

Translocation of a localized maternal mRNA to the vegetal pole of *Xenopus* oocytes

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A prominent hypothesis in embryology is that localized maternal factors are important in specifying cell fate. There are, however, only a few examples of maternal molecules that have been shown to be localized and very little is known about how such factors are physically localized within an egg (for review see ref. 1). Previously, cDNA clones were obtained for a class of localized maternal mRNAs from *Xenopus laevis*². These mRNAs are unusual in that they are concentrated at either the animal or vegetal pole of unfertilized eggs. In the present study the synthesis and intracellular distribution of one of them, Vg1, has been examined during oogenesis. The results show that Vg1 mRNA is localized as a crescent at the vegetal pole of mature oocytes. Surprisingly, this mRNA is uniformly distributed in the cytoplasm of immature oocytes. These findings suggest that a single cell, the frog oocyte, has some mechanism for translocating specific RNAs like Vg1. The process that moves Vg1 mRNA is evidently a cytoplasmic localization machinery which is not directly coupled to the synthesis of Vg1 RNA.

In a previous study, total RNA was isolated from animal or vegetal pole caps cut from frozen unfertilized eggs. Northern blots of these RNA preparations showed that Vg1 mRNA is 20–25 times more abundant in the vegetal pole or presumptive endoderm of the egg². To determine more precisely where Vg1 RNA is located and to investigate how it comes to be there, *in situ* hybridizations were performed on sectioned oocytes. Oocytes are the ovarian cells which undergo a steroid-induced maturation into eggs. The results show that Vg1 RNA is located in a cortical crescent in the vegetal hemisphere (Fig. 1). This thin (~10 μm) crescent begins just beneath the equator and forms a uniform bowl at the vegetal end. Hybridization to serial sections shows that the crescent of Vg1 RNA localization is seen throughout the radially symmetric oocyte (Fig. 1c) and not only below the nucleus. It is noteworthy that the Vg1 RNA is restricted to a thin subcortical shell and does not spread from the vegetal pole in a gradient. These *in situ* hybridizations strongly suggest that the vegetal placement of Vg1 RNA in unfertilized eggs is a direct consequence of its location in fully grown oocytes.

Several kinds of assays show that most oocyte poly(A)⁺ RNAs are not localized and are uniformly distributed in the cytoplasm. Differential screening of cDNA libraries with probes specific for animal and vegetal pole RNAs and *in vitro* translation of messenger RNAs isolated from animal and vegetal sections both support this conclusion^{2,3}. The typical and even RNA distribution of maternal mRNAs is exemplified by histone H4 mRNA. Histone H4 mRNA, which is at least 20 times more abundant than Vg1 RNA, is found throughout the cytoplasm (Fig. 1d) as was previously shown by Jamrich *et al.*⁴. Shorter exposures show that there is a smooth gradient of histone mRNA from the animal to vegetal pole and that the animal pole has about 2–3 times more H4 mRNA. The shallow animal to vegetal gradient of total poly(A)⁺ RNA may simply reflect the distribution of non-yolky cytoplasm in the oocyte^{5,6}.

To learn how Vg1 RNA is localized it is important to know when during oogenesis the RNA is made and whether it is immediately concentrated at the vegetal pole. If this maternal RNA were always found in a crescent at the vegetal pole of the oocyte, that might suggest that the localization of the RNA is coupled to its synthesis and transport from the nucleus. The

