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Whole-mount *in situ* Hybridization of Mitochondrial rRNA and RNase MRP RNA in *Xenopus laevis* Oocytes

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In order to analyze the intracellular localization of specific RNA components of ribonucleoproteins (RNP) in *Xenopus* oocytes, a modified protocol of whole-mount *in situ* hybridization is presented in this paper. Mitochondria specific 12S rRNA probe was used to detect the amplification and distribution of mitochondria in various stages of the oocyte life cycle, and the results were found to be consistent with previously known distribution of mitochondria. The results with other specific probes (U1 and U3 small nuclear RNAs, and 5S RNA) also indicate that this procedure is generally effective in localizing RNAs in RNP complexes even inside organelles. In addition, the RNA component of RNase MRP, the RNP with endoribonuclease activity, localize to the nucleus in various stages of the oocyte life cycle. Some of MRP RNA, however, were found to be localized to the special population of mitochondria near the nucleus, especially in the active stage of mitochondrial amplification. It suggests dual localization of RNase MRP in the nucleus and mitochondria, which is consistent with the proposed roles of RNase MRP in mitochondrial DNA replication and in rRNA processing in the nucleolus.

Xenopus oocytes is an outstanding cell biological system to study the mechanism of nucleo-cytoplasmic transport of ribonucleoproteins (RNPs). It is partly due to the large nucleus (germinal vesicle, GV) which could be easily separated from the cytoplasm by simple manual dissection under a light microscope. Dissected nucleus and cytoplasm from a single cell contain enormous amount of materials, thus routine biochemical analyses are possible from the single cell. Since the oocyte nucleus can be easily visualized as previously stated, *Xenopus* oocytes are excellent for the fine mapping of intranuclear structure compared to mammalian cells. In particular, *Xenopus* oocytes are uniquely advantageous to study due to the nucleolar localization of specific RNA. It is due to the amplification of rDNA as extrachromosomal circles, thus thousands of nucleoli are generated on the periphery of the nucleus (Brown and Dawid, 1968). In addition, mitochondria are also amplified enormously during oogenesis (Billett, 1979), making it possible to study mitochondrial function. However, in spite of these exceptional qualities, nucleolar or mitochondrial localization of specific RNA or protein has not been studied thoroughly. It is partly because intracellular localization of RNA and protein is usually examined by biochemical fractionation followed by Northern blot or Western blot analysis. Such a procedure has limitations when fine localization of the substances is required or the substances are in an easily detachable position, such as the outside of the organelle. To overcome the limitations of cell fractionation and to visualize directly the subcellular distribution of specific nucleic acid sequences, a procedure for whole-mount *in situ* hybridization has been established. In this procedure, the need for embedding and sectioning of samples to observe a three-dimensional expression pattern is obviated, thereby allowing processing of a large number of samples at once; thus making it easier to analyze gene expression during various stages of development. Whole-mount *in situ* hybridization is widely used to visualize the expression of specific genes in *Xenopus* embryos (Harland, 1991). However, the use of this technique with *Xenopus* oocytes and eggs has been problematic due to the large yolky cytoplasm which hinders penetration of a probe (O'Keefe et al., 1991). Here a modified procedure which allows the specific localization of RNAs in oocytes is reported, even inside the nucleus and mitochondria.

In this report, various RNAs were used as probes to detect the nucleus or mitochondria in various stages of oocytes. Especially in post-vitellogenic oocytes, mitochondria are not easily detected either by light microscopy or by fluorescent microscopy due to the abundance and autofluorescence of massive yolk proteins in the cytoplasm. Therefore, in this study, the validity of whole-mount *in situ* procedure was tested as a simple method for mitochondria detection in oocytes. To specifically detect mitochondria in various stages of

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oocytes, mitochondrial 12S rRNA probe was used. In addition, to visualize the nucleus as well as the mitochondria, the RNA component of RNase MRP (MRP RNA) was also used as a probe. RNase MRP (Mitochondrial RNA Processing) is an RNP with site-specific endonuclease activity; it is suggested to process two distinct substrates, mitochondrial RNA and ribosomal RNA (Chang and Clayton, 1987; Schmitt and Clayton, 1993; Jeong-Yu and Clayton, 1996). Since 12S rRNA and MRP RNA are RNA components of ribonucleoprotein (RNP) complexes, it can be concluded that whole-mount *in situ* procedure can be applied to RNA which is complexed with proteins, even inside organelles. Therefore, whole-mount *in situ* hybridization with a specific RNA probe can be used as an easy-to-use procedure to detect mitochondrial and nuclear localization of RNP RNA in *Xenopus* oocytes.

Materials and Methods

Preparation of oocytes

Ovaries were surgically removed from mature females and treated with collagenase as described previously (Jeong-Yu and Carroll, 1992). Oocytes were staged according to Dumont (Dumont, 1972), incubated in OR-2 buffer (Wallace, 1973) and the nucleus was dissected under J-buffer (70 mM NH_4Cl; 7 mM $MgCl_2$; 10 mM HEPES; 0.1 mM EDTA; 2.5 mM DTT; 10% glycerol; 1% polyvinylpyrollidone). At least 20 oocytes were processed for *in situ* hybridization.

