THE BIOSYNTHESIS OF BIOLOGICALLY ACTIVE PROTEINS IN mRNA-MICROINJECTED *XENOPUS* OOCYTES

Author: Hermona Soreq
Department of Neurobiology
Weizmann Institute of Science
Rehovot, Israel

Referee: G. Huez
Department of Biochemistry and Laboratory of Biological Chemistry
University of Brussels
Brussels, Belgium

I. INTRODUCTION

How is the production of biologically active proteins from their specific mRNA species controlled and which processes determine the distribution and the fate of such proteins within the living cell? A prerequisite to approaching these important questions in cell biology is the availability of an assay system in which the authentic protein biosynthetic processes can be quantified. One of the most popular approaches to this issue has been to microinject foreign mRNA into living cells, where the fate of both the injected mRNA and of its encoded protein product may be followed. One of the first and still most widely used types of living cell that serves these purposes is the fully grown oocyte of the South African clawed toad, *Xenopus laevis*.

The value of *Xenopus* oocytes as living "test tubes" for protein synthesis processes first became apparent in 1971 when Gurdon and co-workers discovered that the oocytes constitute an extraordinarily efficient system for translating foreign messenger RNA.[1-3]

At the same time Graessmann demonstrated the translation of mRNA injected into somatic cells.[4] However, the size of *Xenopus* oocytes makes oocyte microinjection experiments much easier. This may be the reason for the wide use of this system.[1-3] Since 1971 microinjected *Xenopus* oocytes have been employed as a translation system for a large variety of mRNAs, from mammals, birds, fishes, insects, viruses, and plants. Numerous comprehensive reviews have been written in which the technical approach[5-7] and the observations made using it[8-14] were covered. The present review is confined to a summary of a relatively recent aspect of this system: namely, the use of mRNA-microinjected *Xenopus* oocytes to study the biosynthesis of biologically active proteins.

Within a living cell, the production of an active protein involves a complex chain of events. First, the translationally active mRNA molecules have to interact with other cellular constituents to form polysomes. In addition specific signals have to be read in order to enable initiation, elongation, and termination of polypeptide chains. Nascent polypeptides are often subjected to various post-translational events, such as cleavage of specific peptide domains, various chemical modifications of distinct amino acids, and assembly into multisubunit structures. Some of these post-translational processes are concomitant with, and necessary for, the transfer of the nascent protein from one site to another within the cell. The biological activity of the resulting mature protein, therefore, depends, in many cases on the availability of all of the different cellular constituents that take part in these processes. Finally, when the mRNA is transferred to a heterologous cell type, a further requirement is apparent, namely, that some rep-

resentatives of each essential component would be interchangeable with no species and/or cell type specificity.

When all of the above requirements for the assay system are fulfilled and specific proteins can be synthesized, two types of biological questions may be studied. First, the fate of a polypeptide within a foreign cellular milieu can be examined with respect to localization of the finished protein as well as the acquisition of biological functions; such studies complement experiments performed in vivo. The *Xenopus* oocyte is a cell specialized for the production and storage of proteins for later use during embryogenesis.[15,16] It satisfies the above requirements; in addition, the complex architecture of the frog oocyte includes the subcellular systems involved in the export and import of proteins.[13,17,18] The mRNA-microinjected oocyte of the frog is, therefore, an appropriate system in which to study the synthesis of specific polypeptides, as well as the storage of particular proteins in various subcellular organelles, and the export of others into the extracellular space. Moreover the subcellular compartmentation, as well as the structure and/or biochemical, physiological, and biological properties of the synthesized protein, may be examined in the injected oocyte.

The second type of questions that may be posed using mRNA-microinjected oocytes concerns the biochemical processes leading to the production of biologically active proteins. In these studies, the injected foreign substance (whether it is mRNA, protein, or another molecule) serves as a probe, and the experiments are aimed to reveal the nature and specificity of the biological pathways and, in particular, the control mechanisms involved in the functioning of this probe within the living cell. Thus, the system has two basic uses: (1) as an assay — to measure the presence or amount of a substance and (2) as a system that can be "probed" by injecting specific macromolecules.

The interpretation of both types of experiments largely depends on the properties of the injected cell, the fully grown *Xenopus* oocyte, on the extent to which the injection of substances perturbs the natural state of the oocyte, on the efficacy of the biosynthetic processes involved, and on the specificity and sensitivity of the detection methods employed. In the following, the use of mRNA-injected oocytes to study the production of biologically active proteins will be discussed in view of these points. Particular attention will be paid to the interaction between the oocyte and the injected mRNA and its protein product, as it affects the biosynthesis and/or detection of biologically active proteins.

II. BIOLOGICALLY ACTIVE FOREIGN PROTEINS DETECTED IN mRNA-MICROINJECTED OOCYTES

When a protein is referred to as "biologically active", it generally means that it is capable of functioning in the same way it does in vivo, in its homologous milieu. It could take part in, or initiate, a measurable biochemical reaction. Alternatively, it could contribute to other specific types of biological activity when expressed in the test assay system. Therefore, determination of the levels of biologically active proteins as produced in mRNA-microinjected *Xenopus* oocytes takes many different forms. Several proteins with various enzymatic activities have been shown to be produced in mRNA-injected oocytes. Other active proteins were detected by their ability to bind labeled ligands. Yet, others were shown to confer changes on the biological properties of the injected oocytes, or on those of other test cells. Table 1 lists some examples for the detection of oocyte-produced active proteins. All of the activities measured reflect quite faithfully the in vivo properties of the studied proteins. Moreover, in several cases, the measurements of biological activities were much more sensitive than the direct detection of newly synthesized polypeptides by amino acid labeling. In other cases, the protein was identified only by its biological activity, and the measurement

Table 1
BIOLOGICALLY ACTIVE PROTEINS PRODUCED IN *XENOPUS* OOCYTES UNDER THE DIRECTION OF FOREIGN mRNA

Protein	Source of messenger RNA expressed in the oocytes	Bioassay	Ref.
Globin	Rabbit reticulocytes	Column chromatography	1,3,4
		Binding of metal porphyrin	13
Interferon	Human fibroblasts	Inhibition of viral infection	23
Beta-glucuronidase	Mouse kidney	Enzyme activity	24
Vitellogenin	*Xenopus* liver	Uptake by oocytes	13
Immunoglobulin	Rat spleen	Antigen binding	25
Thymidine kinase	Herpes virus (DNA)	Enzyme assay	26,27
Cytochrome P-450	Rat liver	Enzyme assay (deethylase activity)	28
Plasminogen activator	Human carcinoma	Enzyme assay	19
	Rat brain	Casein overlay	22
	Rat muscle	Proteolysis in PAA gel	21
Acetylcholinesterase	*Torpedo* electric organ	Enzyme assay	29,38
	Rat brain	Enzyme assay	38,40
	Human brain	Enzyme assay	30
	Ciona intestinalis	Immunoprecipitation	31
Nicotinic acetylcholine receptor	*Torpedo* electric organ	Alphabungarotoxin binding	32
		Electrical stimulation by ACh	50
Erythropoietin	Human kidney	Radioimmunoassay	33
Pro-EGF	Mouse salivary gland	Immunoprecipitation	67
		Binding to A-431 cells	34
2′-5′ Oligoadenylate synthetase	Human fibroblasts	Enzyme activity	35,36
Herpes simplex alkaline exonuclease	Mouse L cells	Enzyme activity	37
	Plasmid DNA	Northern blot hybridization	
Interleukin	Gibbon lymphocytes	Thymidine incorporation	65

of the oocyte-produced active protein was the only way by which the corresponding mRNA could be detected and identified. Altogether, these experiments show that the oocytes, as compared with in vitro translation systems, are well equipped for an extraordinarily efficient production of numerous active proteins.

A. Detection of Oocyte-Produced Enzymatic Activities

The biochemical conversion of substrates provides sensitive ways to detect minute amounts of a newly synthesized enzyme and numerous proteins with authentic enzymatic activities have, indeed, been shown to be produced in *Xenopus* oocytes from foreign mRNAs. These include cytoplasmic and lysosomal enzymes, integral membrane enzymes, and secreted enzymes. The following section covers several examples of foreign mRNA-directed enzymes produced in microinjected oocytes.

1. Mouse Kidney Lysosomal Beta-Glucuronidase

The synthesis of catalytically active mouse beta-glucuronidase (beta-D-glucuronide glucuronosohydrolase, EC 3.2.1.31) in *Xenopus* oocytes microinjected with mouse kidney polyadenylated RNA was the first case in which the production of a foreign active enzyme in mRNA-microinjected oocytes has been reported. In this pioneering work, Labarca and Paigen[24] calculated that most of the mRNA-directed enzyme molecules produced in the oocytes were catalytically active. Glucuronidase was detected by fluorometric measurement of substrate hydrolysis catalyzed by the newly synthesized enzyme. This assay was 100-fold more sensitive than detection of this protein by incorporation of isotopically labeled amino acids. The mammalian mRNA-directed enzyme

produced in the oocytes was much more heat-stable than the endogenous amphibian enzyme, as could have been expected from the protein of a warm-blooded animal. It could be purified by affinity chromatography, using resin-bound antibodies. Gel-electrophoresis under nondenaturing conditions revealed that the purified oocyte-produced enzyme is a multimeric protein, with two different forms. Both of these appeared to migrate slower than the natural mouse lysosomal enzyme. This difference could result from inaccurate glycosylation, defective post-translational processing, or (as discussed in detail in the following sections) assembly of subunits of the oocyte endogenous enzyme with those directed by the injected mRNA.

2. mRNA-Directed Synthesis and Secretion of Mammalian Plasminogen Activators

The development of bioassays for the faithful detection of biologically active enzymes as produced from their specific mRNA species is particularly important when several different proteins display the examined enzymatic activity. Plasminogen activators (PA) are an example for such a family of enzymes. These are highly specific serine proteases that convert the inactive zymogen, plasminogen, into the active protease plasmin. PAs are glycoproteins, found as both secreted and membrane-bound enzymes, which exhibit heterogeneity of molecular weights and immunological properties both among and within species. Biochemical characterization of PA has been difficult, as there are very low amounts of these heterologous enzymes even in relatively rich sources. However, catalytic activity of PA, as measured via the formation of active plasmin, can be measured by a very sensitive assay. The high sensitivity of PA detection was exploited to develop a bioassay to monitor the translation of PAmRNA into active PA in microinjected oocytes. We term this an *in ovo* bioassay.

Newly synthesized plasminogen-dependent PA activity can be measured both in oocyte homogenates and in the incubation medium. It can also be detected visually in single, viable oocytes, covered with casein-containing agar overlay (Figure 1). The mRNA-directed enzyme is selectively inhibited by specific antibodies[19] and it can also be radioactively labeled and immunoprecipitated.[20]

The *in ovo* PAmRNA bioassay has been performed using mRNA from various species, tissues, and cell types.[19,21,22] In all of these cases, the electrophoretic migration properties of the oocyte-produced PAs resembled those of the corresponding activities in the originating tissue. This implies that the heterogeneity of PA activities is not regulated at the post-translational level but extends at least to the level of mRNA. Examples for the heterogeneity of oocyte-produced PAs are presented in Table 2.

3. Cholinesterases: Multiple Enzymes Encoded by Multiple mRNAs

Each of the available *in ovo* bioassays has first been developed using total, unfractionated mRNA as the translated material. It is possible that a single species of mRNA within the injected population of mRNA molecules is responsible for the synthesis of all of the measured activity. In other cases, the protein(s) that display the pursued activity may be produced from multiple mRNA species of different properties. The biosynthesis of cholinesterases in mRNA-microinjected oocytes can serve as an example for this issue.