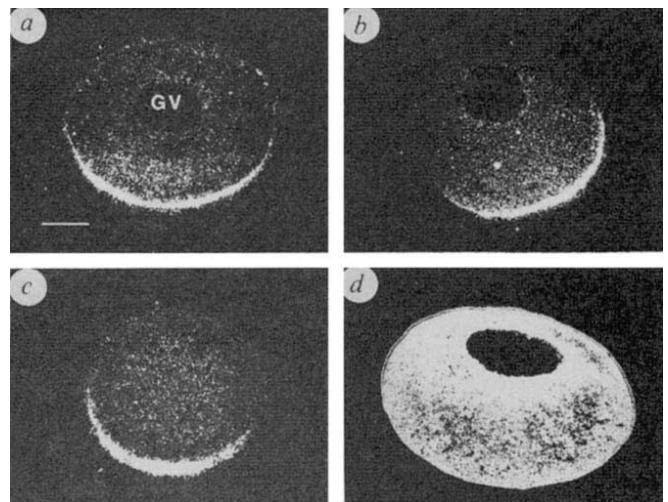


Fig. 1 Localization of Vg1 RNA at the vegetal pole of *Xenopus* oocytes by *in situ* hybridization. Post-vitellogenic (stage V–VI) albino oocytes were fixed in ethanol acetic acid chromium trioxide, dehydrated, embedded in Paraplast and sectioned at 6 μm . Oocytes from albino frogs were used because the pigment granules that mark the animal hemisphere of wild-type oocytes are almost indistinguishable from autoradiograph grains. Following attachment to silane-treated slides, the oocyte sections were treated with proteinase K and prepared for hybridization with single-stranded ³²P-RNA probes (3×10^6 d.p.m. μg^{-1} ; $0.3 \text{ ng } \mu\text{l}^{-1}$) as described elsewhere²⁰. After washing and digestion with RNase A, the slides were dipped in Kodak NTB-2 emulsion, exposed for 3–7 days, developed, stained with Giemsa and photographed under darkfield optics. In each photo the animal pole is at or near the top. The nucleus or germinal vesicle (GV) is seen as a circle nearer the animal pole, except in c where the section is taken at a position past the nucleus. The hybridization probe for a–c was ³²P-labelled antisense Vg1 RNA of 2.3 kilobases (kb), and for d was ³²P-labelled antisense histone H4 RNA (0.4 kb). Controls with sense Vg1 probes do not show any autoradiograph signal above background, nor do sections that are treated with RNase before hybridization with Vg1 antisense RNA probes. All photographs were taken at the same magnification and the scale bar in a is 200 μm .

time during oogenesis at which Vg1 RNA is synthesized was assayed by RNase protection using total RNA extracted from oocytes ranging from small pre-vitellogenic to fully grown, post-vitellogenic oocytes. The results (Fig. 2) show that Vg1 RNA is present in the smallest oocytes tested (stage 1) and that the level of Vg1 RNA remains constant throughout oogenesis. The assay for Vg1 RNA levels was performed five separate times on oocytes from five different females and together these data allow one to conclude that the total amount of Vg1 RNA does not change by more than a factor of two from stage-II to stage-VI oocytes. Although a continual synthesis and degradation of the RNA cannot be ruled out, the simplest interpretation is that all the Vg1 RNA is made early (by stage II) in pre-vitellogenic oocytes and this store of maternal RNA remains in the growing oocyte and is inherited by the egg.

The location of Vg1 RNA in young oocytes is revealed by the *in situ* hybridizations in Fig. 3. The results show that Vg1 RNA is uniformly distributed in the cytoplasm of young pre-vitellogenic (stage-I and stage-II) oocytes. These young oocytes cannot be oriented for sectioning because there is no morphological sign of the future animal or vegetal pole. However, in no case out of more than 50 oocytes tested was any localization of the autoradiographic signal observed. Thus, when the Vg1 RNA is first synthesized, early in oogenesis, the RNA is evenly spread throughout the cytoplasm and shows no sign of localization.