Preparation of in situ probes

Digoxigenin-labeled probes were prepared according to standard Dig labeling kit as described (Jeong, 1997). Antisense mtRNA probe, which contains the 5'-170 nucleotides of 12S rRNA was prepared by *DdeI* digestion of a *Sau*3A I-*Eco*RI subclone of *Xenopus* mtDNA (provided by Dr. D. Bogenhagen) followed by transcription with T7 RNA polymerase. To detect *Xenopus* MRP RNA, either an RNA probe or oligonucleotide probe was used; uniformly labeled antisense RNA probe was prepared from pXLMU by *in vitro* transcription with T7 RNA polymerase and digoxigenin-dUTP (Bennett et al., 1992) and sense-strand RNA probe was transcribed with T3 RNA polymerase. The 3'-end labeled oligonucleotides complementary to nucleotides 65-110 of MRP RNA (5' MRP oligo) were made using terminal transferase and digoxigenin-ddUTP. A human U1 gene was cloned into a pBluescript II-KS vector, linearized with *FokI* and transcribed with T3 RNA polymerase to make a U1 antisense probe of the 175 nucleotides. A digoxigenin-labeled DNA probe for 5S rRNA was prepared from pHU1063 (provided by Dr. D. Carroll) by isolation of a *HindIII* fragment and random primer label.

Whole-Mount in situ hybridization

The method of *in situ* hybridization for whole-mount oocytes was derived from a procedure designed for use with *Xenopus* embryos (Harland, 1991). Oocytes were fixed in BRB buffer (80 mM KPipes, pH 6.8; 5 mM EGTA; 1 mM $MgCl_2$) with 3.7% formaldehyde and 0.1% Triton X-100 at room temperature for 2 to 6 h and stored in methanol at -20°C (Gard, 1991). This fixation allows excellent preservation of organelle structures. After rehydration, stage IV to VI oocytes were bisected with a scalpel, either laterally or equatorially, to visualize the inside of the oocytes. Fixed oocytes were incubated with proteinase K (10 µg/ml) for 10-20 min at room temperature followed by refixing for 20 min with 4% paraformaldehyde. Oocytes were washed twice with PTw (1x Phosphate Buffered Saline; 0.1% Tween 20) after proteinase K treatment and refixing. Oocytes were prehybridized for 4 h in hybridization buffer (50% formamide; 5x SSC; 1x Denhardts; 0.1% Tween-20; 0.1% CHAPS; 5 mM EDTA; 1% blocking reagent; 200 µg/ml yeast tRNA; 200 µg/ml salmon sperm DNA) at 45°C to 60°C. Hybridization was continued overnight at 45°C for oligonucleotide probes and 60°C for RNA probes. Unbound RNA probes were removed by washing with 2x SSC plus 0.3% CHAPS at 37°C for 20 min followed by RNase A incubation (20 mg/ml, 37°C 20 min) and a 0.2x SSC/0.3% CHAPS wash at 60°C for 30 min. Unbound oligonucleotide probes were washed with 2x SSC/0.3% CHAPS at room temperature and 0.2x SSC/0.3% CHAPS at 45°C for 20 min. To prevent non-specific binding, oocytes were preblocked with oocyte extract mixture and antibody (1:600 diluted alkaline phosphatase conjugated antidigoxigenin antibody) was pre-absorbed for 4 h at 4°C with the following solution: TNT (100 mM Tris-HCl, pH 7.5; 150 mM NaCl, 0.1% Tween 20); 10% goat serum; 10% lamb serum; 1% blocking reagent (Boehringer Mannheim); 5% oocyte extract. Pre-absorbed antibody was incubated with preblocked oocytes at 4°C overnight, and then washed with TNT plus 2 mM Levamisol (Sigma) at room temperature three times for 1 h each. Before the alkaline phosphatase reaction, samples were preincubated with 100 mM Tris-HCl (pH 9.5), 100 mM NaCl, 50 mM $MgCl_2$, and 5 mM Levamisol for 30 min. The color reaction was initiated by adding X-phosphate and nitro-blue tetrazolium chloride to buffer C and continued for 1-2 h until color was apparent. The reaction was stopped by washing with TE and oocytes were postfixed with 3.7% formaldehyde in PBS for 20 min. In some experiments, the black pigments on oocytes were bleached overnight with 10% H_2O_2. Oocytes were dehydrated with methanol, cleared and mounted with benzyl benzoate: benzyl alcohol (2:1) solution. Rhodamine 123 (10 µg/ml) was incubated with unfixed oocytes in OR-2 buffer for 30 min at room temperature followed by 1 to 2 h of wash with OR-2 buffer. Zeiss Axiophot microscope was used

to examine stained oocytes and images were photographed either with Kodak Ektachrome P800/P1600 or with Ektachrome 64T films.