Cholinesterases (ChEs) are serine esterases hydrolyzing acetylcholine, abundant in nervous system and muscle. ChEs exist in multiple molecular forms, differing in their sedimentation in sucrose gradients, tissue localization, and mode of association with the surface membrane. Mammalian ChEs can be classified by their substrate specificity and sensitivity to inhibitors into acetylcholinesterase (acetylcholine hydrolase, AChE, EC 3.1.1.7) and pseudocholinesterase (acylcholine acylhydrolase, pseudo ChE, EC 3.1.1.8). ChEs are scarce proteins even in enriched sources like the brain tissue, where they altogether constitute about 0.001% of total tissue protein. This complicated the

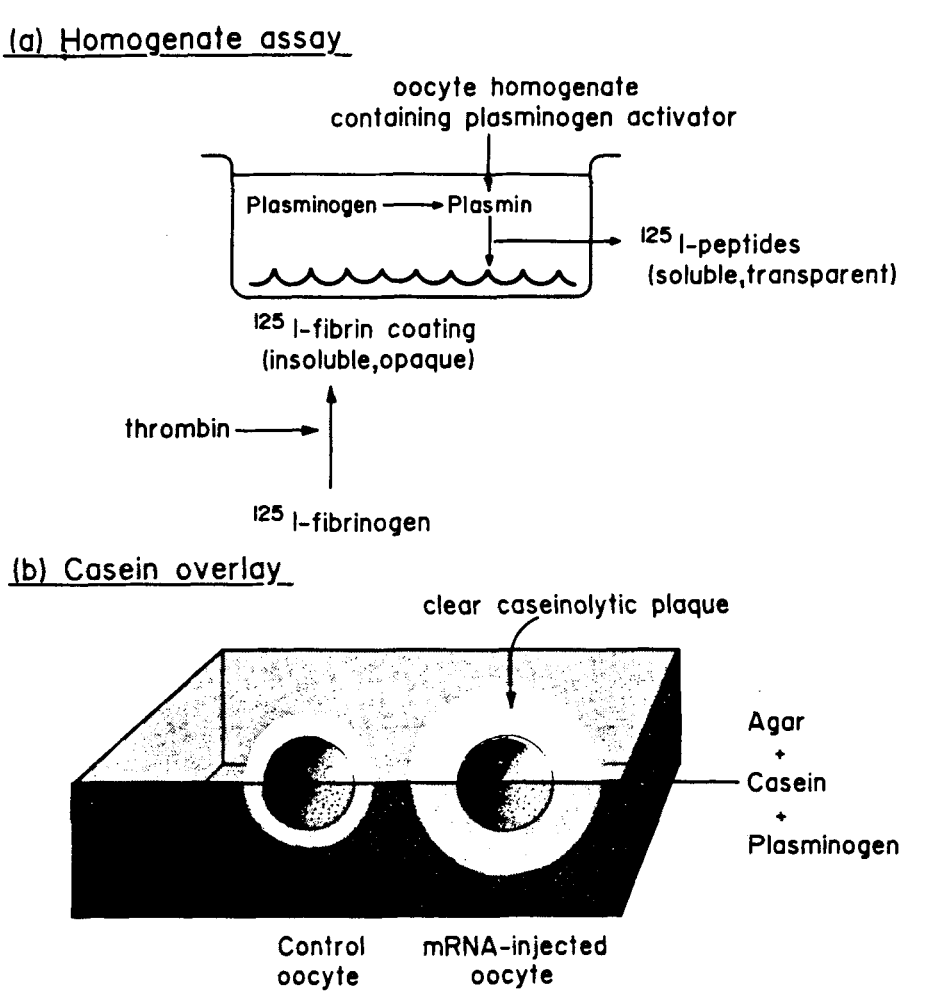

FIGURE 1. Detection of oocyte-produced PA. Plasminogen activators (PA) convert the inactive zymogen plasminogen into plasmin, a trypsin-like protease of broad specificity. Plasmin is a potent fibrinolytic and caseinolytic protease that digests insoluble fibrin or casein into soluble peptides. This reaction is exploited to assay PA activity. The plasminogen-dependent proteolytic activity induced by PA in mRNA-injected oocytes can be quantitatively measured in oocyte homogenates, by counting the solubilized radioactivity (a). It can also be qualitatively detected as secreted from single, viable oocytes covered with opaque casein-containing agar overlay where transparent plaques are formed by PA-induced proteolysis (b).

study of ChEs by conventional biochemical approaches and prevented detection of brain ChEmRNA by radioactive labeling of the mRNA-directed enzyme. However, the high turnover number of ChEs (*circa* 10^4 molecules of substrate degraded per second by a single catalytic site) makes it possible to detect minute quantities of the active enzyme. The combination of this property with the efficiency of the oocyte system led to the development of an *in ovo* bioassay for ChEmRNA.[38] *Xenopus* oocytes express and secrete acetylcholinesterase activity of their own.[39]

When injected with poly(A)-containing RNA from cholinesterase-producing tissues, the oocytes synthesize and secrete catalytically active ChE.[29,31,40] The foreign enzyme differs from the amphibian endogenous ChE in that it is not sensitive to parachlorom-

Table 2
HETEROGENEITY OF OOCYTE-PRODUCED PLASMINOGEN ACTIVTORS

Source of mRNA	Molecular weight of PA	Type of activity	Ref.
Human epidermoid carcinoma (HEp3,tumor transferred in nude mice)	55,000	Urokinase	19
Human melanoma cell line (Bowes)	70,000	Tissue activator	20, 21
Rat muscle tissue	80,000 (Minor) 50,000 (Major)	Tissue activator Urokinase	21
Rat cerebellum	80,000 (Major) 50,000 (Minor)	Tissue activator Urokinase	22
Mouse neuroblastoma cell line	80,000	Tissue activator	208

ercurophenyl sulfonic acid.[7,30,38] Sucrose gradient fractionation of dimethylsulfoxide-denatured mRNA from embryonic human brain revealed three differently migrating size classes of mRNAs that are capable of inducing ChE activity in oocytes. These mRNAs sedimented at about 32S, 20S, and 9S and were all present in primary gliomas, meningiomas, and embryonic brain, each of which expresses ChE activity with distinct substrate specificities and molecular forms.[30]

To distinguish between ChEs having different substrate specificities, radiometric activity determination of the oocyte-produced ChEs was carried out in the presence of selective inhibitors. Both acetylcholinesterase and pseudocholinesterase multimeric activities were found to be produced in the oocytes injected with mRNA. Moreover, the size distributions of the human brain mRNAs inducing these two ChE activities were different, indicating that different mRNAs are translated into various types of ChEs.[30] The *in ovo* bioassay for ChEmRNA, thus, served to prove that the heterogeneity of ChEs in the human nervous system is not caused by post-translational modifications, but reflects heterogeneity of mRNAs.

4. (2′-5′) (A)n Synthetases: Molecular Heterogeneity of mRNAs Related to Subcellular Localization of their Products

The molecular heterogeneity of several enzymes, produced by various mRNA species, has been suggested to be related to different cell type specificities and/or subcellular locations. (2′-5′) (A)n Synthetases represent an oocyte-studied example for such heterogeneous subcellular distribution. The synthetases convert ATP to 2′-5′ linked oligoadenylates with 5′-terminal triphosphate residues. These enzymes are believed to be involved in the mechanism of viral response. When injected with total mRNA from interferon-treated mouse L cells, the oocytes produce (2′-5′) (A)n synthetase activity.[35] Sucrose gradient fractionation of mRNA from Ehrlich ascite tumor cells revealed that two mRNAs (sizes 1.5 and 3.8 Kb) can be translated in microinjected oocytes into (2′-5′)(A)n synthetases of 20,000 to 30,000 and 85,000 to 100,000 daltons, respectively. (2′-5′)(A)n Synthetases of corresponding sizes appear in the nuclear fraction and in the cytoplasm of these cells.[36] It has not been checked, however, whether the two enzymes migrate into their correct subcellular compartments in the injected oocytes.

5. Cytochrome P-450: A Foreign Enzyme Inserted into the Oocyte Membrane

The insertion of foreign, mRNA-directed enzymes into the membranes of microinjected oocytes is neither cell-type nor species specific. This is indicated from the finding that functional rat liver enzymes are found inserted in the endoplasmic reticulum of the frog cell. Nonetheless, insertion is highly selective, since albumin and several other proteins made under the direction of the injected liver RNA are sequestered within membrane vesicles and are then secreted by the oocytes, while epoxide hydratase and cytochrome P-450, with deethylase activity, are inserted into membranes but are not secreted.[28]

6. Production of Functional Viral Enzymes in DNA and mRNA-Injected Oocytes

Many of the DNA viruses infecting mammalian cells have been characterized in detail and sequenced. Particular fragments of these sequences were inserted into bacterial plasmids. The polypeptide products encoded by these viral DNA fragments could be indentified by hybridization-selection experiments, followed by *in vitro* translation. In parallel, the enzymatic activities induced by these viruses when infecting cells in culture have been tested in cell extracts. Microinjected *Xenopus* oocytes could then be employed to identify a specific viral-directed polypeptide with a distinct viral-induced enzymatic activity. This has been done in the case of herpes simplex alkaline exonuclease.

Infection of cells with herpes simplex virus (HSV) induces the production of a viral-coded alkaline exonuclease. Microinjection of HSV-infected cell mRNA into *Xenopus* oocytes results in the biosynthesis of active alkaline exonuclease. The *in ovo* bioassay for this mRNA species has facilitated studies on the size and genome map location of the viral exonuclease-directing sequences. Hybrid-arrested translation showed that it was virus-coded, mapping at 0.080 to 0.185 genome map units. In vitro translation experiments revealed that exonuclease mRNA has a size and genome location equivalent to the mRNA encoding a well-defined HSV protein, V185, in reticulocyte lysate. This suggested that V185 is the exonuclease. Two plasmids containing HSV DNA fragments directed the synthesis of exonuclease when microinjected into oocyte nuclei. These observations placed the coding and control sequences for this gene between 0.155 to 0.185 genome map units.[37]

Oocytes injected with HSV-derived plasmids have also been shown to produce catalytically active thymidine kinase. In this case, the mRNA species directing thymidine kinase synthesis has also been detected by "northern" blot hybridization. This analysis revealed that sequences present in thymidine kinase mRNA were highly represented in stable HSV transcripts.[27]

7. General Considerations for the Evaluation of mRNA-Directed Synthesis of Enzymes in Oocytes

In evaluating the results of *in ovo* bioassays for the production of active enzymes several points should be taken into consideration.

1. The injected mRNAs compete with the oocyte mRNAs, and with other injected mRNAs, for formation of polysomes in the oocyte. This competition is more crucial when mRNAs for secretory or membrane-bound proteins are injected, since the amount of polysomes for such mRNAs is rather limited in the oocytes.[41] It is not known how such mRNAs are distributed in different tissue sources and/or different size fractions of mRNA. Hence, the competition within different injected mRNAs might not be equal.
2. It is possible that more than a single mRNA species might be required to produce the catalytically active enzyme pursued. The additional mRNA(s), which could

be either an oocyte or a foreign mRNA species, might then become the limiting factor in the biosynthesis of the enzyme.

3. The determination of the newly synthesized enzymatic activity, and, hence, the calculations of the abundance of its mRNA species, are based on the assumption that the newly synthesized enzyme possesses a fully functional catalytic site, with similar kinetic properties to those measured for this enzyme in its originating tissue. However, it is possible that the oocyte-produced enzyme might not be identical to the original tissue protein, due to improper post-translational event(s) (see following sections for further details). In this case, such calculations would be underestimates.

For these reasons, the *in ovo* bioassays cannot be used for quantification of absolute levels of specific mRNA species, directing the synthesis of catalytically active enzymes, and their major value lies in the qualitative nature of the enzymes produced.

B. Interaction of mRNA-Induced Proteins with Labeled Ligands

The first mRNA-directed protein produced in microinjected *Xenopus* oocytes was rabbit hemoglobin. The biosynthesis of globin in oocytes microinjected with reticulocyte 9S mRNA was reported first in 1971.[1] Recently, it has been reported that within the oocytes metalloporphyrin is added to mRNA-directed globin tetamers,[13] indicating that the oocyte-produced globin is similar to the original reticulocyte protein. In many other cases as well, the biological activity of a particular protein is displayed by its interaction with a specific ligand. In these cases, the production of the pursued proteins in their biologically active forms can best be followed by labeling their particular ligands and measuring the interaction. Immunoglobulins and surface membrane receptors are good examples for such functional proteins.