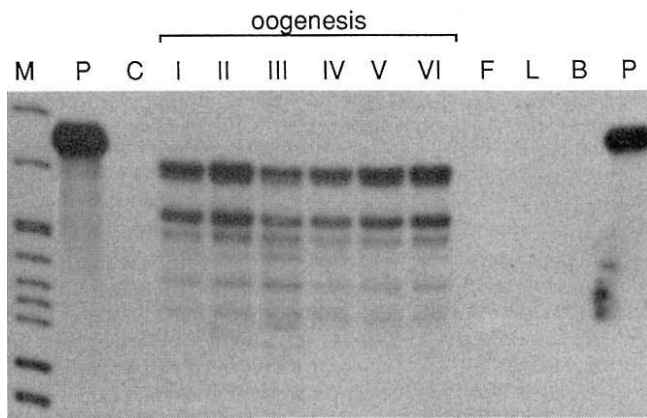


Fig. 2 Vg1 RNA is synthesized in young pre-vitellogenic oocytes. Total RNA was extracted from various stages (stages I-VI as in ref. 21) of oocyte development, pre-vitellogenic to fully grown post-vitellogenic oocytes. Because of the difference in the amount of yolk present at these different stages, RNA recovery was monitored by mixing in ^{32}P -labelled synthetic globin RNA with the oocyte homogenates. The ^{32}P label recovered was used to normalize the amount of total RNA used for the RNase protection assay. RNase protection assays²² were performed with one oocyte-equivalent of RNA. Total RNA from the follicles (F) surrounding 100 stage-IV to stage-VI oocytes and a comparable amount of total RNA from liver (L) or blood (B) were tested. Each RNA sample was hybridized to a uniformly ^{32}P -labelled antisense Vg1 RNA synthesized with SP6 RNA polymerase from *Dde*I-cut pSP65-Vg1 DNA². Following hybridization and digestion with RNase A and RNase T1, the products were fractionated in an 8 M urea 6% polyacrylamide gel. Lane P is full-length probe (370 nucleotides) carried through the entire hybridization procedure, but not digested with RNases. Lane C is the probe digested with RNases following hybridization to tRNA. The antisense probe is protected from RNase digestion by Vg1 transcripts to give two major bands as shown previously². Markers (M) are *Hpa*II-cut pBR322 DNA.

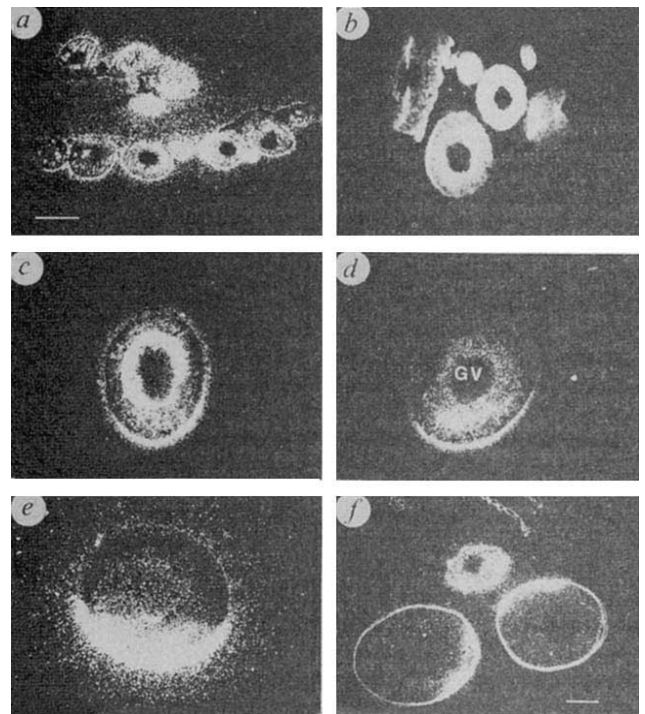


Fig. 3 Translocation of Vg1 RNA during oogenesis. *In situ* hybridizations were performed with an antisense Vg1 RNA probe as described in Fig. 1 legend. Albino oocytes at various stages of growth are shown. The black hole present in the centre of most sections is the nucleus or germinal vesicle (GV). The stages according to Dumont²¹ are I, II, III, III and IV for a-e, respectively. The scale bar in a is 200 μm and applies to a-e. A stage-II and two stage-IV oocytes from a wild-type female are shown in f, where the scale bar is 200 μm . Note that the white ring around the pigmented oocytes is due to the pigment granules in the cortex.

As oocytes grow they take up vitellogenin by pinocytosis from the blood and store derivatives of this protein in yolk platelets⁷. It is during this period (vitellogenesis) that the Vg1 RNA becomes localized. Figure 3c-e shows the beginning of Vg1 RNA localization. The first sign of localization is the concentration of Vg1 RNA near the cortex at one end of the oocyte. In some cases (Fig. 3c) the RNA seems to concentrate around the nucleus as well. In other cases, however, this perinuclear concentration is not observed (Fig. 3d and e). In contrast to Vg1 RNA and as observed in fully grown oocytes (Fig. 1) histone H4 mRNA is uniformly distributed at all these early oocyte stages. These data with histone H4 probes (not shown) confirm studies⁴ which demonstrated the even cytoplasmic distribution of histone RNA during early oogenesis.