Results

Localization of mitochondrial RNA during oogenesis

The number of mitochondria is greatly increased during *Xenopus laevis* oogenesis. Most of the mitochondrial amplification occurs during previtellogenesis (stage I), generating a cytologically distinct spherical mitochondrial mass (Callen et al., 1980). The distribution of mitochondria in small stage I oocytes was visualized with rhodamine 123, which specifically stains mitochondria due to its affinity for the negative transmembrane potential of the organelle (Johnson et al., 1980; Ronot et al., 1986). The stage I oocyte has a mitochondrial mass (arrowhead) and a mitochondrial network (arrows) around the nucleus, as shown by rhodamin 123 staining (Fig. 1A). Mitochondrial 12S rRNA was used as a probe to test whether the present *in situ* hybridization procedure is suitable to obtain enough permeability to detect RNA in the RNP particle, especially inside a double-membrane organelle such as a mitochondrion (Fig. 1B-G). 12S rRNA was chosen as a mitochondria specific probe, because it has no detectable homology to cytoplasmic rRNA and it is the most abundant mtRNA in the cell. In addition, it has been shown to be localized to mitochondria in sectioned *Drosophila* ovary tissues at the electron microscopic level (Binder et al., 1986).

Examination of stage I oocytes demonstrates the specific staining of the mitochondrial mass (arrowhead) and punctuated (Fig. 1B) and locally accumulated (Fig. 1C) perinuclear network (arrows) of mitochondria, similar to rhodamine 123 staining (Fig. 1A). Higher magnification shows more clearly the homogenous staining of the mass and differential staining of the mitochondrial network around the nucleus (Fig. 1C). During early vitellogenetic stages the mitochondrial mass is dispersed, resulting in a heterogenous distribution of mitochondria in the cytoplasm; however, accumulation of mtDNA continues until stage IV (Webb and Smtih, 1977). Similar results were shown in Fig. 1D-F. At the onset of vitellogenesis, the mitochondrial mass is dissociated and diffused throughout the cytoplasm, but some of the perinuclear mitochondria remain near the nucleus. Stage II oocytes display a diffused mitochondrial network (Fig. 1D), and the intensity of the staining is much higher in stage II oocytes than in stage I oocytes (Fig. 1D). Such a difference of rRNA staining in stage I and stage II oocytes is likely to be caused by the fact that mitochondria are amplified at the end of stage I or in early stage II. By stage III-IV, most of the amplified mitochondria diffuse in the cytoplasm and congregate toward the periphery of the oocyte membrane with some remaining associated with the nucleus (Fig. 1F). Bisected stage IV oocytes show a characteristic crowning of mitochondria (arrowhead) around the vegetal side of the nuclear envelope (arrowhead in Fig. 1G; Tourte et al., 1984).

Fig. 2A-D shows the mitochondrial staining of stage V-VI oocytes with 12S rRNA probe; mitochondria are distributed in the subcortical layer (arrowheads) as well as in the proximal region to the nuclear membrane (arrows in Fig. 2A). Some of the nucleus-associated mitochondria are found at the yolk-free area near the vegetal side (arrows in Fig. 2B). A three-dimensional view of mitochondria in stage VI is shown in Fig. 2C; the arch-like area near the animal pole is enriched in mitochondria as described previously (Tourte et al., 1984), and as are the sites near the nuclear membrane and in the subcortex of the vegetal side. Inspection of the animal pole shows that mitochondria are aligned and some are associated with the nucleus (arrows in Fig. 2D).

Other RNAs which are in the RNP complex were used as probes to show the general applicability of this technique for localization of RNA. Hybridizations with U1 snRNA and 5S rRNA probes are shown in Fig. 2E and 2F, respectively. A relatively high level of U1 snRNA is localized in the nucleus at stage I, but a lower level was found in stage II oocytes (Fig. 2E). The U1 snRNA localization is consistent with the previous localization data using Sm antibody which specifically recognizes the protein component of U1 snRNP (Zeller et al., 1983). The U3 snRNA probe showed nucleolar localization, as well as other nucleoplasmic signals (data not shown); 5S rRNA was dispersed throughout the nucleus and cytoplasm as expected (Fig. 2F; De Robertis et al., 1982; Guddat et al., 1990). The same procedure was performed with sense probes of RNA to confirm the specificity of the probe used. The sense probe staining did not show any specific staining (data not shown).

These data clearly demonstrates that the whole-mount *in situ* hybridization could be used to follow the intracellular localization of specific RNA. The mitochondrial probe (12S rRNA) can specifically hybridize to mitochondria without any significant background signal in other cellular organelles, and its localization pattern in different stages of oocytes is consistent with the previously determined distribution of mitochondria (Tourte et al., 1984). In addition, it is presented here that the localization patterns of other RNAs using whole-mount *in situ* hybridization are consistent with known intracellular locations of RNAs with other detection methods.