1. Production of Functional Immunoglobulins

Several independent criteria indicate that functional immunoglobulins are produced in mRNA-microinjected oocytes: (1) MOPC-321 kappa chain immunoglobulin, labeled with ^{3}H-amino acids, can be immunoprecipitated from extracts of oocytes injected with plasmacytoma mRNA,[42] whereas injection of mRNA, or even of cell homogenates, from IR2 myeloma results in the production of immunoglobulin G;[43](2) the amino acid sequence of the NH_2-terminal end of the purified kappa chain produced in MOPC-321 mRNA-injected oocytes was found to be identical to that of the mature, secreted form of the protein;[42](3) fully assembled 7S H_2L_2 immunoglobulin molecules could be detected in medium of oocytes injected with rat spleen mRNA.[45] When the rats had been hyperimmunized with ferritin, specific antiferritin antibodies could be purified from the microinjected oocytes by affinity chromatography on Sepharose-antigen columns;[45] (4) the secretion of immunoglobulins from mRNA-microinjected oocytes depended on the presence of both heavy and light-chain immunoglobulin molecules, and the synthesis of either of these by itself was not a sufficient requirement for secretion, similar to the in vivo situation.[44]

Correct processing and subcellular segregation were also reported for the HLA-DR antigens, related to the immune response. Microinjection of mRNA from human B cells resulted in the production of all three polypeptide chains (alpha, beta, and gamma) of HLA-DR proteins in immunoprecipitable forms. Of these, the alpha and beta chains needed detergent to be solubilized from the oocyte membrane, as expected from their in vivo membrane association, whereas the cytoplasmic gamma chain was soluble in the oocyte cytosol. Furthermore, all three chains were correctly glycosylated and appeared to be assembled in the right equimolar ratio.[46]

2. mRNA-Directed Biosynthesis of Receptors and Hormone-Binding Proteins

Several receptors and hormone-binding proteins have been synthesized in mRNA-microinjected oocytes. The first to be reported was rat prostatic binding protein (PBP), a steroid-binding protein produced in the rat ventral prostate. Both subunits of PBP (Mr 16,000 and 18,000) appear in the oocyte cytosol in immunoprecipitable forms.[47] Reduction of S-S bonds releases a single, small, glycosylated polypeptide from both subunits.[48] NH_2-terminal signal peptides of about Mr 1000 are removed from the two larger subunits, which are thereafter assembled and secreted as a mature PBP from the microinjected oocytes.[49]

The glycosylation of PBP C_3 subunit is not complete in the oocytes. An oligomannosyl core chain is added to C_3 peptides, but the complete profile of oligosaccharides attached to secreted PBP molecules appears to be heterogeneous, and different from that of PBP in the native tissue. However, glycosylation is neither a prerequisite for secretion nor for the assembly of PBP in oocytes, as has been shown by inhibition of glycosylation in the oocytes by tunicamycin.[49]

When injected with mRNA from the *Torpedo* electric organ, the oocytes produce all four subunits of the nicotinic acetylcholine receptor. The mRNA-directed receptor appears to be correctly assembled and inserted into the oocyte membrane and displays specific binding of ^{125}I-alpha-bungarotoxin.[32] Furthermore, mRNA microinjection led to the appearance of fully functional acetylcholine receptors in the oocyte membranes. When activated by acetylcholine, these *Torpedo* acetylcholine receptors in the oocyte membrane opened channels whose ionic premeability resembled that of nicotinic receptors in the original tissue.[50]

C. Other Biological Activities Displayed by Foreign Proteins Produced in Microinjected Oocytes

1. Inhibition of Viral Infection by Oocyte-Produced Interferons

The advantages of the oocyte system in producing biologically active proteins have been used extensively in studying the biosynthesis of interferons, where the biological activity was for a long time the criterion known by which this protein could be identified. Interferons (If) are secretory glycoproteins that impart an antiviral state in cultured cells. Experiments done in cell cultures revealed that the production of If can be induced by poly(rI)-poly(rC) complexes, and "superinduction" can be conferred on induced cells by several metabolic inhibitors. When mRNA from If-producing human fibroblasts was injected into *Xenopus* oocytes, they produced biologically active human If. The oocyte-produced If protected cultured fibroblasts against viral infection and its activity could be blocked by specific antibodies.[23] The antiviral effect was both species[51,52] and cell-type[53]specific. The mode of appearance of interferon mRNA (If mRNA) in cells was investigated using the *in ovo* bioassay. It was found that If induction occurred by increase in transcription of If genes. In contrast, the level of If mRNA, as measured in microinjected oocytes was not increased by superinduction:[51] the incremental effect of superinduction on If level was, therefore, suggested to be caused by a decreased rate of IfmRNA degradation.[53,54] It should be noted that this result was not confirmed in other cells and appears to be peculiar to the oocyte system.

Interferon mRNAs were found to be heterogeneous both within and between cell types and species in terms of their length [57,58,60] and mode of induction.[55-57] The use of the *in ovo* IfmRNA bioassay initiated the detection of a new variety of If activities, translated from multiple IfmRNAs[60] and differing in their activity on different cell lines, their electrophoretic mobility in gels, and their immunogenic properties.[59] For example, when mRNA was prepared from human splenocytes, it induced the synthesis of gamma interferon. This activity could be selectively blocked by antigamma If antiserum, and heterologous gamma IfmRNAs, with different lengths, could be ob-

served.[61] The use of the oocyte system to investigate the biosynthesis of various If species has been reviewed extensively (for a recent example, see Pestka, 1983).[66]

Similar to the case of PBP (see Section II.B.2), the post-translational proccessing of oocyte-produced interferon appears to be incomplete. When the translation product of mouse IfmRNA was compared with the native mouse protein, it was found to display similar immunogenic activity and to be similarly retained on poly(U)-Sepharose columns. However, the majority of the oocyte-produced If migrated as a 21 to 22,000 protein on denaturing gels, whereas the majority of the native mouse If migrated as a 35 to 40,000 protein.[62] As in the case of oocyte-produced enzymes, there are several possible explanations for this phenomenon. The different migration of the oocyte-produced If could be due to selectively high efficiency of the oocytes in translating particular species of IfmRNA. Alternatively, it could indicate incomplete glycosylation or other defective post-translational events.

The *in ovo* bioassay for IfmRNA was a prerequisite for the molecular cloning of the human If genes (for example, see Weissenbach et al.).[55] More recently, the *in ovo* production of If has been studied using cloned DNA probes. Capped transcripts were prepared from a plasmid containing the human fibroblast interferon gene, cloned under the control of a prokaryotic promoter. The translation of such transcripts in microinjected oocytes revealed their functional properties and indicated the necessity of particular sequences for correct transcription.[63] The coordinate transcription of alpha and beta If genes in virus-infected human lymphoblastoid cells was studied by hybridization with cloned cDNA probes in parallel with microinjection experiments. It was shown that the kinetics of accumulation and decay of both alpha and beta IfmRNAs, as assessed by cDNA hybridization, were very similar to those of the corresponding translatable IfmRNA species, as measured in microinjected oocytes.[64]

2. The Production and Secretion of Growth Factors

After it had been shown that the oocytes can produce and secrete rare proteins that display their correct biological activities, a natural choice for further study were growth factors. This group of nonabundant proteins may be distinguished by their high potency in inducing complex arrays of biological processes in specific cell types. Furthermore, the production of the secretory growth factors must involve post-translational processing and sequestration events. All of these properties complicate studying the biosynthesis of growth factors by in vitro translation and make the oocyte system particularly suitable for developing bioassays for mRNA-directed growth factors. Two different examples for such proteins are Interleukin and epidermal growth factor.

Interleukin 2, or T-cell growth factor, is essential for the proliferation of activated T cells. It is produced by stimulated lymphocytes, splenocytes, and several tumor types, and its activity is assayed by stimulation of DNA synthesis and maintenance of viability in activated lymphocytes. When mRNA from Interleukin-producing cells was injected into oocytes, they secreted Interleukin for as long as 96 hr. The oocyte-produced activity could be neutralized by anti-Interleukin monoclonal antibodies. The sedimentation coefficient of the corresponding mRNA species was determined by sucrose gradient fractionation, followed by microinjection of the fractionated mRNA.[65]

Epidermal growth factor (EGF) is a 6045-dalton mitogen produced in the submaxillary gland of male mice. When total, unfractionated mRNA from androgen-induced submaxillary glands was injected into *Xenopus* oocytes together with [^{35}S]-cysteine, newly synthesized 9000-dalton pro-EGF was detected in oocyte extracts and media by immunoprecipitation. Sucrose gradient fractionation of the gland mRNA revealed that two EGF-containing proteins of Mr 125,000 and 110,000 were translated from the 28S mRNA fraction. Of these, the 110,000 protein was secreted.[34] These proteins, there-

Table 3
PROCESSES IMPLICATED IN THE PRODUCTION OF BIOLOGICALLY ACTIVE PROTEINS

Process fate of injected mRNA	Molecules measured	Ref.
Distribution of injected mRNA in the oocytes	Immunoglobulin mRNA	70,89
	SV_{40} RNA	86
	Histone mRNAs	87
	HeLa cells RNAs	90
Competition with oocyte mRNAs	Globin	71
	Alpha-crystallin	72
	Protamine	91
Competition with other injected mRNAs	Globin	73
	Maize	92
mRNA degradation	Globin	13,74,94,95, 97—99,103
	Interferon	54,101,102
	Various	75
	Histones	76,100
	Alpha 2U microglobulin	96
	Mengovirus proteins	103
Fate of mRNA-directed protein		
Recruitment into polysomes	Membrane-bound mRNAs	110,111,157
Initiation (capping involvement)	Reovirus	78,107
	Globin	109
	Alpha-crystallin	79
Elongation	Ovalbumin	80
Termination	Readthrough proteins	81,82,112
Translocation	Immunoglobulin	70,134,136
Proteolytic degradation of protein product	Albumin, ovalbumin, chemotrypsinogen, beta-lactoglobulin, lysosyme, pro-EGF	67,77
Pynocytosis of exported proteins	Vitellogenin, albumin	13,114,115

fore, appear to be processed to yield the small pro-EGF by the products of gland-specific mRNA(s), smaller than 28S.[67] The oocyte-synthesized processed pro-EGF displayed specific binding to A-431 cells, which contain high amounts of EGF receptor.[34]

The post-translational processing of EGF is not complete in the oocytes. Pro-EGF is not cleaved further to yield the mature EGF. In this it behaves similarly to pro-insulin.[68,69] Furthermore, pro-EGF is not associated with a high molecular weight complex, as occurs in the original tissue. It, therefore, appears that the production of this growth factor requires more than the two tissue-specific mRNAs that are responsible (1) for the translation of the large precursor protein and (2) for its processing into pro-EGF, and that the final cleavage of the "pro" sequence and the association into a high molecular weight complex are not essential for either secretion or binding to EGF receptors.

III. BIOSYNTHETIC PROCESSES IMPLICATED IN THE PRODUCTION OF BIOLOGICALLY ACTIVE PROTEINS IN OOCYTES

What happens to the mRNA molecule once it is injected into the oocyte, and what happens to its polypeptide product until it becomes a correctly processed and sequestered active protein? The major processes involved in these two pathways are common to most molecules. Several examples for such processes and the studies in which these were investigated are summarized in Table 3. Some of these examples will be described in the following section, according to the probable order of events involved.

A. The Fate of Foreign mRNA in Microinjected Oocytes

1. Intracellular Partitioning of Foreign RNAs

There is a widely accepted view that foreign mRNA, when microinjected into *Xenopus* oocytes, distributes itself evenly throughout the oocyte. However, there is no compelling evidence to support this view and very little is known, in physical terms, regarding the fate of microinjected mRNA. Is simple diffusion the first rate-limiting step in recruitment of microinjected mRNA to engage translational machinery within the oocyte? A recent review[83] points out that most of the cytoplasmic material in a cell cannot diffuse freely. This effect would be most apparent in cells with high concentration of proteins, like *Xenopus* oocytes. The rate of diffusion of a particular species of mRNA within a microinjected oocyte would, therefore, depend on additional factors, such as the structural properties of the injected molecule. Indeed, coinjection of radioactively labeled amino acids with mRNA suggests that mRNAs for secretory[34,67] and membrane proteins[70] are preferentially translated at the site of injection. It is, therefore, possible that such mRNAs diffuse at a much slower rate than those coding for cytoplasmic proteins.

a. Nuclear-Cytoplasmic Transport of RNAs

The selective intracellular partitioning of foreign mRNAs in the oocytes has recently been investigated by nuclear injection of DNA from simian virus 40 (SV40). Only those RNAs with a mature 3′ terminus and poly(A) are located in the cytoplasm, but correct splicing is not an obligatory requirement for mRNA transport into the cytoplasm.[86] Earlier measurements of histone gene transcripts have also indicated that only mature mRNAs, with proper 5′ and 3′ termini, enter the cytoplasm.[87] However, histone mRNAs differ from SV40 transcripts, and most other mRNAs, in that they do not undergo RNA splicing or polyadenylation. It is, therefore, not clear yet how mRNA maturation affects its distribution in the oocyte.

Experiments carried out with [^{32}P]-labeled RNA from HeLa cells indicate that microinjected nuclear RNAs migrate from the cytoplasm into the cell nucleus, where they become 30 to 60-fold more concentrated than in the cytoplasm. Other HeLa cell RNAs, such as tRNA and 7S RNA, remain in the oocyte cytoplasm and 5S RNA concentrates in the nucleolus. Furthermore, migrating foreign RNAs become selectively associated with oocyte RNA-binding proteins.[90] Partitioning of these RNA species, therefore, operates against concentration gradients and is not determined by free diffusion. It would be interesting to examine whether the intracellular distribution of different messenger RNA species in the oocytes is similarly specific and selective.

b. Fate of Globin mRNA in Embryos Developed from Microinjected Xenopus Eggs

Microinjection of globin mRNA into fertilized *Xenopus* eggs has been used as an approach to investigate the post-injection distribution of foreign mRNAs in the frog cell. In these experiments the embryos were allowed to develop and then labeled with [^{3}H]histidine, dissociated, and fractionated. The biosynthesis of total endogenous proteins and of separated globin was then measured. Tail-bud embryos and swimming tadpoles were first examined. There was no apparent difference in globin synthesis between the ventral half (containing blood islands) and the axial region of the embryos. These results were interpreted as suggesting that the injected mRNA was evenly distributed in the developing embryos and that translation efficiency was similar in various differentiating tissues, independent of their function.[88] However, different results were later obtained when an earlier stage of development (i.e., gastrulae) was studied. All cell fractions were, in this experiment as well, capable of synthesizing globin protein, but in animal hemisphere cells globin represented a larger fraction of total synthesized protein,[89] suggesting an unequal distribution of globin mRNA to the different cell

types. Microinjected polyriboadenylic acid was distributed in *Xenopus* gastrulae in an opposite concentration gradient to that formed by globin mRNA, indicating that the partitioning pattern was not a consequence of microinjection.[89]

2. Competition of mRNAs on the Formation of Polysomes

The large variety of heterologous proteins made in microinjected oocytes, the competition seen between different injected messengers,[8,73] and the competition between injected and endogenous mRNAs[72] suggest that all messengers use at least some common machinery. However, the different competition effects observed were not equal. Thus, Laskey et al.[71] showed that while injecting increasing amounts of globin mRNA into oocytes resulted in more globin synthesis, endogenous protein synthesis decreased with essentially reciprocal kinetics. It is, therefore, difficult to saturate the oocytes' capacity to translate globin message, although the oocyte has no spare translational apparatus. The membrane-free protamine mRNA is similar to globin mRNA in this respect,[91] but the capacity of oocytes to translate the membrane-associated Zein mRNAs is saturated at relatively low amounts of injected mRNA.[92] Hybridization of RNA from injected oocytes to cDNA probes revealed that there was no difference in the stability of globin and Zein mRNAs and that most of the globin mRNA was associated with polysomes, whereas most of the Zein mRNA was not. Thus, the oocyte appears to be limited in rough endoplasmic reticulum (RER). Indeed, microinjection of RER induced Zein synthesis in the oocytes.[92]

Are species-specific factors required for translation of particular classes of mRNAs? This is difficult to conclude, since crude mRNA preparations were used in most of the bioassays. Thus, other heterologous mRNAs, injected into the oocyte together with the pursued one, might code for such factors. The specific differences that are seen in the oocyte proteins that bind different kinds of mRNA are consistent with this possibility.

