., and Worthington, W. M. (1992) Dev. Biol. 153, 283–290
37. Bouvet, P., Matsumoto, K., and Wolffe, A. P. (1995) J. Biol. Chem. 270, 28297–28303
38. Tata, J. R., and Smith, D. F. (1979) Recent Prog. Hormone Res. 35, 47–95
39. Dingwall, C., Dilworth, S. M., Black, S. J., Kearsey, S. E., Cox, L. S., and Lasky, R. A. (1987) EMBO J. 6, 69–74
40. Dingwall, C., Schirnick, S. V., and Lasky, R. A. (1982) Cell 30, 449–458
41. Deshamps, S., Viel, A., Garrigues, M., Denis, H., and Le Maire, M. (1992) J. Biol. Chem. 267, 13799–13802
42. Sealy, L., Cohen, M., and Chalkley, R. (1986) Biochemistry 25, 3064–3072
43. Philpott, A., and Leno, G. H. (1992) Cell 69, 759–767
44. Philpott, A., Leno, G., and Lasky, R. A. (1991) Cell 65, 569–578
45. Hiyoishi, H., Yokota, T., Katagiri, C., Nishida, H., Takai, M., Agata, K., Eguchi, G., and Abe, S. (1991) Exp. Cell Res. 194, 85–90
46. Murray, M. T., Schiller, D. L., and Franke, W. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 11–15
47. Tafuri, S. R., and Wolffe, A. P. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 9026–9032
48. Tafuri, S. R., and Wolffe, A. P. (1992) New Biol. 4, 349–359
49. Davidson, E. H. (1986) Gene Activity in Early Development, 3rd Ed., Academic Press, San Diego
50. Dilworth, S. M., Black, S. J., and Lasky, R. A. (1987) Cell 51, 1009–1018
51. Woodland, H. R., and Adamson, E. D. (1977) Dev. Biol. 57, 118–135
52. Dimitrov, S., Dasso, M., and Wolffe, A. P. (1994) J. Cell Biol. 126, 591–601
53. Moreau, N., Angelier, N., Ronanfont-Jaia, M. L., Geunon, P., and Kubisz, P. (1986) J. Cell Biol. 103, 683–690
Regulated Unmasking of in Vivo Synthesized Maternal mRNA at Oocyte Maturation: A ROLE FOR THE CHAPERONE NUCLEOPLASMIN
Funda Meric, Ken Matsumoto and Alan P. Wolfe

J. Biol. Chem. 1997, 272:12840-12846.
doi: 10.1074/jbc.272.19.12840

Access the most updated version of this article at http://www.jbc.org/content/272/19/12840

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 52 references, 15 of which can be accessed free at http://www.jbc.org/content/272/19/12840.full.html#ref-list-1