Localization of RNase MRP RNA during oogenesis

It has been previously shown by immunofluorescent microscopy that the RNase MRP particle is localized to the granular component of the nucleolus in mam-

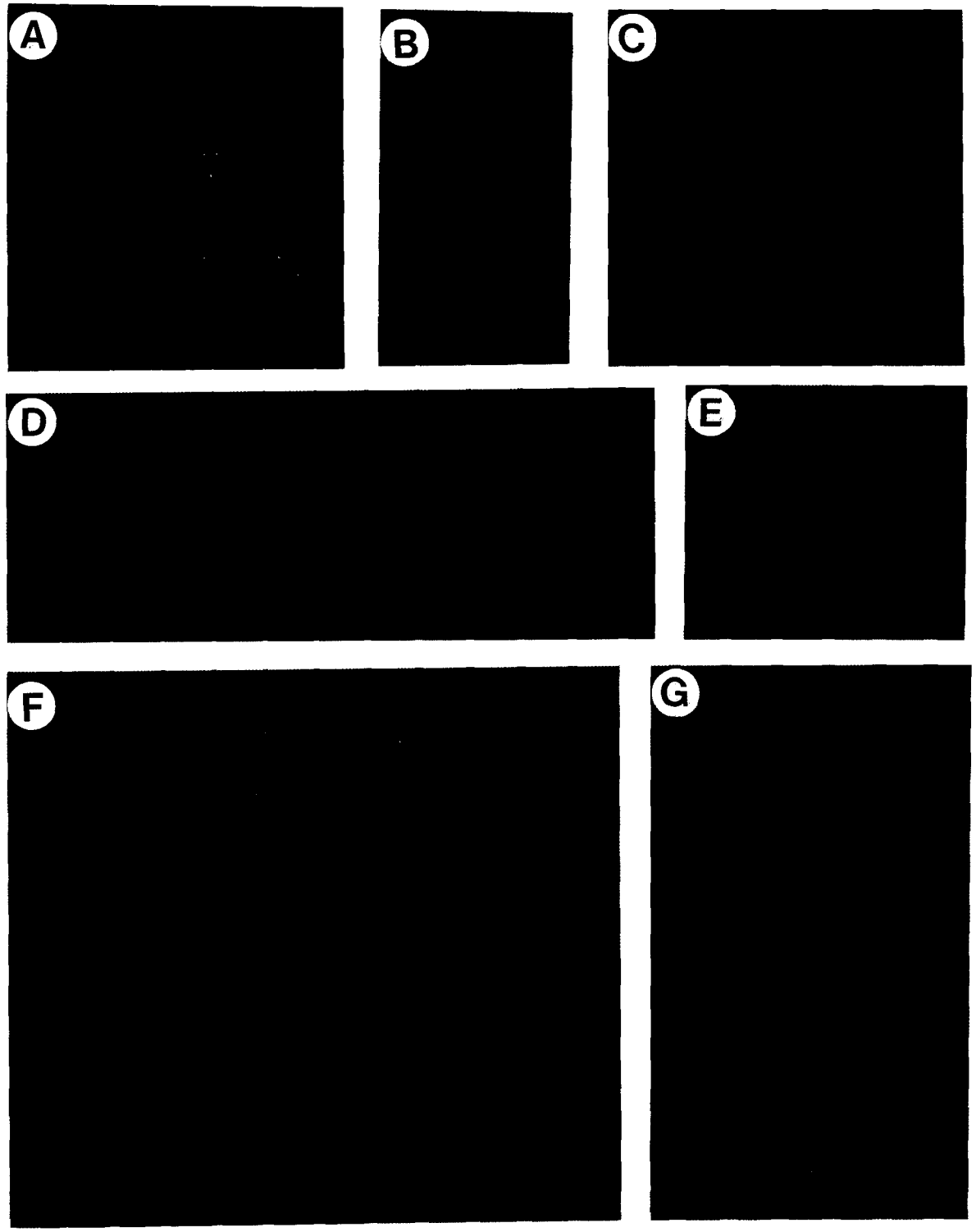

Fig. 1. The distribution of mitochondria in stage I-IV oocytes visualized by whole-mount *in situ* hybridization with DIG-labelled mitochondrial 12S rRNA probe. Nucleus (N) and cytoplasm (C) are indicated. A, Rhodamine 123 stained stage I oocytes. Arrowhead represents the mitochondrial mass; arrows indicate the perinuclear mitochondrial network. B, Stage I oocyte. C, Higher magnification of stage I. D, Stage I and stage II oocytes. E, Stage II oocyte. F, Stage III and IV oocytes compared to stage I. G, Laterally bisected stage IV oocyte showing crowning of mitochondria around the nuclear envelope (arrowhead). Scale bars=50 μm (B) and 100 μm (A, C-G).