3. Degradation of Foreign mRNAs in Microinjected Oocytes

What is the rate of degradation of foreign mRNA in microinjected oocytes? Is it different from the rate of degradation of endogenous oocyte mRNAs and of other types of RNA? Does it depend on specific structural properties and/or on functional activity of the mRNA species? All of these questions were approached during the last decade, mostly by using RNA molecules that had been labeled or modified in vitro. Although the effects observed are not necessarily of physiological significance, they are of considerable interest.

a. Role of Poly(A) Tail in mRNA Degradation

When different types of radioactive RNAs were injected into *Xenopus laevis* oocytes and their degradation followed with time, it was found that ribosomal RNAs were degraded rapidly, with apparent first-order kinetics and 1- to 6-hr half-lives. Whole ribosomal particles were rather stable for 20 hr and polyadenylated mRNAs displayed biphasic kinetics, with about 60% degrading rapidly and the rest rather stable for 20 hr at least.[75] The complex pattern of mRNA degradation was further analyzed by using mRNA preparations from which precisely determined sequences had been removed from the 3'-OH terminus by polynucleotide phosphorylase.[74] In agreement with earlier findings,[93] native globin mRNA appeared to be very stable for over 70 hr. However, deadenylated globin mRNA displayed a half-life of 5 to 10 hr, whereas readenylated molecules became as stable as the native material.[94] Similar results have been obtained using human globin mRNA.[95] A minimal length of 30 adenylate residues was found to be sufficient to maintain the full stability of globin mRNA chains, whereas poly(A) tails shorter than 30 did not have any stabilizing effect.[74] Indeed, experiments involving microinjection of alpha-2U microglobulin mRNA fractionated on oligo(dT)-cellulose failed to reveal differences in stability between molecules with an average of 175 ter-

minal adenylate residues and those with 40 such residues.[96] Thus, there is no change in the half-life of globin mRNA until the poly(A) tails are shortened below a critical length.

The accelerated degradation of the deadenylated mRNA in microinjected oocytes is responsible for its reduced translation, as has been shown by hybridization to a labeled globin cDNA probe.[97] In the absence of a poly(A) tail and/or the proteins bound to it, rapid destruction of translationally active globin mRNA takes place in the oocytes.[98] The stability of deadenylated globin mRNA is much lower than that of native polyadenylated mRNA in microinjected HeLa cells as well,[99] which indicates that this finding is not an oocyte peculiarity, but holds true in somatic cells as well. Furthermore, enzymatic polyadenylation of poly(A)-deficient histone mRNAs increased their stability in oocytes. The same is true for mRNA4 from the alfalfa mosaic virus.[104] These experiments indicate that the poly(A)-stabilizing effect is not unique to globin mRNA and may even effect mRNAs naturally lacking poly(A).[76]

When histone mRNA from sea urchin embryos was injected into *Xenopus* oocytes, most of it appeared to decay with first-order kinetics, with a half-life of about 3 hr, whereas 5% remained stable for weeks.[100] Therefore, it appears that compartmentalization of unused stored mRNA protects it from nucleolytic degradation.

The effect of poly(A) on mRNA stability is not a general one. The physical and functional stability of IfmRNAs appeared to be unaffected by the removal of their poly(A) tails.[54, 101, 102] The same is true for mengovirus RNA.[103] Hence, it appears that individual mRNAs are selectively stabilized to different extents by their poly(A) tails.[103]

b. Other Structural Elements Involved in mRNA Stability

Another structural element that has been implicated in the stability of mRNA in the oocytes is the 5′-terminal "cap".[105] Removing or breaking open the cap structure of globin mRNA causes a complete reduction (>95%) in its ability to direct globin synthesis in oocytes.[106] Reovirus RNA has been initially reported to be rather unstable in the oocytes and to become even less stable upon cap removal.[78] More recently, it has been shown that the capped species are, in fact, quite stable and that cap removal greatly destabilizes all ten reovirus messengers.[107] Thus, direct and indirect evidence suggests that in most cases, mRNAs have to be capped to be translated in the oocytes. However, as in the case of the poly(A) tail, this is not a general rule, and naturally occurring uncapped eukaryotic mRNAs, such as satellite tobacco necrosis virus RNA, RNA[13] CPMV RNA,[104] or EMC viral RNA[71] may both persist and function in the frog cell.

As emphasized by Lane (1983),[13] there has, to date, been no systematic and general study of mRNA stability in oocytes. mRNA stability appears to be, in some cases, related to the presence of poly(A) or cap structures; in other cases, it is not. In some cases, mRNA stability has been found to be inversely related to translational efficiency;[98] in other cases,[107] it is not. With the rapid advance of molecular cloning studies, it is possible that common structures will be found in different mRNAs that confer stability on these molecules in all living cells, perhaps through the binding of developmentally regulated proteins.[108]

B. The Fate of mRNA-Directed Proteins in Microinjected Oocytes

As has been concluded before, it is reasonable to assume that the different mRNAs expressed in microinjected oocytes use translational machinery that is common to all eukaryotic cells. The oocyte system is, therefore, particularly suitable for studying the various processes involved in protein synthesis and turnover, as is demonstrated in Table 3. This pathway includes several key steps, each of which might be important in regulating the mRNA-directed production of active proteins in microinjected oocytes.

1. Recruitment of Foreign mRNA and Reading of Protein Synthesis Signals

Several studies indicate that components of the protein synthesis machinery other than mRNA availability are the limiting factors that restrict use of mRNA in the oocyte.[71] It appears that the oocytes have a limited amount of membranes, or membrane binding sites, with which membrane-bound mRNAs can interact. When injected, such mRNAs remain in a preinitiated form. Similar to the in vitro situation, microinjection of RER or of RER salt-wash facilitated translation of such mRNAs. The generality of recognition of membrane-bound mRNAs is further indicated from the observation that microinjected synthetic leader peptide inhibits the translocation of nascent membrane and secretory proteins in the oocytes.[70]

When the amount of polysome-recruited ovalbumin and Zein mRNA in the oocytes was compared to the absolute amount of the radioactive proteins produced, it was concluded that most of the mRNA found on polysomes was, indeed, translated.[110] It, therefore, appears that initiation of translation in the oocytes in *not* the rate-limiting step. Rather, it seems that a preinitiation step of recruitment is limiting, as had been suggested earlier.[111] Microinjected mRNAs are, according to this hypothesis, first recruited from an inactive pool, and this step involves binding (or removal) of protein(s) that recognize specific classes of mRNAs. The nature of these proteins, as well as their mode of action, remains to be revealed.

Both endogenous protein synthesis and translation of foreign mRNA were unaffected by the presence of injected cap analogs such as $m^7G5'p$ or $m^7G5'pp5'G$.[79] In contrast, microinjection of aurintricarboxylic acid, which inhibits elongation, strongly inhibited oocyte protein synthesis.[79] This situation is consistent with the observation that initiation is not the rate-limiting step in mRNA-microinjected oocytes.

Are all mRNAs correctly read in the oocytes, so that all proteins are terminated at their natural termination sites? Apparently, this depends, to a certain extent, on the available tRNAs. This issue has been extensively studied by Grosjean and co-workers. They found that when they injected "nonsense" suppressor tRNAs from yeast (amber, ochre, and opal) together with purified mRNAs, the oocytes produced large "readthrough" proteins that were not terminated at their normal sites.[81] Readthrough endogenous proteins were also produced, although with a rather low abundance. Thus, it appears that natural termination codons are rather resistant against readthrough, probably via specific context sequences around the termination codon.[82]

The termination codons used in the cytoplasm of eukaryotic cells differ from those employed in mitochondria. For example, yeast mitochondrial $tRNA^{Trp}$ contains the anticodon sequence U*CA where U* is an uridine derivative of unknown structure which would function as an *opal* suppressor in the cytoplasm. However, when a mitochondrial $tRNA^{Trp}$ was injected into the oocytes, it needed a coinjected acetylating enzyme to produce the U*CA sequence and act as a suppressor.[112] The absence of the mitochondrial acetylase would, therefore, prevent accidental readthrough if a tRNA exchange between the two cellular compartments occurred in vivo. This suggested that there is a biological barrier for the use of mitochondrial tRNA species in the cytoplasm.

2. Turnover of mRNA-Directed Proteins in Microinjected Oocytes

What determines the half-life of mRNA-directed proteins in microinjected oocytes? Should the rate of turnover of such proteins be similar to the natural ones or should it be different in the foreign milieu of the microinjected oocyte? These questions are difficult to answer, particularly when biologically active proteins are discussed. The fate of nascent chains is often different in the oocyte from that of processed, completed proteins, and protein turnover within the oocyte is different from that in the incubation medium and is affected by other elements.

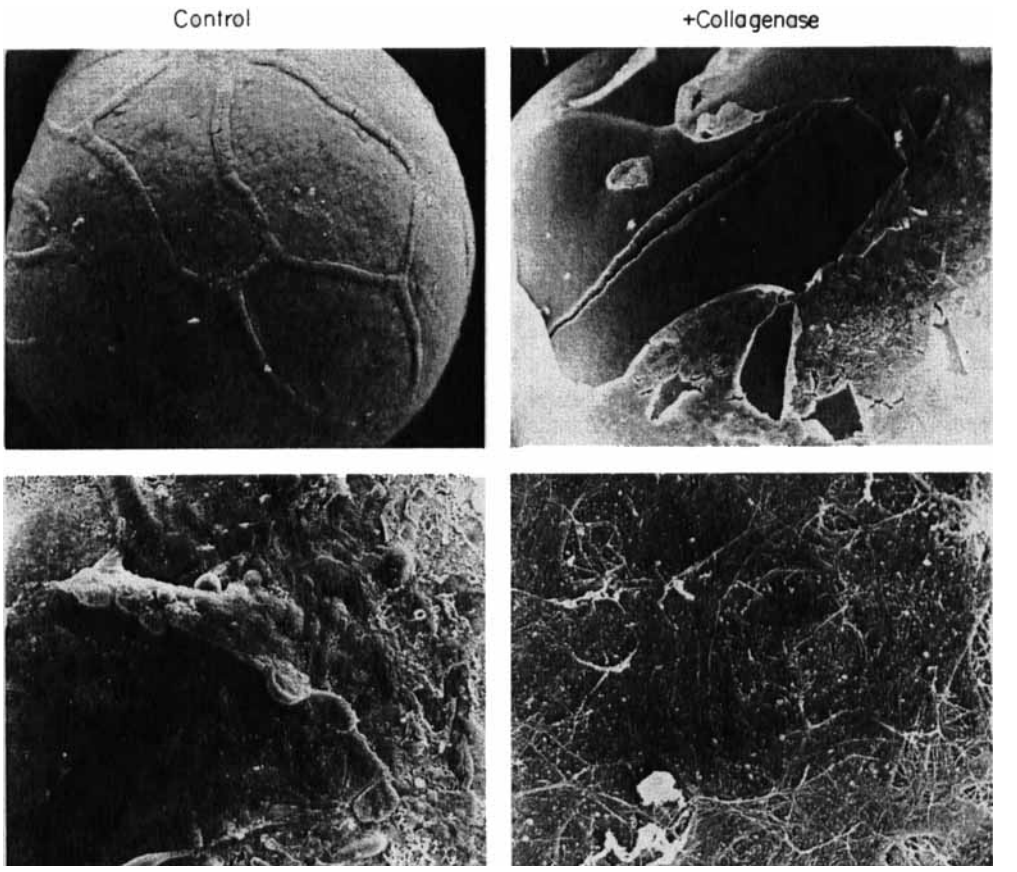

FIGURE 2. Collagenase defolliculation of *Xenopus* oocytes. Scanning electron microscopy reveals the presence of follicle cells covering the oocytes and the existence of blood vessels surrounding each oocyte (upper left). Collagenase treatment peels off these covering cells (upper right). Higher magnification photographs show that below the follicle cell layer (lower left) the vitellin membrane forms an intricate net covering the oocyte surface membrane (lower right).

a. Degradation of mRNA-Directed Secreted Proteins in the Medium of Microinjected Oocytes

It is important to remember that the oocyte is not a closed system. Continuous export and import of proteins occur throughout the incubation period. Both endogenous oocyte proteins and exogenous, mRNA-directed proteins are involved in these processes. The rate of degradation of oocyte-produced secretory proteins is determined by intra- and extracellular proteolytic activities.

