Review–Hypothesis

Mobilization of maternal mRNA in amphibian eggs with special reference to the possible role of membraneous supramolecular structures

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Current knowledge of the mechanism of mobilization of maternal mRNA is summarized herein and a working hypothesis has been constructed to explain the mechanism on the assumption that the mRNA enters the cytoplasm in association with the cytoplasmic membraneous structures and is then stored in the structures until liberation and relocation at the step of oocyte maturation. An extensive turnover of poly(A) sequences as well as the occurrence of repetitive sequences in the maternal mRNA may be relevant to mRNA activation.

Maternal mRNA *Poly(A)+RNA* *Oocyte maturation* *Repetitive sequences* *Annulate lamellae* *Microinjection*

1. INTRODUCTION

Maternal mRNA is the storage form of mRNA which accumulates during oogenesis within the cytoplasm and which is required for early embryonic development [12,14]. In amphibian embryos, the activation of the translation of maternal mRNA takes place at the stage of oocyte maturation and is induced by a gonadotropic hormone. Thus, the rate of the synthesis of proteins, in particular histones, is accelerated at maturation.

This article is devoted mainly to factors related to activation of maternal mRNA in amphibian eggs at the stage of oocyte maturation. The importance of the membraneous supramolecular structures within the cytoplasm will be focussed on, and translational-related factors such as ribosomes, tRNAs, messages, or soluble regulative factors will be given attention to.

2. TRANSLATIONAL EFFICIENCY OF PROTEIN SYNTHETIC FACTORS DOES NOT APPEAR TO INCREASE AT OOCYTE MATURATION

Historically, studies on the mechanism of the activation of maternal mRNA in fertilized eggs began using sea urchin eggs. Two theories have been put forward: One is the alteration of protein synthetic factors and the other concerns structural changes in mRNAs. In the case of the sea urchin, it was found [20] that the efficiency of translation, defined as the number of proteins produced/mRNA molecule/unit time, did not change after fertilization. Thus, a plausible explanation for the acceleration of protein synthesis at fertilization is the translation of additional mRNA molecules, rather than an increase in the translational efficiency of protein synthetic factors or ribosomes.

In *Xenopus* embryos, it was shown [36] that both polysomal size and ribosomal transit time on mRNA do not change appreciably after oocyte maturation. It has been shown [47] that sea urchin

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histone mRNA which was microinjected into *Xenopus* oocytes or fertilized eggs, was translated and degraded in a similar manner. These authors stated that if the machinery for protein synthesis itself is in some way limiting in oocytes, but not in eggs, the translation of the injected histone mRNA would be much higher in the eggs, since histone synthesis is activated about 50-times in normal maturation processes [46]. Thus, it may be deduced that it is the availability of mRNA that is limiting, also in amphibian eggs.

3. POLYADENYLATION OR CAPPING OF mRNA MOLECULES DOES NOT APPEAR TO BE THE CAUSE OF mRNA ACTIVATION

In the sea urchin, the amount of poly(A) sequences increased about 2-fold shortly after fertilization, and this was expected to relate to the increase in the amount of available mRNA [43]. The experiments in [30] with an analogue, 3′-deoxyadenosine (cordycepin), however, ruled out this possibility, since the drug did not inhibit increases in protein synthetic activity, although it did suppress the increase in poly(A) content. Therefore, it was assumed that capping of mRNA might be insufficient in the unfertilized sea urchin eggs. However, it was shown that cap structures are already present in most of the maternal mRNA [35]. In [28] the size and sequence homology of maternal and embryonic mRNAs for all five classes of histones were compared and no changes were found after fertilization. Thus, the possibility of mRNA activation through alteration in molecular structures was put in doubt.