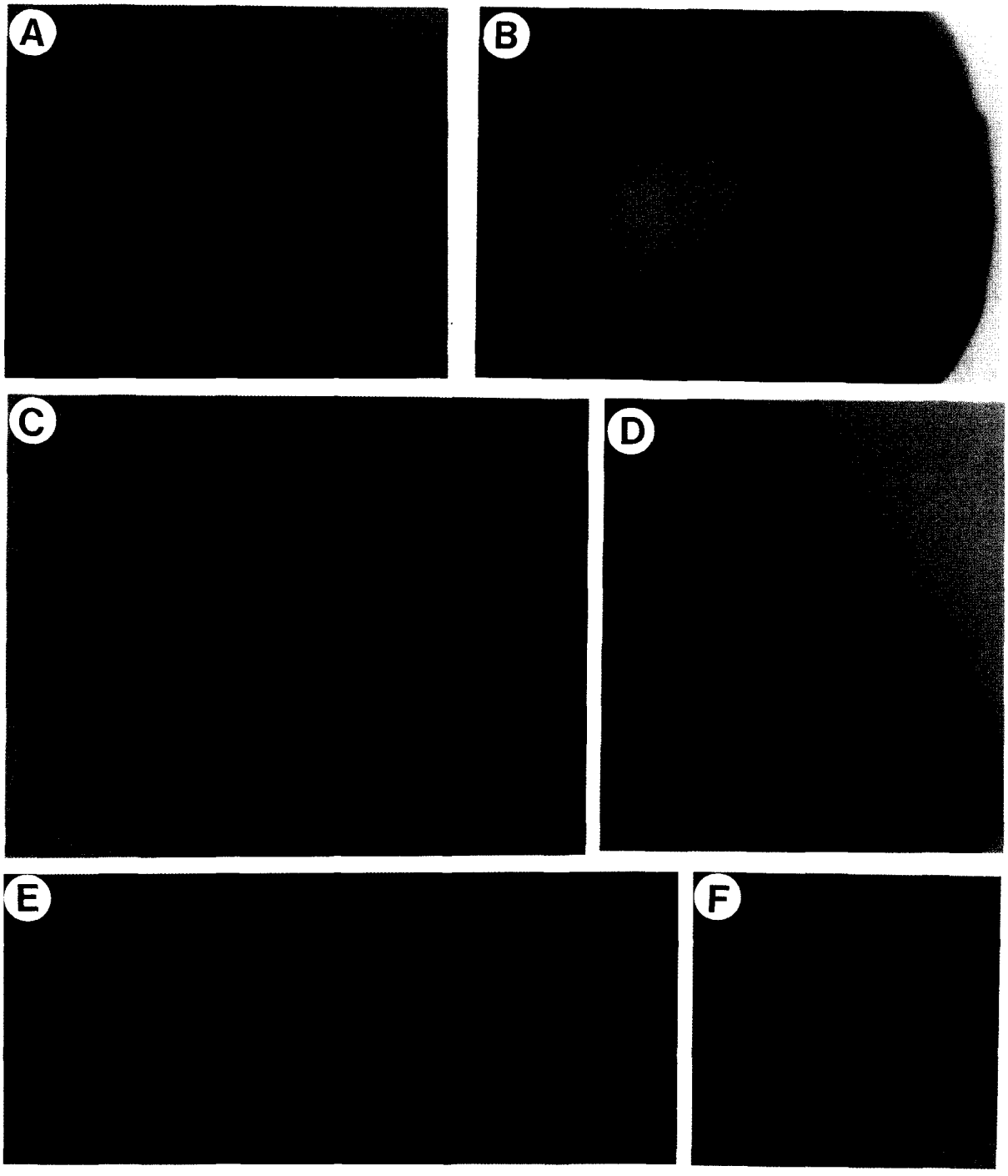

Fig. 2. Whole-mount *in situ* hybridization with DIG-labelled mitochondrial 12S rRNA (A-D), U1 snRNA (E) and 5S rRNA (F) probes. Stage V-VI oocytes in a-d are laterally bisected to show the inside of the cells. Animal pole (a), nucleus (N) and cytoplasm (C) are indicated. A, Stage V oocyte showing perinuclear (arrow) and subcortical (arrowhead) mitochondria. B, Nucleus associated mitochondria (arrows) in stage VI oocyte. C, Stage VI oocytes showing three-dimensional view of mitochondrial distribution. D, Animal pole region of stage VI oocyte with nucleus associated mitochondria (arrow) and intense mitochondrial staining in cytoplasm. E, U1 snRNA probe staining on stage I-II oocytes. F, 5S RNA staining on stage III oocyte. Brownish coloration on the cell surface is due to pigments on the animal hemisphere. Scale bars=100 μm (A-F).

malian tissue culture cells (Reimer et al., 1988). Since *Xenopus oocytes* have thousands of amplified nucleoli in the germinal vesicle (nucleus) and the size of the nucleus is large, it is possible to use light microscopy to detect nucleollar localizations.

Fig. 3A-G shows the results of *in situ* hybridization with antisense MRP RNA probe. A high level of MRP RNA can be found in the nuclei of previtellogenic