A survey of oocytes from many frogs revealed a considerable heterogeneity, both qualitative and quantitative, in the level and properties of oocyte proteases, in the ratio between intracellular and secreted proteases and in the dependence of oocyte protease activity on plasminogen.[77] The average specific activity of oocyte proteases appeared to decrease with development. This stresses the importance of using single, selected mature oocytes rather than unseparated ovarian fragments for microinjection experiments involving secretory proteins.

A layer of follicle cells surrounds each oocyte, which can be removed manually or by collagenase treatment (Figure 2). The follicle cells also secrete proteolytic activities, but defolliculation does not greatly decrease oocyte proteolysis.[77] Foreign mRNA-directed secretory proteins are, therefore, exposed to proteolytic degradation by oocyte

proteases. To minimize such degradation, it is possible to employ mixtures of protease inhibitors.[7,30,34,77] These should first be tested to ensure that they do not affect protein synthesis in the oocytes. Different mixtures of protease inhibitors were selected for various studies so that they would not affect the bioassay of the mRNA-directed protein investigated. Thus, phenylmethylsulfonylfluoride (PMSF) was found advantageous to protect interferon in the oocyte medium,[77] whereas a mixture containing trasylol, soybean trypsin inhibitor, 6-aminocaproic acid, leupeptin, and antipain protects bovine serum albumin, ovalbumin, chemotrypsinogen, beta-lactoglobulin, lysosyme, and Pro-EGF,[34,67,77] and another mix, including leupeptin, pepstatin, bacitracin, and trasylol, protects secreted cholinesterases.[7,30] In cases where the injected mRNA codes for secretory proteases,[9,21,34,67] we find that these inhibitors also protect the oocytes themselves against rapid decay.[208]

b. Incorporation and Breakdown of Xenogeneic Proteins by Oocytes

Like all oocytes of oviparous vertebrates, *Xenopus* oocytes grow primarily by the pinocytotic incorporation of hepatically synthesized vitellogenin from the bloodstream.[113] During the micropinocytotic uptake of vitellogenin, the oocyte is also able to incorporate adventitiously other proteins from the incubation medium, although at a much slower rate. This implies that a certain fraction of the secretory proteins exported from the oocyte might be taken back by pinocytosis. What would be the fate of such proteins? It appears that the specifically bound and incorporated vitellogenin is transferred from coated vesicles via fused endosomes to yolk platelets, where it is converted into the stable yolk proteins, lipovitellin and phosvitin.[114] In contrast, nonspecifically incorporated proteins are degraded, without the formation of fused endosomes. The rate of degradation of proteins incorporated from the oocyte incubation medium depends on their chemical structure, as suggested from the finding that proteins iodinated by the chloramine-T method are degraded much more rapidly after endocytosis than in vivo ^{3}H-labeled proteins.[115]

What, then, happens when mRNA coding for *Xenopus* vitellogenin is injected into the oocyte? It has recently been shown by Lane and co-workers that mRNA-directed vitellogenin goes through a complex route. First, the oocyte translates the injected mRNA and secretes the translation product, as would have happened in the originating liver tissue. The *Xenopus* vitellogenin in the oocyte medium is then recognized by the oocytes vitellogenin-specific binding sites, incorporated by endocytosis, and transported through fused endosomes into yolk platelets, where it is cleaved into phosvitin and lipovitellin.[116] This process is highly specific and does not take place when mRNA encoding locust vitellogenin is injected.

IV. POST-TRANSLATIONAL PROCESSING EVENTS INVOLVED IN THE PRODUCTION OF FOREIGN PROTEINS IN mRNA-INJECTED OOCYTES

Perhaps the most compelling evidence for the fact that oocytes treat injected mRNA species, as well as their translation products, similarly to the originating tissues is the large variety of post-translational processing events carried out by the oocytes. Examples to these processes are summarized in Table 4 and will be discussed in the following sections.

A. Cleavage of Polypeptides from Foreign mRNA-Directed Translation Products

1. Removal of Signal Peptide

The cotranslocational removal of the NH_2-terminal signal peptide is an obligatory requirement for secretion of exported proteins. Since the oocytes correctly secrete such

Table 4
POST-TRANSLATIONAL PROCESSING OF FOREIGN PROTEINS PRODUCED IN *XENOPUS* OOCYTES

Modification	Protein	Ref.
N-acetylation	Calf lens alpha$_2$crystallin	79
Hydroxylation	Mouse fibroblast collagen	13
Glycosylation	Mouse plasmacytoma immunoglobulin	44
	Rat immunoglobulin	25,45
	Chicken ovalbumin	80,132
	Rat prostatic binding protein	48,49
	Human chorionic gonadotrophin	131
Removal of signal sequence	Pre-pro insulin	68
	Mouse plasmacytoma immunoglobin light chain	44
	MOPC 321 kappa chain	42
	Honey bee venom gland promelittin	129
	Pre-pro EGF	34,67
	Uteroglobin	84
Removal of other "Pro" sequences	Pre-pro EGF	34,67
Phosphorylation	Trout testis protamine	91
	cAMP-dep. kinase — synthetic peptide	133
Cleavage of polyprotein	Viral proteins	86
	EMC	85,123
	BLV	120,122
	MMTV	124
	MLV	127
	Lipovitelin, phosvitin	13
Formation of S-S bond	Rabbit uteroglobin	85
	Immunoglobulins	25,45
Noncovalent assembly of protein subunits	Calf alpha- and beta-crystallins	79
	Mouse kidney	24
	Beta-glucuronidase acetylcholine receptor	32
Metalloporphyrin addition	Rabbit hemoglobin tetramer	13

proteins and since protein processing enzymes are often topologically segregated, correct processing of foreign proteins implies correct interaction with the macromolecular sorting machinery of the frog cell.

The removal of NH_2-terminal signal peptide was first demonstrated for mouse plasmacytoma immunoglobulin light chain, as produced in mRNA-microinjected oocytes.[44] Many more cases of removal of signal peptide have been reported since and it appears that the nature of the injected mRNA determines this step in the processing pathway and that the oocytes' capacity to remove the signal peptide is not limited. Two recent examples are the processing of pre-alpha-2U-globulin to yield the mature alpha-2U-globulin in microinjected oocytes[117] and the correct processing of the entire array of secretory chorion proteins-of *Bombix mori*.[118]

That recognition of signal peptide by the oocyte machinery is not cell-type or species specific is also indicated from experiments where synthetic signal peptide was injected into the oocytes.[70] It was found that the injected peptide competitively inhibits the *de*-novo synthesis of secretory and membrane proteins, but not of cytoplasmic proteins. This inhibition occurs both with oocyte proteins and with proteins directed by coinjected myeloma mRNA. The inhibition appears to be mediated through the cell membrane, since ^{125}I-labeled signal peptide concentrates into the membrane fraction of microinjected oocytes simultaneously with the interference with methionine incorporation.[70]

A second effect found to be asserted by microinjected signal peptide was a highly specific, rapid acceleration in the rate of export of preexisting secretory proteins from the oocyte. Thus, signal peptide sequences appear to be involved in both translocation of nascent chains and in later step(s) in the secretory pathway in microinjected oocytes, in a manner that is neither cell type nor species specific.

2. Cleavage of Viral Polyproteins

Several virion RNAs direct the synthesis and the post-translational cleavage of precursor polypeptides of viral core proteins, or group-specific antigens, when injected into *Xenopus* oocytes. These include the virion RNAs of avian myeloblastosis virus,[119,120] Rauscher murine leukemia virus,[121] bovine leukemia virus,[122] encephalomyocarditis virus, [123] mouse mammary tumor virus,[124] avian sarcoma virus,[125] and mouse hepatitis virus.[126] The kinetics of translation and the kinetics of cleavage of several precursor polypeptides from murine leukemia virus to yield mature viral proteins in microinjected oocytes were studied extensively.[127] It was found that three large precursor polypeptides (Mr 77,000, 75,000, and 65,000) were initially synthesized. These were progressively processed to yield, first, several intermediate proteins and, finally, mature viral core proteins. It was further shown that the rate of synthesis of the precursor polypeptides decreased gradually postinjection, while immunoprecipitable viral proteins continued accumulating. This was interpreted to suggest that the viral core polypeptide products regulate processing or synthesis of the group-specific antigen precursors.[127]

3. Tissue-Specific Removal of "Pro" Sequences

Xenopus oocytes cannot remove *all* "pro" sequences from mRNA-directed translation products. The frog cell fails to remove the pro sequence from insulin.[69,128] Bee promelittin is not processed to yield mature melittin in the oocytes, since the removal of dipeptides from the NH_2 terminus of promelittin and the transamidation of its C terminus to become glutamine amide are both defective.[129] In contrast to proinsulin and pro-EGF, which are both secreted unprocessed, promelittin remains inside the oocytes. Thermodynamic calculations and the results of subcellular fractionation experiments suggested that the hydrophobic promelittin molecule is trapped within the oocytes' intracellular membranes,[13] perhaps due to the defective processing of the C terminus. Altogether, the failure of the oocyte to remove "pro" sequences from a number of secretory proteins suggests that this removal is performed by tissue-specific cleavage enzymes and that these enzymes cannot be produced in the oocytes in their active forms, either because their mRNAs are missing from the injected preparations or because of defective post-translational processing events.

B. Site-Specific Modifications of mRNA-Directed Polypeptides

The mRNA-injected oocyte can carry out a large range of site-specific enzymatic modifications of nascent translation products, including phosphorylation, hydroxylation, glycosylation, and acetylation (see Table 4 for examples). It appears that all these reactions are carried out by endogenous oocyte enzymes, since they also take place when purified mRNAs are injected, and yet, the foreign-mRNA-directed heterologous proteins are all modified in the manner expected of their parental cell types.[13] This suggests that it is the nature of the injected mRNA and the structure of its polypeptide products, rather than the spectrum of oocyte enzymes, that determines the major steps in the processing pathway. A few examples for such processes are covered in the following sections.

1. Glycosylation of mRNA-Directed Proteins

Immunoglobulin kappa chains directed by mRNA from mouse spleen or plasmacytoma cells are glycosylated in the oocytes,[44] but a mutant kappa chain remains unglycosylated.[42] Many other mRNA-directed proteins were also found to be glycosylated in the oocytes. However, the glycosylation was, in many cases, incomplete. Thus, the glycosylation of the alpha subunit of human chorionic gonadotropin (HCG) was found to be incomplete in the oocytes as compared to the human placenta.[131] Several [^{3}H]mannose residues were incorporated into the oocyte-produced HCG, as expected. However, in the oocyte the HCG-bound oligosaccharide was sensitive to alpha-mannosidase, whereas the native HCG ends with sialic acid and is insensitive to alpha-mannosidase.[131] This is true, also, for the oligomeric rat prostatic binding protein (PBP), where the intracellular oocyte product contained an incompletely processed oligomannosyl core unit; upon secretion, this sugar core was shown to be processed further, probably at random, since the resulting secreted PBP molecules displayed a heterogeneous pattern of carbohydrate chains attached to the exported molecules. Furthermore, tunicamycin experiments showed that glycosylation was neither a prerequisite for secretion nor for the assembly of PBP in oocytes.[49,131]

2. Phosphorylation of Oocyte Proteins, of mRNA-Directed Proteins, and of Injected Substrates

When mRNA from trout testis was injected into the oocytes, it induced the formation of phosphorylated protamine.[91] A protein kinase in the oocytes is also capable of phosphorylating injected substrates, such as the synthetic peptide Leu-Arg-Arg-Ala-Ser-Leu-Gly, which closely resembles the phosphorylation site sequence in porcine hepatic pyruvate kinase.[133] Kinase activity in the oocytes is regulated by progesterone, as indicated from the finding that the S6 ribosomal protein from oocytes is differently phosphorylated after progesterone stimulation of the oocytes.[135] However, similar to the other post-translational processing activities, there is evidence for tissue specificity resulting in deficient phosphorylation of mRNA-directed proteins. Thus, the oocytes lack significant casein kinase activity and the proteins made from injected mammary gland mRNA cannot be labeled with ATP or phosphates. Furthermore, the oocyte-produced caseins will only electrophorese similarly to guinea pig milk caseins following phosphatase treatment of the latter, indicating that in the oocytes caseins remain underphosphorylated.[13]

C. Assembly of Multisubunit Proteins in mRNA-Microinjected Oocytes

As already mentioned, several mRNA-directed translation products have been shown to assemble into multisubunit proteins in microinjected oocytes. These include enzymes such as beta-glucuronidase and acetylcholinesterase, structural proteins like alpha- and beta-crystallin, and receptors such as prostatic binding protein and the nicotinic acetylcholine receptor. The mode of assembly within *Xenopus* oocytes was analyzed in considerable detail for several mRNA-directed proteins. It was shown that beta-crystallin fails to assemble correctly, and that beta-glucuronidase subunits may form hybrids with the cognate endogenous subunits of the oocyte enzyme. Thus, the oocyte capacity to sort and assemble foreign proteins in a correct manner is limited. It is possible that such hybrids may form from many proteins of many cell types if the opportunity exists and that a tissue-specific barrier for the selective assembly of proteins has not been needed and, therefore, did not evolve. This would imply that the evolutionary change responsible for the appearance of cell-type specific isoforms of such multisubunit proteins did not involve structural modifications in the protein domains that are important for the intersubunit interactions.