4. MESSAGE MICROINJECTION EXPERIMENTS REVEAL THAT EFFECTIVE CHANGES MAY OCCUR BEFORE MESSAGES CONTACT PROTEIN SYNTHETIC FACTORS

Message microinjection experiments provided important clues. First, [25], rabbit globin mRNA was injected into *Xenopus* oocytes and it was found that the exogenous mRNA is translated at the expense of the translation of endogenous mRNA. Some message-non-specific component seemed to be required for each mRNA to be translated. In [29] the authors also injected oocytes with rabbit globin mRNA and compared the size of globin-synthesizing polysomes at the subsaturating (5 ng) and oversaturating (40 ng) level of the injected mRNA. They found that the size remained unaltered. These authors interpreted the results in the following way. If the process for the initiation of the formation of translational complex is limiting, the oversaturating level of mRNA would reduce the chance of the binding of ribosomes to mRNA and result in the formation of smaller polysomes. If elongation and/or termination is limiting, the oversaturating level of mRNA would result in increases in the length of time of ribosomal stay on each mRNA molecule, and increases in size of the polysomes would follow as a matter of course. Since neither of these events occurred, they considered that the limiting step takes place before binding of the ribosome to mRNA [29]. The authors postulated the occurrence of a recruitment or a stabilization factor which provides mRNA with the opportunity for translation.

A *Xenopus* oocyte contains about 40–80 ng of maternal poly(A)$^+$ RNA [11,17,39,40]. The amount of the mRNA injected in the above experiment was comparable to the content of existing poly(A)$^+$ RNA. During oocyte maturation only a small percentage of the total maternal mRNA is translated [37] and the majority of the injected histone mRNA is translated, and decays in a relatively short time (the half-life was 3 h). Thus, while all the injected mRNA does reach the protein synthetic machinery, the majority of maternal mRNA does not; rather, the maternal mRNA appears to be sequestered in a cellular compartment. In [3] the efficiency of translation of microinjected mRNAs of several different origins was studied and the possibility that maternal mRNA may be sequestered within the cytoplasm was pointed out.

5. MATERNAL mRNA MAY BE LOCALIZED IN THE OOCYTE CYTOPLASM

Recent advances in the field of cytoplasmic localization of maternally inherited poly(A)$^+$ RNA within the oocytes and eggs are significant. In [7] it was shown, by in situ hybridization of [^{3}H]poly(U), that poly(A)$^+$ RNA in full-grown oocytes is mainly localized in the region of the subcortical area at the vegetal pole. A similar localization of

maternally inherited poly(A)$^+$ RNA has also been demonstrated in eggs of the insect *Oncopeltus fasciatus* [6] and an ascidian *Styela partita* [23]. Here, the question arises as to the reality of the localization. It may be argued that the poly(A)$^+$ RNA might not be completely conserved during fixation and embedding. However, the poly(A)$^+$ RNA in the conserved area is stably bound to cytoplasmic structures. These authors also showed that in *Xenopus* embryos shortly after fertilization, poly(A)$^+$ RNA is distributed uniformly within the cytoplasm. Thus, the localization observed in oocytes may not be just an artifact. According to [7] the distribution of maternal mRNA appears to change completely. This indicates that mRNA relocates from the place of sequestration to the area of its physiological requirement during maturation.

Our own assessment of changes in the amount of poly(A)$^+$ RNA in the particulate and soluble fractions of oocytes revealed that about 50% of the total oocyte poly(A)$^+$ RNA contained in the extranuclear particulate fraction and sedimentable at 10000 rpm for 10 min disappears at the stage of oocyte maturation [33]. In [44] it was shown that poly(A)$^+$ RNA shifts its location from the particulate to the soluble fraction with resumption of development in *Artemia salina* embryos. These results favor the idea of mRNA relocation at the stage of activation.

6. THE POSSIBLE INVOLVEMENT OF MEMBRANES IN mRNA TRANSPORT AND THE RELEVANCE TO MATERNAL mRNA

The mechanism of nuclear-cytoplasmic transport of mRNA will now be given attention. Gene expression is now better understood and clear ideas have emerged on the processes involved in transcription and translation. However, little is known of mRNA transport, the process connecting these two. This point has been discussed in [2,13].

In [42] it was proposed that mRNAs are transported from the nucleus to the cytoplasm, not in the form of free particles, but rather in some association with the membraneous structures connecting intranuclear and cytoplasmic membrane systems. Since little is known of the process, the proposal that outer and inner nuclear membrane systems might be involved in the transport of mRNA remains questionable.