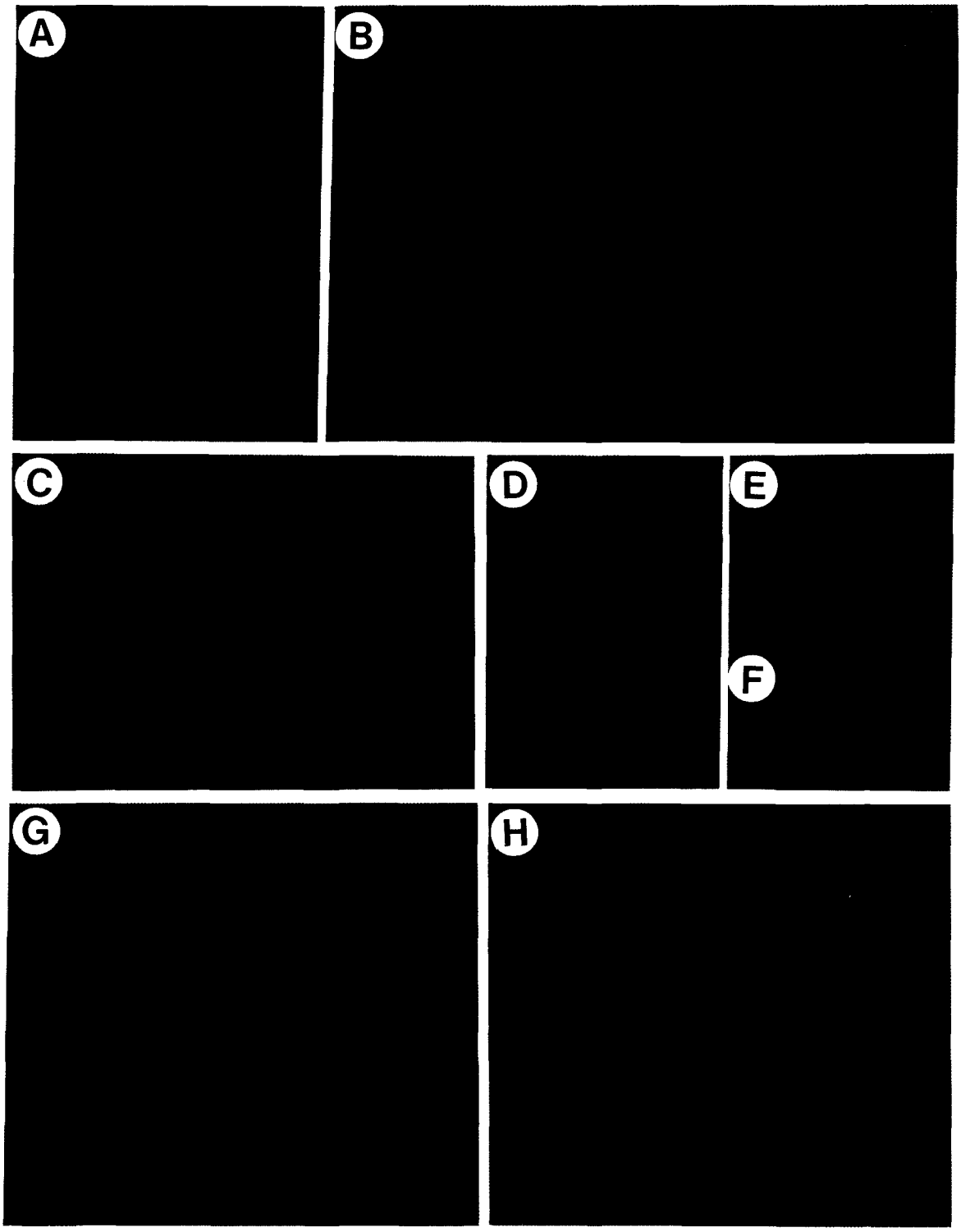

Fig. 3. MRP RNA expression. Nucleus (n) and cytoplasm (c) are indicated. A, Stage I oocytes showing intense nuclear staining. B, Stage I-IV oocytes as indicated. Stage II oocytes show diffuse nuclear outline (thick arrow) than others. Thin arrows indicate perinuclear network. C, Higher magnification of stage I oocytes. Larger oocytes with a mass (arrowhead) and perinuclear network of mitochondria (arrows) shows the MRP RNA staining around the network as well as in the nucleus. Smaller oocyte shows tight nuclear localization. D-F, Stage II oocyte with diffuse cytoplasmic staining; perinuclear mitochondrial network was also stained in D and F (arrows). G-H, Stage VI oocytes showing nuclear staining with MRP RNA. Nucleoli staining is shown better in H (arrows). The cell surface coloration (thin arrows) is not from a MRP RNA stain, but from pigments on the animal hemisphere. Scale bars=50 μm (C) and 100 μm (A, B, D-H).

oocytes (Fig. 3A), including less than 50 μm-sized stage I oocyte or oogonium (data not shown). Fig. 3B also shows that stage I oocytes have highly localized MRP RNA in the nucleus, but stage II oocytes (middle) have a rough outline of staining around the nuclear periphery (arrow) compared to the smaller previtellogenic (stage I) or vitellogenic (stage III and IV) oocytes. A higher magnification of different stage I oocytes shown in Fig. 3C reveals that the smaller (200 μm) oocyte shows MRP RNA staining inside of the nucleus. The larger (250 μm) oocyte in Fig. 3C has a distinct mitochondrial mass (arrowhead) and a perinuclear mitochondrial network (arrows); the majority of MRP RNA is present inside of the nucleus, but a small amount is seen in the perinuclear network of mitochondria and on the surface of the mitochondrial mass as dots. It should be noted that this oocyte is about the same size and morphology as the one in Fig. 1C, which exhibited homogenous staining of the mitochondrial mass with the mitochondrial 12S rRNA probe. The diffused cytoplasmic staining around the nucleus is more prominent later in early stage II oocytes, which are 350-450 μm size at the onset of vitellogenesis (Fig. 3D-F). They all show an almost circular (round) distribution of the staining in the cytoplasm, but never completely extending to the plasma membrane. The oocyte in Fig. 3F clearly shows that the MRP RNA signal is higher in the perinuclear network of mitochondria. Later, in stage V-VI oocytes, MRP RNA is only detected in the nucleus, nucleoplasm and nucleoli (arrow), but it is not found in the cytoplasm (Fig. 3G and 3H). The same procedure was performed with a sense probe of MRP RNA to confirm the specificity of the probe used. The sense strand results in a very low level of staining on the nuclear membrane but no staining in the nucleus or cytoplasm (data not shown).