1. Interactions of Mouse Immunoglobulin Complexes within Xenopus Oocytes

mRNA from several immunoglobulin-producing cell lines was injected into oocytes in order to investigate various aspects of immunoglobulin assembly. Both heavy and light chains should be synthesized in the oocytes to be secreted. Thus, covalently bound H_2L_2 tetramers, but not the L_2 dimers, are efficiently secreted, both in their originating cell lines and in microinjected oocytes.[136] Immunoglobulin assembly, therefore, appears to be a necessary prerequisite for secretion.[44] Messenger RNA for the complementary chain may, however, be injected 24 hr later, indicating that nascent immunoglobulin subunits either remain marooned on the secretory route, or continue to be translated and are available for assembly with newly forming complementary chains.[134] Furthermore, tetrameric H_2L_2 immunoglobulin molecules were produced in oocytes into which heavy and light chain mRNAs were injected separately, at diametrically opposed sites. This indicated that diffusion of either the mRNAs or their membrane-sequestered products must occur throughout the cytoplasm, since microinjection was performed beneath the oocyte plasma membrane.[14]

Recently, microinjection of heavy and light chain mRNAs, purified by hybridization selection, was shown to be sufficient to induce correct assembly and secretion of H_2L_2 complexes. This indicates that other tissue-specific mRNAs are not involved in this process. When injected with mRNA from a mouse hybridoma cell line producing monoclonal antibodies against chick albumin, the oocytes secrete such antibodies. Coinjection, or even a later injection of ovalbumin mRNA into these oocytes, resulted in the formation of antigen-antibody complexes within the internal membranes of the oocyte, and in a severe reduction in secreted antibody.[202] This elegant experiment clearly indicated that monoclonal antibodies and their antigens can become spatially apposed in the oocyte before being exported, even if their synthesis is temporally separated.[14]

2. Antigen-Binding Activity of Suppressor T-Cell Factor

Antigen-specific factors from suppressor T lymphocytes possess Ig determinants with antigen binding affinity and I-J products of genes in the H_2-complex. Each of these is synthesized independently, but they are secreted as an associated complex, capable of suppressing antibody response. When injected into oocytes, mRNA from T-cell hybridoma induces the secretion of active antigen-specific suppressor complexes; furthermore, the mixture of the translation products of the separate mRNA fractions coding for the two protein components was shown to reconstitute the antigen-specific suppressing activity.[137] Covalent S-S bonds were shown to be formed between the two protein components and the *in ovo* reconstituted factor displayed full biological activity in suppressing hybridoma cells in culture.[138]

D. Segregation of Foreign Active Proteins into Various Subcellular Compartments in *Xenopus* Oocytes

What are the mechanisms controlling the sequestration of proteins in particular subcellular compartments? When, in the route of production, does the sorting of nascent proteins take place? These and related questions in cell biology have been asked during the last decade with an increasing use of mRNA-injected oocytes, in combination with subcellular fractionation techniques. Various mRNA-directed proteins were, thus, found to segregate, as they do in their orginating tissues, into secretory vesicles on their way to be exported into the surrounding medium or into the oocyte membrane, nucleus, protein storage bodies, or yolk platelets. Examples for such segregation processes are presented in Table 5 and will be discussed in the following sections.

Table 5
SEGREGATION OF FOREIGN ACTIVE PROTEINS INTO VARIOUS SUBCELLULAR COMPARTMENTS IN *XENOPUS* OOCYTES

Segregation	Protein	Ref.
Export	Guinea pig milk proteins	140
	Plasminogen activator	19
	Acetylcholinesterase	29,30
	Pro-insulin	68
	Pro-EGF	67
	Interferon	62,77
	Plant storage globulin	203
Sequestration in vesicles	*Xenopus* liver albumin	139
	Mouse immunoglobulins	43
	Synthetic leader peptide	70
Insertion as integral membrane protein	Rat liver epoxide hydratase	
	Cytochrome P-450	28
	Acetylcholine receptor	192
Assembly into yolk platelets	*Xenopus* liver phosvitin and lipovitelin	13
Assembly into ribosomal subunits	*E. coli* and *Artemia salina* acidic proteins	148
Assembly into protein storage bodies	Maize storage proteins	146
Entry into nuclei	*Xenopus* nuclear proteins	155,156
	Karyophilic proteins	154,156

1. Export of mRNA-Directed Proteins into the Oocyte Incubation Medium

Sucrose gradient centrifugation of oocyte homogenates gave the first sign that nascent chains of albumin, milk proteins, and vitellogenin are sequestered in membranous vesicles.[139] Mouse interferon was, subsequently, found in the incubation medium of mRNA-microinjected oocytes.[52] Using coinjection experiments, Colman and Morser established that only the secretory proteins were exported from the oocytes and combined the various bits of information into a clear picture of a secretory system.[140] Since then, it has been established that mRNA-microinjected *Xenopus* oocytes secrete proteins made by mRNAs from many tissue sources (see Table 5 and previous sections for examples). Thus, microinjection of mRNA from a secretory tissue such as the mouse submaxillary gland results in the appearance of several mRNA-directed proteins in the incubation medium of the oocytes, while others remain in the cytosol (Figure 3). The sorting mechanism is highly selective, so that oocyte-secreted albumin comigrates with liver albumin on two-dimensional gel electrophoresis,[142] and miscompartmentalized proteins are rapidly degraded.[141] The secretion mechanism requires cotranslational events since nascent chains translated in vitro cannot be exported.[143]

The oocytes themselves, as well as their surrounding follicular cells, also secrete specific proteins via the exocytotic release of cortical vesicle content (Figure 4). However, the follicle cells are not required for secretion of either oocyte or mRNA-directed proteins.[18]

What are the factors governing access to the export pathway? It appears that transfer of nascent ovalbumin chains through the endoplasmic reticulum, but not glycosylation, is a prerequisite for secretion.[145] Immature growth factors, such as proinsulin[68] and Pro-EGF,[67] are also secreted, indicating that complete processing is not necessary for secretion. This conclusion is in line with the recent observation that the precursor polypeptides of the major bean storage globulins, legumin and vicilin, are secreted from bean mRNA-injected oocytes without proteolytic cleavage of the leg-

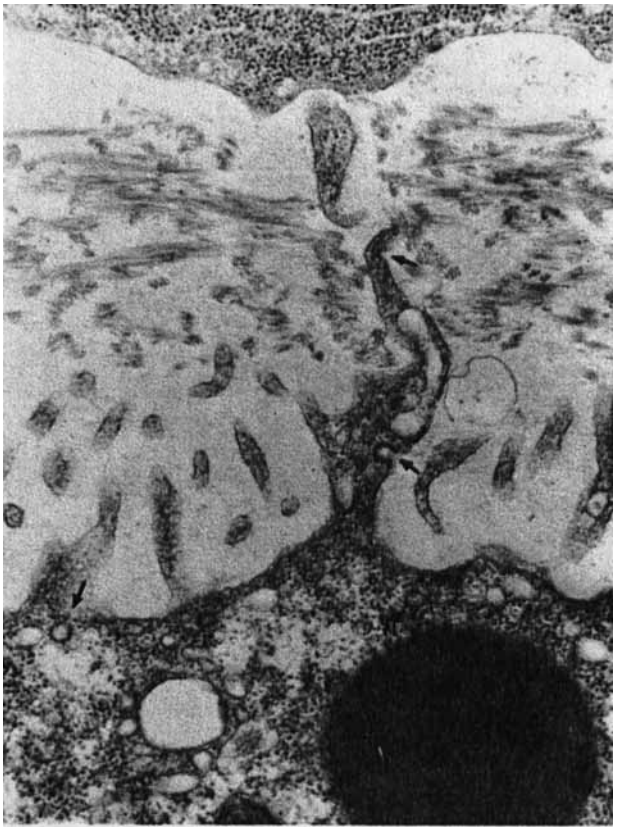

FIGURE 3. Secretion of mouse submaxillary gland proteins from mRNA-injected oocytes. Oocytes were microinjected with [^{35}S]-cysteine and Barth medium (−), sucrose-gradient fractionated mRNA, sedimenting as 7-12 S (A), or total poly(A$^+$)RNA (B). Incubation was for the noted time periods. Samples from the incubation medium (right) and oocyte homogenates (left) were separated by gradient polyacrylamide gel electrophoresis and the gels were autoradiographed. Cysteine-labeled products of a reticulocyte lysate primed with the same 7-12S fraction of mRNA are shown for comparison (in vitro). Note the selectivity of the secretion process and the relatively delayed accumulation of foreign mRNA-directed polypeptides in the incubation medium.

umin propolypeptides into the mature disulfide-linked alpha and beta chains, and that secretion is not affected by tunicamycin.[203] Secretion appears to be delayed in time compared with the appearance of mRNA-directed proteins in the oocyte cytosol (e.g., see references[11,13], and Figure 3) and the rates of secretion of different proteins, such as ovalbumin and lysozyme, for example, are remarkably different.[13] The signal peptides of nascent secretory polypeptide chains operate as regulatory elements in the pathway for secretion, by competing for available membrane sites. In addition, the signal peptide selectively accelerates the rate of secretion of already processed and sequestered secretory proteins[70] (see, also, Section IV. A.1). Colchicine, in contrast, inhibits the export of heterologous proteins from mRNA-microinjected oocytes and its inhibition is enhanced synergistically by cytochalasin, suggesting that microtubules stablized by microfilaments are required for the release of foreign proteins from oocytes.[145] Export of proteases via secretory membrane vesicles (Figure 4) appears to take

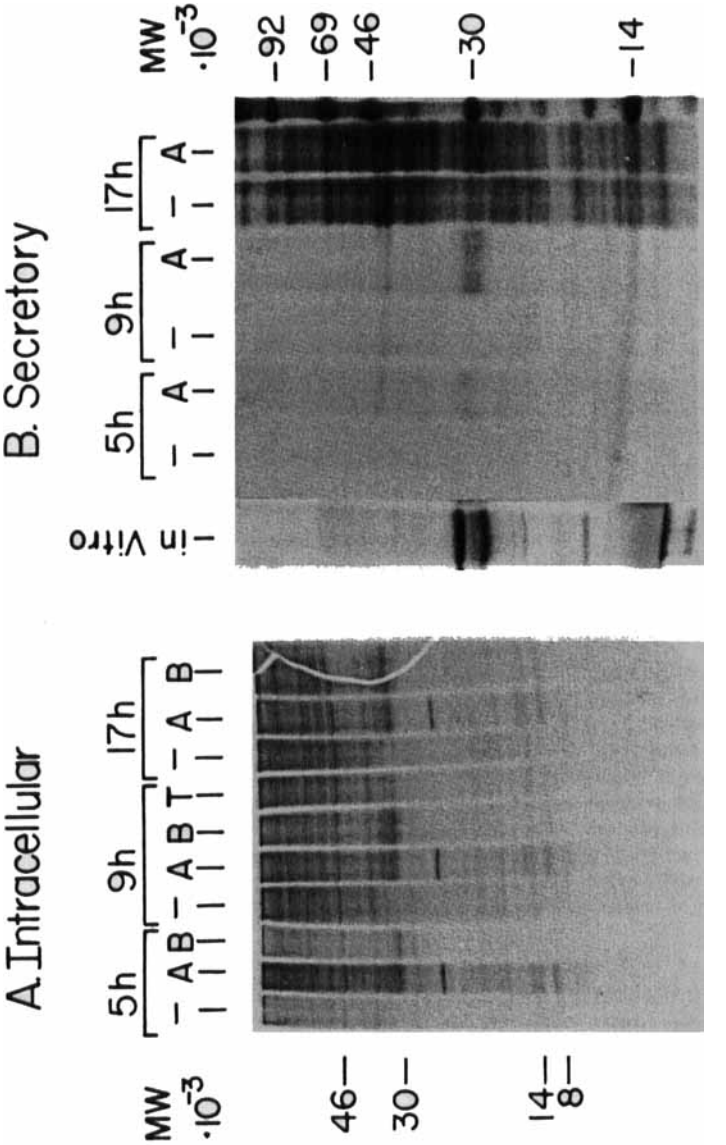

FIGURE 4. Electron micrograph of coated vesicles at the vicinity of the oocyte surface membrane. See text for details.