The albino oocytes used for the *in situ* hybridizations in Fig. 3a-e lack cortical pigment granules. In wild-type oocytes, cortical pigment granules first appear over the entire surface (at stage III) and are later concentrated at the animal hemisphere (stage IV). Thus, in addition to cell diameter, the appearance of cortical pigment helps mark the stage of oocyte development. The hybridizations in Fig. 3f were performed with pigmented (wild-type) oocytes and these sections highlight the difference in Vg1 distribution between early and later stages in oogenesis. By the time cortical pigment granules have appeared (the white ring on the perimeter of larger oocytes in Fig. 3f) the Vg1 RNA signal is localized at one end.

At a superficial level some aspects of Vg1 RNA synthesis and localization parallel the biogenesis of yolk platelets⁸. Both are initially found throughout the cytoplasm and later form a gradient along the animal-vegetal axis. There are however,

several important differences. Vg1 RNA is synthesized before vitellogenesis begins and the localization of Vg1 RNA is nearly complete at a time when the yolk platelets are still of a uniform size and radially distributed in the cytoplasm (see Fig. 3e and ref. 8). In addition, the final gradient of yolk in the oocyte is not nearly as steep as the quantitative localization of Vg1 RNA. Nevertheless, it is not unreasonable to ask whether Vg1 RNA is imported into oocytes as is vitellogenin. A sensitive test for Vg1 RNA in the follicle, liver and blood (the latter tissues being sites for vitellogenin synthesis and transport, respectively) proved negative (Fig. 2). A layer of follicle cells envelopes every oocyte until maturation at which time the oocyte is released from the follicular layers. In these assays (Fig. 2) RNA from the follicle cells surrounding 100 oocytes and an equal amount of RNA from liver or blood is compared to the RNA found in just one oocyte. The results clearly show that there is at least 100 times more Vg1 RNA in the oocyte than in the other tissues tested. Although the possibility that follicle cells or other tissues synthesize Vg1 RNA and quickly transport it into the oocyte cannot be absolutely ruled out, the results strongly suggest that Vg1 RNA is synthesized by the oocyte itself.

The results of the *in situ* hybridizations (Fig. 3) show that the Vg1 RNA is first found throughout the cytoplasm and is subsequently localized at one end. To test whether this change in location corresponds to any substantial change in the structure of Vg1 RNA, Northern blots containing RNAs isolated from oocytes before, during, and after the localization process were probed for Vg1 sequences. The results (Fig. 4) show that Vg1 RNA is in the form of a mature (spliced) polyadenylated message at all oocyte stages. There is a trace of unadenylated

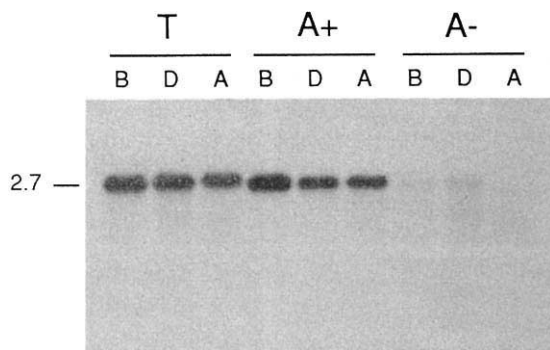


Fig. 4 Mature Vg1 mRNA is present at all stages of oogenesis. RNA was extracted from oocytes before (B), during (D), and after (A) the localization of Vg1 RNA. Using the *in situ* hybridizations shown in Figs 1 and 3 as a guide, this required RNA from stage I-II, stage III-IV and stage V-VI oocytes, respectively. Total RNA (T) was fractionated into poly(A)⁺ and poly(A)⁻ fractions by oligo-(dT)-cellulose chromatography. The RNA samples were electrophoresed in formaldehyde-agarose gel, blotted to GeneScreen, and hybridized with ³²P-labelled antisense Vg1 RNA as described previously². Unpublished data, including the DNA sequence of a Vg1 cDNAs, shows that the gene that encodes Vg1 is interrupted by introns and that the mature spliced Vg1 mRNA is about 2.7 kb long.