Thus, in early stage I oocytes, MRP RNA is only localized in the nucleus; but at or near the onset of vitellogenesis, a small portion of the MRP RNA population exits from the nucleus and appears to become localized to the mitochondrial network. The timing of the localization of MRP RNA in the mitochondria is within a developmental window when mitochondrial biogenesis is very active. Later in development, all detectable MRP RNA is found in the nucleus, not only in the nucleoli, but also in the nucleoplasm.

Discussion

In this study, simple whole-mount *in situ* hybridization technique was used to assess the distribution of mtDNA-encoded RNA, as well as to test the distribution of nuclear gene products in the cell. Our knowledge of mitochondrial organelle morphology and content in any cell type is limited to assays detecting membrane proteins. While the latter may serve as a useful diagnostic for overall organelle architecture in a given cell (and variations between cell and tissue types), it cannot answer the question of intramitochondrial distribution of mtDNA and RNAs. The localization of intraorganellar nucleic acid has been explored by the cell fractionation method, however, the possibility of contamination should be kept in one's mind when analyzing the data. These limitations can be partially complemented by the *in situ* approach, and such knowledge is important in understanding mitochondrial structure and function in the cell. However, the application of *in situ* has been problematic in oocytes, due to the large amount of yolk proteins present in the cytoplasm. Therefore, a modified version of whole-mount *in situ* hybridization method was presented to facilitate the penetration of the probe even inside organelles, to preserve intracellular structure and to reduce background staining. The use of proper fixative and preincubation with an oocyte extract mixture enhanced the signal-to-noise level using this modified procedure for oocytes. Other fixatives have been employed successfully, for example DMSO:methanol (1:4) (Dent et al., 1989), but in our experience these result in less preservation of organelle structure and less efficient penetration of the probe through the yolk-filled cytoplasm (data not shown). The sensitivity of *in situ* hybridization is even higher than that of a northern blot, thus it allows the detection of low levels of MRP RNA expression shortly after injection (Jeong, 1997).

In situ hybridization with the mitochondrial 12S rRNA probe showed not only specific staining of the mitochondria, but also developmental stage specific localization of 12S rRNA. The rRNA staining pattern corresponds well to previously known distribution of mitochondria in various stages of oocytes, which was shown by the other method (Mignotte et al., 1987). In stage I oocytes, mitochondrial staining was found in the perinuclear network, and in the cytologically distinct region of the mitochondrial mass as shown in Fig. 1. The perinuclear mitochondrial network has been suggested to be composed of a specific set of mitochondria which is more active in mtDNA replication (Tourte et al., 1984; Mignotte et al., 1987). It was also clearly shown that there was a clear increase in mitochondrial staining at the onset of vitellogenesis and its intensity remained high at later stages of oocyte maturation (Fig. 1 and 2). Such an increase of mitochondrial staining has been expected because it is known that the number of mitochondria is dramatically increased before or at the onset of vitellogenesis (Marinos, 1985). Therefore, it could be concluded that the modification of the *in situ* approach with mitochondria specific rRNA can be utilized to monitor distribution of mitochondria even in cells with large yolk protein contents which would impede the penetration of the probe. It is also interesting to note that the probe can successfully penetrate the double membranes of organelles and hybridize to the RNA component of RNP, such as mitochondrial 12S rRNA. To my know-

ledge, this is the first systematic study of mitochondrial distribution using a mitochondrial specific RNA in oocytes.

To test the applicability of this procedure, MRP RNA probe was also used in this study. RNase MRP (Mitochondrial RNA Processing) is a RNP endoribonuclease that processes mitochondrial RNA (mtRNA) transcripts (Chang and Clayton, 1987; Lee and Clayton, 1997). Although it was originally thought that RNase MRP activity was required to generate the RNA primer for mitochondrial DNA (mtDNA) replication, it was later found that this activity is also required for rRNA processing in the nucleolus (Chang and Clayton, 1987; Karwan et al., 1991; Schmitt and Clayton, 1993). In fact, immunocytochemical detection of a protein component of the RNase MRP and fluorescent RNA cytochemistry of MRP RNA showed a predominant localization of MRP within the nucleoli (Reimer et al., 1988; Jacobson, 1995). Since nucleoli are the sites of ribosome biogenesis, these experiments suggest that RNase MRP might be required for pre-rRNA processing and/or pre-ribosome assembly and transport; in the case of yeast, RNase MRP has been implicated in the late stages of 5.8 S rRNA processing (Schmitt and Clayton, 1993). It is also interesting to note that RNase MRP and tRNA processing RNase P share extensive homology not only in RNA but also in several protein components in both RNP, suggesting extensive evolutionary conservation of RNP (Dichtl and Tollervey, 1997; Chu et al., 1997; Chamberlin et al., 1998).