place, also, in noninjected oocytes and may affect the apparent rate of turnover of mRNA-directed secretory proteins.[77]

2. Incorporation of mRNA-Directed Proteins into Oocyte Organelles

How is the entry of proteins into various subcellular organelles in the oocyte regulated? Is mouse beta-glucuronidase correctly inserted into the oocyte lysosomes and, if so, is this process regulated at the cotranslational level like the secretion pathway or the insertion of membrane proteins? The few studies in which the issue of subcellular sorting mechanisms has been approached indicate that the recognition signals are general, and that the sorting of proteins may be regulated, either by the protein or by the mRNA. Thus, barley seed storage proteins remain within the oocyte, whereas secretory plant proteins are exported.[146] and maize storage proteins form structures in the oocytes that resemble plant protein bodies.[143] It, therefore, appears that the mRNA coding for these proteins also includes the information required to form such storage bodies. It would be interesting to compare these intracellularly deposited proteins with the secretory proteins directed by bean mRNA.[203] In contrast, the subcellular distribution of nucleic acid binding proteins seems to be determined by the properties of the mature protein itself. When poly(A)-binding protein from *Artemia salina* was injected into the oocytes, it accumulated in the nucleus.[148] Although this protein was labeled by methylation, which would be expected to alter its normal turnover rate in the oocyte,[113] this should not affect the sorting machinery of the oocyte. That nuclear proteins in various species share common structural properties is also indicated from biochemical, immunological, and cytochemical studies.[150,152] Thus, sorting mechanisms to the various subcellular compartments are regulated at different steps in the gene expression pathway, and *Xenopus* oocytes are particularly promising as a model system in which these sorting mechanisms can be studied.

V. INTRINSIC PROPERTIES OF *XENOPUS* OOCYTES: POTENTIAL INVOLVEMENT AND IMPACT ON PRODUCTION AND DETECTION OF mRNA-DIRECTED PROTEINS

All of the steps leading to the formation and the correct deposition of processed active proteins can be studied in the oocytes. Particularly advantageous is the generality of this system, leading to the presence of protein synthesis machinery that lacks both cell-type and species specificity. However, the oocyte should by no means be regarded as an inert system. Like other cell types, it has its own specialized properties. These might interfere with specific steps in the pathway of gene expression, and alter the level of detectable mRNA-encoded protein. The interference might occur in various ways, part of which are summarized in Table 6 and discussed in the following sections.

A. Oocyte Constituents Affecting the Rate of mRNA-Directed Protein Synthesis

When injecting mRNA into oocytes, one assumes that the capacity of the oocyte to translate this injected mRNA is the same between different frogs and in different seasons. However, this general assumption is not justified, and both the rate of oocyte protein synthesis and the different pools of protein synthesis machinery items are prone to various modes of regulation. Furthermore, proteins directed by the injected mRNA itself might evoke a chain of reactions leading to changes in the rate and nature of oocyte protein synthesis.

1. Composition of Oocyte RNA

The specialized nature of the *Xenopus* oocyte is clearly reflected in the composition of its RNA species and in the developmental and maturation-related changes occurring

Table 6
PROPERTIES OF *XENOPUS* OOCYTES WHICH MIGHT AFFECT THE PRODUCTION AND/OR DETECTION OF mRNA-ENCODED PROTEINS

Property	Regulated by	Might affect	Ref.
Pool of oocyte RNAs and tRNAs	Oocyte development Hormones	Translation efficiency	152,160,161,165
Segregation of oocyte mRNAs	Oocyte development	Competition on polysomes	152 — 159
Nonribosome-bound 5SRNA	Oocyte development	Translation efficiency	161
Lysosomal enzymes, hydrolases, proteases	Maturation	Product measurement	77,181
Maturation of oocytes	Season Hormones Carbon dioxide	Rate of protein synthesis	165,190 176,187 178
Amino acid pool	Amino acid uptake	Intensity of labeling	187
Endocytosis	Maturation	Measurements of produced proteins	176 115
Phosphorylation-related enzymes			
Adenylate cyclase AMP-phosphodiesterase Phosphatase 1	Maturation of oocytes	Phosphorylation of foreign proteins	177 179 190
Membrane channels	Maturation	Rate of protein synthesis	201
Intracellular pH change	Maturation	Rate of protein synthesis	199
Acetylcholine receptors	Season	Rate of protein synthesis	190 196
Acetylcholine esterase	Season (possibly, hormones)	Measurement of ChE induced	7

in this RNA pool.[85] Screening of a *Xenopus* cDNA library revealed that most of the oocyte mRNAs cease accumulation early in oogenesis and remain at a constant steady-state level for the remainder of oocyte development, while mitochondrial mRNAs accumulate throughout oogenesis.[152] *In situ* hybridization, employing [^{3}H]-poly(U) as a probe was performed to examine the spatial distribution of poly(A)$^+$RNA species within developing oocytes. It was found that early in oocyte development most of the poly(A) sequences were localized in the nucleus, whereas in fully grown oocytes most of the poly(A) was detected in the cytoplasm.[153]

The spatial distribution of poly(A)$^+$RNA in the oocyte cytoplasm was studied in further detail and was found to undergo developmental changes. Poly(A)$^+$RNA is uniformly distributed in the cytoplasm of previtellogenic oocytes, whereas distinct cytoplasmic regions exhibiting high concentrations of poly(A)$^+$RNA were detected in vitellogenic and fully grown oocytes. These regions change with the oocytes' state of development. In stage 6 oocytes, which are commonly used for microinjection, the area most enriched in poly(A)$^+$RNA was found to be the vegetal subcortical region. Upon oocyte maturation, uniform distribution of poly(A)$^+$ reappears.[154] It has recently been suggested that supramolecular membranous structures are implicated in the mobilization of maternal mRNA in the oocytes[155] and that actin and tubulin are involved in poly(A) metabolism of oocyte mRNA via modulating the activity of poly(A) metabolizing enzymes.[156] In particular, it was postulated that the nucleo-cytoplasmic transport of poly(A)-containing mRNA is mediated by nuclear envelope nucleoside triphosphatase, stimulated by poly(A) and modulated by microtubule protein.[156] RNA binding proteins are also developmentally regulated during oogenesis and have been suggested to be involved with regulating the stability or translatability of oocyte mRNAs.[157]

The presence of complementary, interspersed repetitive sequences on different

poly(A)⁺RNA molecules of maternal origin in the oocyte cytoplasm (but not in *Xenopus* tadpoles) was shown by electron microscopy of reannealed oocyte mRNA. This large pool of poly(A)⁺RNA appears to turn over slowly throughout oogenesis[158] and to be stored in an untranslatable form.[159] In addition to stored mRNA, the previtellogenic oocyte contains large pools of stored 5S RNA and tRNA. These are kept for use in later oogenesis for protein synthesis and ribosome assembly and are stored in ribonucleoprotein particles.[160] Most of the 5S RNA stored during oogenesis is incorporated into ribosomes at later stages. However, about 20% of it remains in the form of 7S ribonucleoprotein particles in full-grown oocytes.[161]

The 5S RNA in oocytes is transcribed from oocyte-specific genes, which are not expressed in somatic *Xenopus* cells. In contrast, the genes coding for somatic-type 5S RNA are expressed in both oocytes and somatic cells. The finding of a species whose oocytes do not express these genes ("nonactivating" oocytes) made it possible to examine the mechanism of regulation of the 5S RNA genes. It was found that the expression of the oocyte-type 5S RNA genes is regulated by nuclear protein(s), since developmentally inactivated oocyte-type genes were reactivated when erythrocyte nuclei were injected into "activating" oocytes or when nuclear extracts from "activating" oocytes were injected into "nonactivating" ones.[162] The transcriptional changes that occurred in somatic nuclei injected into oocytes were highly selective and only affected a specific minority of genes,[163] but did not cause gross chromosomal changes.[164] The selectivity of transcriptional changes induced by specific nuclear proteins in the oocytes raises several questions related to the functioning of *Xenopus* oocytes as a heterologous system for gene expression. Thus, translation products of injected mRNAs might have regulatory functions that modify the rate of transcription and/or translation by endogenous oocyte mRNAs in an unexpected manner.

2. Oocyte Development and Maturation

What are the natural environmental conditions that induce transcriptional and translational changes in the oocytes? The most obvious inducers are, of course, oocyte development and hormonal-induced maturation. Oogenesis of *Xenopus* oocytes is accompanied by accumulation of enormous amounts of mitochondria. Mitochondrial DNA is mostly synthesized in previtellogenic oocytes,[177] where most of the mitochondria are aggregated in a spherical mass. In contrast, the mitochondrial population of vitellogenic oocytes consists mainly of isolated organelles, which continue replicating throughout the vitellogenesis period.[179]

Injection of *Xenopus* females with human chorionic gonadotropin (HCG), or incubation of oocytes in vitro in the presence of progesterone, induces the resumption of meiosis from prophase I to metaphase II (meiotic maturation). This maturation is under post-transcriptional control and is inhibited by carbon dioxide.[178] Oocyte maturation results in considerable induction in synthesis of both stable and unstable RNA species,[165] in parallel to the appearance of a maturation-related protein designated Maturation Promoting Factor. The numerous effects of hormonal-induced maturation are reflected in changes in various activities in the oocyte. Some of these have possible implications in the pathway for production of active proteins. Others might interfere with measurements of mRNA-induced proteins (see Table 6).

Maturation can be induced, even in the absence of progesterone, by drugs that bind to calcium-regulating proteins, such as Trifluoperazine,[167] by drugs that increase K^+ influx via the inhibition of adenylate cyclases,[168] and by agents that stimulate Na^+-K^+ pump activity, such as bee melittin.[169] These observations indicate that the induction of oocyte maturation is affected by ion influx through the oocyte surface membrane. Indeed, *Xenopus* oocytes possess steroid receptors on their surface membrane.[170] Furthermore, the follicular envelope is involved in the initiation of oocyte maturation; in

defolliculated oocytes, gonadotropins do not induce maturation and the rate of progesterone-induced maturation is considerably decreased.[166] Following the binding of progesterone to the surface membrane or to intracellular membrane sites,[171] a rapid and persistent decrease occurs in cyclic AMP (cAMP) concentration and the synthesis of maturation-promoting factor takes place.[172] The progesterone inhibition of oocyte adenylate cyclase is dependent on guanine nucleotides[173] and is associated with calmodulin synthesis.[174] The newly synthesized calmodulin, in turn, activates soluble cyclic nucleotide phosphodiesterases in the oocytes.[175] The increase in calmodulin levels enhances the rate of vitellogenin endocytosis; calmodulin inhibitors such as stelazine inhibit considerably the uptake of vitellogenin.[176] Most of the protein stored in the oocytes, including vitellogenin, is accumulated in yolk platelets. These are related to lysosomal structures, enriched in acid hydrolases,[180] and it has been suggested that fusion of lysosomes with yolk platelets is involved in yolk digestion. Release of hydrolases, in turn, will induce proteolysis. From the above it is clear that development and maturation-related processes in the oocytes[186] might have serious effects on the entire array of biosynthetic processes leading to the mRNA-directed production of biologically active proteins.

3. Maturation-Related Effects on mRNA Recruitment

The endogenous oocyte RNA is markedly deficient in abundant mRNA species in comparison with somatic mRNAs, as revealed by cDNA hybridization. In fully grown oocytes, less than 10% of the mRNA is actively engaged in protein synthesis.[186] Upon hormonal-induced maturation, the general rate of protein synthesis increases considerably.[181,183] This reflects an overall increase in the synthesis of individual polypeptides, as has been shown by two-dimensional gel electrophoresis of oocyte-produced proteins.[187] The average size of oocyte polysomes and the ribosome transit time of oocyte mRNAs remain apparently unchanged in mature oocytes,[188] indicating that the maturation-related increase in oocyte protein synthesis is due primarily to mRNA recruitment, rather than a change in translational efficiency. This process results in a preferential increase in synthesis of several specific proteins, such as ornithine decarboxylase[184] and the protein kinase phosphorylating ribosomal protein S6.[185]

4. Other Alterations in Oocyte Protein Synthesis

Hormonal-induced maturation is not the only cause for changes in the rate and pattern of protein synthesis in the oocytes. Oocyte protein synthesis also depends on environmental conditions, such as CO_2 concentration.[178] The oocytes respond to high (>31°C) temperature by the synthesis of one major (Mr 70,000) protein, and by a gradual reduction in the rate of normal protein synthesis. This heat-shock response is controlled exclusively at the translational level, since enucleated or alpha-amanitin-injected oocytes synthesize normal levels of heat-shock protein. It is, therefore, an example of a specific species of mRNA, which is synthesized in the oocytes under normal conditions and stored in an untranslatable state unless released by the induction of high temperature.[191] One may, therefore, postulate that the oocyte contains other such stored mRNAs, which might be induced by yet unknown signals, such as, for example, the translation product(s) of an injected mRNA species. In addition to this selective mode of induction, it is possible that certain translation products have a similar function to that of the maturation-promoting factor and enhance the general rate of protein synthesis in the oocyte. Indeed, microinjection of adenovirus RNA was shown to stimulate the rate of oocyte protein synthesis.[188] As in the case of the heat-shock response, this induction does not require nuclear transcription in the oocyte. Similar to the maturation-related enhancement in protein synthesis, it does not involve changes in the efficiency of translation. In contrast to the heat-shock response, it did

not induce the synthesis of particular proteins but appeared to operate as a general enhancer, suggesting that it affected a protein operating as a regulatory element in translational processes. Maximal induction was observed 18 hr postinjection, indicating that the enhancement was caused by a translation product of the injected mRNA.