However, the idea may be worth considering here with regard to mechanisms related to mRNA sequestration, since a number of investigators have reported the occurrence of direct binding of mRNA to cytoplasmic membranes [8,31,32]. The most pertinent for the present discussion is the discovery [32] that poly(A)$^+$ RNA, which is stocked in encysted *Artemia* embryos in dehydrated state, is in membraneous structures. Thus, it seems feasible that maternal mRNA is carried on some membraneous structures from the nucleus to the cytoplasm.

7. MATERNAL mRNA MIGHT BE CONSIDERED IN THE SAME LIGHT AS NUCLEAR PRE-mRNA

Maternal mRNA in *Xenopus laevis* embryos [1] as well as in sea urchin [9] contains repetitious sequences. In [9] it was found that about 70% of the egg poly(A)$^+$ RNA in sea urchin contains interspersed repetitive sequence elements and other studies from the same laboratory [19,41] showed the absence of such sequences in polysomal mRNA. Since repetitious sequences are rather characteristic of nuclear pre-mRNA, and since maternal mRNA contains repetitious sequences, the latter seems to be a special form of mRNA that has to be processed within the cytoplasm.

A discussion of unprocessed or incompletely processed mRNA within the cytoplasm may be odd in the light of previous studies. However, if we assume that maternal mRNA is still in the process of transport and that the transport is somehow coupled to the processing, the occurrence within the maternal mRNA of the structures resembling nuclear pre-mRNA would not be surprising. Thus it was suggested [1] that the cytoplasmic processing of mRNA precursor could be either a qualitative or quantitative determinant of mRNA prevalence.

8. NATURE OF THE STRUCTURES THAT MAY CARRY AND SEQUESTER MATERNAL mRNA

Concerning the putative cytoplasmic anchorage structures that carry and sequester maternal mRNA, the discussion in [7] is of interest. These

investigators postulated that mRNA may be carried to the area of sequestration via membraneous structures. One candidate was the mitochondria-associated endoplasmic reticulum. However, this was ruled out because there was no evidence of [^{3}H]poly(U) grains in autoradiographs of the mitochondrial region. They stated that more probable candidates of the mRNA vehicle may be pitted membranes or annulate lamellae, as in [4,45]. The idea that annulate lamellae serve as mRNA carriers is compatible with the idea that mRNA may be transported from the nucleus to the cytoplasm via membraneous structures [42].

In the case of *Rana pipiens*, ultrastructural changes of the annulate lamellae in maturing oocytes were examined [24]. They found that when maturation occurs in vitro, as induced by progesterone, annulate lamellae are remarkably deformed and eventually dispersed into the cytoplasm in the form of numerous small vesicles. Imoh [22] also observed such a drastic disappearance of annulate lamellae along with the oocyte maturation in the newt. In [18] it was quite clearly shown that annulate lamellae are formed at the outside of the nuclear membranes and separated into the cytoplasm. According to these authors, the structures are occasionally connected to endoplasmic reticulum within the cytoplasm [18].

Thus, it is attractive to assume that the putative mRNA carrier might be annulate lamellae. This means that maternal mRNA might be sequestered in some way to the annulate lamellae. Since maternal mRNA is expected to be localized in oocytes, as shown in [7], annulate lamellae might somehow be connected to cytoplasmic structures such as the cytoskeleton in [26].

9. AN INTERPRETATION OF THE TURNOVER OF POLY(A) SEQUENCES IN THE CONTEXT OF THE PRESENT ASSUMPTIONS OF mRNA MOBILIZATION

There is still no consistent explanation for the role of poly(A) sequences in mRNA [21]. Thus, in sea urchin eggs, addition of poly(A) stretches to mRNAs is probably not essential for mRNA mobilization, since cordycepin did not inhibit the activation of protein synthesis [30].

In *X. laevis* oocytes, a precipitous decrease (almost 50%) of poly(A) content occurred during maturation [11,39,40]. The change did not accompany changes in the amount and quality of mRNAs. In [5], no difference in the in vitro translation products of poly(A)$^+$ RNA before and after the maturation was found. However, the change was accompanied by lengthening of poly(A) sequences from shorter oocyte-type (~60 nucleotides) to longer embryo-type (over 100 nucleotides), as well as by extensive incorporation of adenosine into poly(A) sequences.