The gene for MRP RNA has been isolated from *Xenopus laevis* and used in this study to examine the cellular localization of RNase MRP (Davis et al., 1995; Jeong-Yu and Clayton, 1996). Northern analysis shows that MRP RNA is expressed throughout development; the level is relatively high even at early stage I, slightly increases up to stage IV, and reaches a plateau at stage VI. Considering the large differences in size between previtellogenic and fully grown oocytes, the relative level of MRP RNA in stage I oocytes should be much higher than that of fully grown oocytes. *In situ* hybridization results confirm this prediction; nuclear signals with the MRP RNA probe are intense in small previtellogenic oocytes, but less so in the larger ones (Fig. 3). The most prominent and consistent localization of MRP RNA during oogenesis is in the multiple nucleoli, which are one of the most distinctive cytological features of the oocyte nucleus (germinal vesicle). Very early in oogenesis (mostly during the pachytene stage of meiosis), the genes for rRNA are selectively amplified as extrachromosomal copies of DNA and form multiple nucleoli in the periphery of the nucleus (Brown and Dawid, 1968; Bird and Birnstiel, 1971). After the onset of vitellogenesis (predominantly during the lampbrush chromosome stage), amplified ribosomal genes support a high rate of rRNA synthesis in the nucleoli. Recent data suggest that RNase MRP might function in rRNA processing at the late stage of maturation of 5.8S rRNA (Schmitt and Clayton, 1993; Chu et al., 1994; Lygerou, 1996). The above suggestion might be appropriate in later stage oocytes. However, in stage I oocytes, rRNA precursors are not yet highly expressed, and RNase MRP as a rRNA processing entity does not need to be highly localized. In fact, U3 RNA is not expressed until the oocytes reach stage III, when rRNA transcription starts (Caizergues-Ferrer et al., 1991). In the nucleus of early vitellogenic oocytes, MRP RNA is not highly localized to the nucleoli, but is distributed throughout the nucleoplasm (see Fig. 3). Even in stage VI oocytes, where nucleolar localization of MRP RNA is distinct, the nucleoplasm also contains a considerable level of MRP RNA. Thus it remains an open question what MRP RNA does in the nucleoplasm of previtellogenic oocytes; it may be required for other functions aside from rRNA processing, including ribosomal DNA amplification and the communication between the nucleus and mitochondria.

The evidence for involvement of RNase MRP in mitochondrial primer RNA metabolism and DNA replication has come from several biological species and studies of conserved substrate requirements of the enzyme and certain features of the organization of the MRP RNA gene in mammals (Bennett et al., 1992; Lee and Clayton, 1997). Previous works have suggested that replication of mtDNA is especially active in previtellogenic (stage I) and early vitellogenic (stage II through stage IV) oocytes, but is largely absent at later stages (Callen, 1983; Webb and Smith, 1977; Chase and Dawid, 1972). In the active mitochondrial proliferation stages, the distribution of organelles in the cytoplasm is also dramatically changed. In previtellogenic oocytes, the mitochondrial mass is a prominent cytoplasmic entity. Since the size of the mitochondrial mass increases (10 to 70 μm), as does the size of oocytes (50 to 300 μm), it has been suggested that growth of the mitochondrial mass is due to a general increase of mitochondrial components; thus the mitochondrial mass would be a site of mitochondrial biogenesis, especially in stage I oocytes (Callen et al., 1980; Marinos and Billett, 1981). However, a more detailed microscopic analysis of the mitochondrial mass showed that it not only consists of mitochondria, but also contains the precursor of germinal granules. In later oogenesis (from stage II) when the mass is dispersed, the mitochondria follow the localization of germinal granules and become quiescent in mtDNA replication (Haesman et al., 1984). More importantly, the mitochondrial mass is not the only source of mitochondria, but a perinuclear network of mitochondria is also the foci of proliferation in previtellogenic stages. This network continues to grow in later vitellogenic oocytes and contributes to the significant amount of mitochondria in the full grown oocyte (Haesman et al., 1984; Tourte et al., 1984; Mignotte et al., 1987). An autoradiographic study showed that mitochondria

proximal to the nucleus are more active in incorporating nucleotide precursors into mtDNA than those distant from the nucleus (Tourte et al., 1984; Mignotte et al., 1987). This raises the interesting possibility that in the proximity of the nucleus or nuclear materials, which can be exported to the nearby mitochondrial arrays, mitochondrial biogenesis may be regulated in some way. According to this model, mitochondria physically distant from the nucleus might be unable to amplify, considering the large size of growing oocytes. The data presented in this paper show developmental stage specific temporal localization of MRP RNA in the perinuclear network of the mitochonchondria; such mitochondria in previtellogenic and early-vitellogenic oocytes are in the special stage of active mitochondrial replication. In addition to the minor population of MRP RNA in the perinuclear mitochondria, the majority of RNA is in the nucleus throughout development. Such a dual localization of MRP RNA in *Xenopus* oocytes is in good agreement with its multiple activities shown in yeast and mammalian cells.

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