Vg1 RNA at all stages, though this could be a result of inefficient binding to oligo(dT)-cellulose. It can therefore be concluded that the localization of Vg1 mRNA does not involve a significant change in the size or structure of Vg1 transcripts.

The data presented here suggest two possible mechanisms for the localization of Vg1 RNA. One is that Vg1 RNA is specifically degraded (by a localized RNase activity) in the animal pole and most of the vegetal end of the oocyte. However, because the total amount of Vg1 RNA levels remains relatively constant during oogenesis (Fig. 2) such a localization mechanism would require a coordinated synthesis and degradation of Vg1 transcripts in different regions of late-stage oocytes. A more likely explanation is that all the Vg1 RNA is translocated by an active process into a crescent at the vegetal end of the oocyte. Although the rate of this localization process cannot be measured from the *in situ* hybridizations, the results suggest that the movement of the Vg1 RNA takes place over many days, perhaps even a few weeks. The growth of a stage-II to a stage-IV oocyte, the period during which Vg1 RNA is localized, can take from two to four weeks *in vitro*⁹.

With respect to the function of the Vg1 RNA and the protein it encodes, the data show that Vg1 mRNA is not distributed like the germinal granules associated with germ cell formation. Germinal granules are closely associated with the mitochondrial cloud and do not extend up to the oocyte equator as does Vg1 RNA (ref. 10). It remains to be determined when Vg1 RNA is translated and whether its protein product is also localized. Nonetheless, the extent of the subcortical distribution of Vg1 RNA (Fig. 1) suggests that the Vg1 protein may be involved in a developmental process common to all vegetal cells of the developing embryo. Two obvious possibilities are that the Vg1 protein is involved in the specification of vegetal cells as endoderm or it may be involved in the induction of mesoderm by endoderm¹¹. Alternatively, a regional activation of the Vg1 gene product could be involved in polarizing the dorsal-ventral axis¹².

It is not known how the animal-vegetal axis is established in an oocyte and the mechanism by which Vg1 RNA is localized remains a puzzle. Wylie and his colleagues¹⁰ noted that the mitochondrial cloud lies on what will become the vegetal pole side of the nucleus in pre-vitellogenic oocytes and the alignment

of a centriole pair and the nucleus¹³ might also mark the beginnings of the animal-vegetal axis. It is possible that the relative positions of the nucleus and mitochondrial cloud set up the animal-vegetal axis and other cellular components or processes might be aligned to this initial polarity. For example, cytoskeletal elements polymerized along the animal-vegetal axis might have an intrinsic polarity which is in turn used to localize Vg1 RNA, yolk platelets⁶, and so on. In any case, it is reasonable to assume that the translocation of Vg1 RNA involves interaction with cytoplasmic proteins and it may be possible to identify these components of the localization machinery by using Vg1 RNA as a probe. The oocyte localization process itself warrants further investigation because it may shed light on how other cells localize RNAs¹⁴⁻¹⁹ and how eggs localize developmental signals.

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The human tumour-associated epithelial mucins are coded by an expressed hypervariable gene locus *PUM*

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A single highly-polymorphic autosomal gene locus *PUM* codes for a family of mucin-type glycoproteins, separable by SDS-gel electrophoresis, which we first identified in human urine^{1,2}. The locus also codes for glycoproteins which are abundant in several other normal epithelial tissues and body fluids, including milk, and in tumours of epithelial origin³. These mucin-type glycoproteins seem to be very immunogenic in rodents and, in a search for epithelial specific or tumour-associated antigens, a large number of related antibodies have been isolated⁴⁻⁹ which bind to the *PUM*-coded mucins³. Many of the antibodies show a pronounced tumour specificity on immunohistology¹⁰⁻¹³ and are being used