This type of extensive poly(A) turnover is not restricted to amphibians: it occurs in sea urchin [16], in mouse embryos [34], in cultured cells of stable cell lines [15] and even in *Xenopus* oocytes not exposed to progesterone, although at a much reduced rate [39].

In amphibians, it has been shown that while oocyte histone mRNAs are largely polyadenylated [27], embryonic histone mRNAs are not [38]. However, since the amount of histone mRNA assumed to be functioning in the early embryos is only about 200 pg [47], elimination of poly(A) from histone mRNAs explains only a small portion of the change. (An oocyte contains 40–80 ng of poly(A)$^+$ RNA, about half of which appears to lose poly(A) sequences [11,39,40].)

Thus, an explanation for the poly(A) turnover remains obscure. Since mRNA may change its location during maturation, poly(A) sequences may be involved in the sequestration and/or relocation of mRNA.

10. A WORKING HYPOTHESIS

Fig.1 shows a working hypothesis concerning the mechanism of storage and mobilization of maternal mRNA at the step of oocyte maturation. This thesis is all-inclusive of reported data. The main points are:

(i) Maternal mRNA is transported in association with cytoplasmic membraneous carrier structures;
(ii) mRNA is bound to the membraneous structures and is sequestered here and separated from the protein synthetic machinery;
(iii) The mRNA might not be completely processed; e.g., it contains repetitious sequences which appear unnecessary for translation;

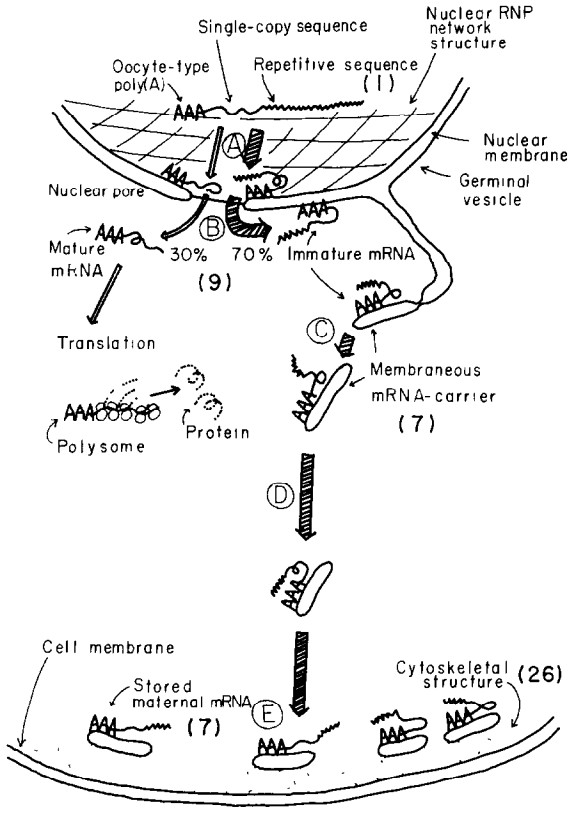

Fig.1. A working hypothesis which may explain synthesis, transport and sequestration of maternal mRNA during oogenesis. (A) Long hnRNA is synthesized and then partially or completely processed within the nucleus. In about 70% of the hnRNA repetitive sequences are partially eliminated, whereas in 30% of the hnRNA, they are completely eliminated. The separation of mRNA into 30% *vs* 70% is based on the results in [9]; (B) While completely processed, mRNA is transported to the cytoplasm to be immediately translated; incompletely processed mRNA is bound to nuclear outer membranes; (C) mRNA to be stored is fixed on membraneous structures, which serve as mRNA carriers to the place, where mRNA is sequestered. Thus, nuclear outer membranes give rise to carrier structures, possibly annulate lamellae; (E) mRNAs are sequestered in the form of the membrane–mRNA complex, possibly connected through poly(A) sequences. (Numbers in parentheses correspond to references.)

(iv) Poly(A) sequences might be involved in the fixation of mRNA in the area of sequestration.

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