The enhancement effect on oocyte protein synthesis is not unique to adenovirus mRNA. A reproducible general enhancement in ^{35}S-methionine incorporation into oocyte proteins was also observed upon the injection of poly(A)$^+$RNA from the human epidermoid carcinoma HEp_3.[19,208] It is, therefore, possible that common mechanisms operate in different types of eukaryotic cells, whereby the rate of protein synthesis may be changed on demand. It is not clear at this stage whether similar protein(s) are involved in the induction of protein synthesis in maturing oocytes, in viral-infected cells, and in transformed cells. However, the *Xenopus* oocyte is clearly a useful system to approach this issue.

It is interesting to note that the average rate of protein synthesis may vary considerably between oocytes derived from different frogs and is variable with seasons.[209] Is it possible that the seasonal changes in the rate of oocyte protein synthesis are related to hormonal regulation, occurring at the reproductive season? That this is, indeed, the case is suggested from the seasonal changes that were observed in specific membrane constituents of the oocytes, as described in Section V.B.

B. Oocyte Surface Membrane Properties as Implicated in Protein Synthesis

1. Ion Channels in the Oocyte Surface Membrane

The surface membrane of *Xenopus* oocytes contains a variety of ion channels, controlled by binding of specific ligands to receptors residing in this membrane.[192] Activation of cholinergic, adrenergic, and dopaminergic receptors leads to the transient opening of Cl^- and K^+ channels in the membrane. The oocyte membrane also contains Cl^- channels that open and close "spontaneously", perhaps owing to the buildup and decay of an activating substance within the cell.[199] In addition, changes in the membrane potential of the oocytes can activate electrical channels in its membrane. These include, for example, a calcium-dependent transient outward current[192] and an electrically gated Na^+ channel, blocked by tetrodotoxin,[201] which controls the transport of amino acids such as leucine[193] and alanine [194] into the oocytes.

The role of these various membrane channels is still unclear. Hormonal-induced maturation of *Xenopus* oocytes was found to be accompanied by delayed, transient changes in the membrane input resistance, potential, and capacitance.[197] In sea urchin eggs, a strong correlation has been demonstrated between intracellular pH and the rate of protein synthesis after activation[198] and progesterone-induced maturation of *Xenopus* oocytes results in a transient average increase of about 0.18 pH units. Furthermore, lowering the oocyte intracellular pH inhibits maturation.[199] pH changes may, therefore, induce the movement of ions in and out of the maturing oocyte and be, thus, involved in controlling the level of protein synthesis.

2. Cholinergic Elements in the Oocyte Surface Membrane

The fully grown *Xenopus* oocyte is by no means a "dormant" cell, nor is it an unspecialized one. The contribution of the oocyte to the measured biosynthetic processes should be carefully considered in interpreting microinjection experiments, particularly those aimed at the production of biologically active proteins. An example for such a case is the expression of cholinergic proteins in control and mRNA-microinjected oocytes.

As mentioned above, the oocyte surface membrane displays an electrical muscarinic response to acetylcholine.[195,196] This response may be separated into four components, each with a different sensitivity to acetylcholine, a different dependence on the extra-

cellular calcium concentration, and a different seasonal variation.[196] The maximal amplitude of the responses was much lower in winter (October to March) than in summer. Further, the minimal concentration of acetylcholine necessary to elicit a detectable response was higher in winter.[196] This is particularly interesting in view of the parallel changes we found in the specific activity of oocyte acetylcholinesterase, which competes with the receptor for the applied acetylcholine. Significant seasonal differences were observed in the levels of both the secreted and intracellular enzyme. These were reproducibly much higher in summer than in winter in experiments carried out during 3 successive years (Figure 5). Furthermore, summer oocytes, which display higher AChE levels and more intense cholinergic responses, are also more efficient in translating brain mRNA to yield active mammalian AChE.[209] Since seasonal variations in the efficiency of translation have not been reported or observed for other injected mRNAs, it is most likely that the limiting step is not translation itself, but post-translational processing of the nascent product of AChEmRNA to yield the active enzyme. A seasonal limitation in the level of oocyte processing enzyme(s) could, thus, be responsible for both the change in the level of endogenous AChE and the difference in the apparent efficiency of translation of AChEmRNA.

What are the hormonal changes underlying these seasonal variations in oocyte gene expression and membrane properties? The content of leuteinizing hormone-releasing hormone (LHRH) in the hypothalamus of *Xenopus* frogs appears to vary in relation to season and reproductive state. Hypothalamic LHRH content was low in sexually quiescent frogs, during the nonbreeding season, whereas LHRH concentration was high in reproductively active frogs (October to March). These observations led to the suggestion that environmental cues stimulate the reproductive system in the frog by increasing LHRH production and secretion.[189] There is, therefore, an inverse relation between the level of *Xenopus* LHRH and the appearance of cholinergic elements in *Xenopus* oocytes. The muscarinic response in mammalian oocytes has been shown to disappear upon fertilization[190] and it is possible that a parallel process occurs in amphibian oocytes, decreasing the level of cholinergic elements during the reproductive period. If, indeed, the decrease in occyte AChE is hormonally regulated at the post-translational level, it would explain the inefficiency of winter oocytes in translating AChEmRNA. Similar seasonal limitations might exist and should be taken into account in studying the synthesis of other active proteins.

VI. CONCLUSIONS

In introducing *Xenopus* oocytes as a translation system, Gurdon stated (1971) that mRNA-microinjected oocytes "provide a very sensitive assay system for the identification of messenger RNA, and permit the study of translational control in living cells."[1] Since then, the oocytes have amply fulfilled this and much more. The large number of studies reviewed above summarize the many sorts of problems that have been attacked by mRNA microinjection into oocytes. The diversity of questions that can be asked profitably is extended by the ability of these cells to utilize, in addition to mRNA, injected DNA and protein. Furthermore, the fact that the intact oocyte produces biologically active proteins makes it advantageous for studying the roles of any of these diverse materials in inducing or modifying the ultimate product of the genetic determinant (e.g., see Jones et al.).[206]

The rapid advance of molecular genetics has led to the development of several other heterologous systems for the expression of foreign genes and mRNAs (for representative examples, see Lane).[13] One naturally tends to ask what the major benefits are and the deficiencies of the oocyte system as compared to others. In answering these questions, one should distinguish between the use of the oocytes as a translation system per

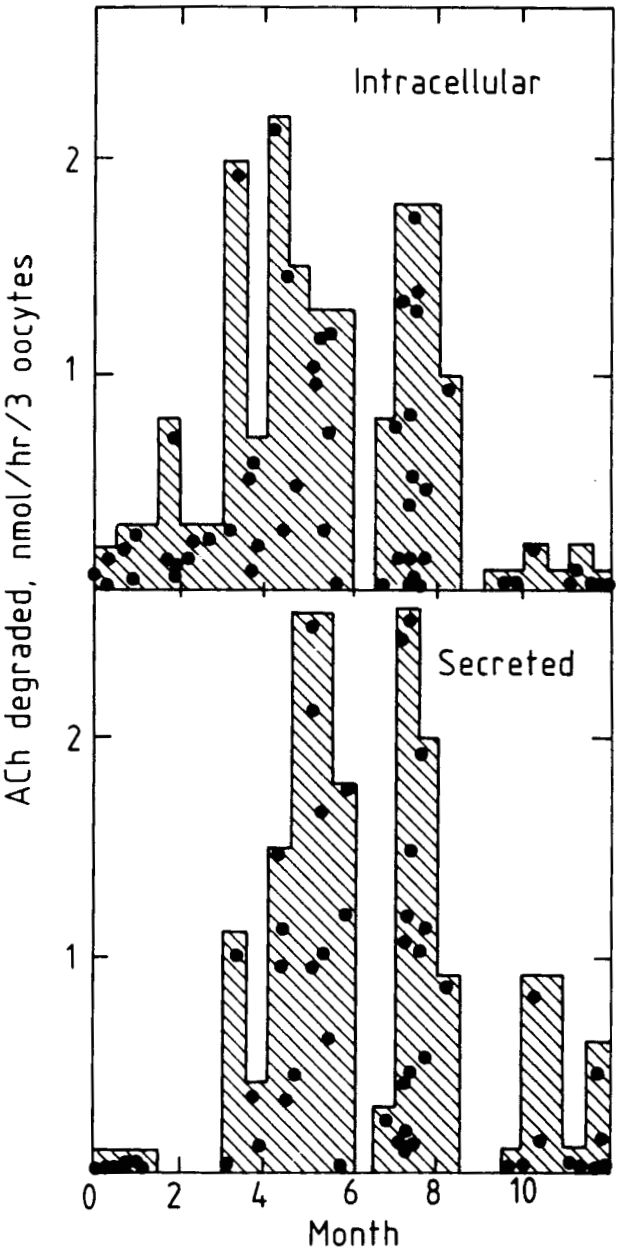

FIGURE 5. Seasonal variations in the level of intracellular and secreted cholinesterase activities of stage VI *Xenopus* oocytes. Cholinesterase activity was measured radiometrically in samples of oocyte homogenates and incubation medium.[38] Experiments were carried out during 3 successive years, on ten oocytes/sample and in triplicates.

se and their application to study regulatory mechanisms. Throughout all applications, the technical requirements involved with oocyte microinjection, as compared to other methodologies, should be evaluated.

The most obvious advantage of the oocytes is their large size, which makes microinjection an easier and faster process than similar manipulations with mammalian cells.[206] An equally important technical consequence of their dimension is the amount

of material that can be accommodated in the oocyte and the corresponding large amount of product synthesized by single cells. It has been demonstrated with globin mRNA and the RNA_4 from alfalfa mosaic virus that it is possible to detect the proteins translated on these mRNAs in microinjected mammalian cells by the classical two-dimensional gel electrophoresis.[99,104] However, the detection of protein products in injected mammalian cells is often limited to histochemical techniques. Alternatively, elaborate micromethods have to be employed. Thus, only when the signal is a replicating one, such as in the case of microinjecting retroviral mRNAs,[205] is it feasible to monitor such proteins with conventional biochemical techniques. In contradistinction, the amount of foreign protein produced in mRNA-microinjected *Xenopus* oocytes is conveniently sufficient to perform detailed biochemical studies.

The technical advantages of the oocytes are equally important for both qualitative demonstration of gene products and analyses of regulation of gene expression. Until now, the former application has dominated the field. The number of studies focusing on mechanisms regulating specific steps in the gene expression pathway is still rather limited. This could simply be due to the fact that qualitative studies are relatively easy to perform compared with experiments aimed at resolving regulatory mechanisms. However, it is possible, as already suggested,[207] that the oocytes are inherently limited as a surrogate system for gene expression. That this is, indeed, the case is indicated from the many examples of defective post-translational processing events that have been detected in the oocytes. Thus, it is likely that mRNA translation per se is an interchangeable process, similar in different cell types. On the other hand, it appears that the regulation of other steps of gene expression might be tissue or cell-type specific. This, in turn, leads to the concern that the oocyte as host cell might interfere with the faithful expression of foreign molecules to a much greater extent than was originally assumed.

The indications are that relatively late events in the pathway for production of active proteins are mainly involved with tissue specificity of particular post-translational processes. For example, signal peptides appear to be regularly removed from nascent polypeptide chains and the core chain of glycosylic residues is generally added. In contrast, additional chains are almost always incorrectly glycosylated and other post-translational modifications are defective as well. Secretory proteins are generally exported but exceptions have been recorded. Though correct insertion of membrane proteins takes place, it has not yet been established how many of the nascent protein molecules are, indeed, inserted in a correct manner.

In spite of these serious problems and perhaps *in view* of them, it seems to be very important to elucidate the common elements in the pathway of gene expression. This obviously requires a detailed understanding of the processing and distribution of RNA and protein in representative cells. The progress already made in studying gene expression in the oocytes makes it imperative that these will continue to be a major cell type in studies to determine which steps are interchangeable and which are tissue or cell-type specific — and in which way. To exploit best the oocytes for such experiments, their inherent properties as a cell type should be further systematically pursued. The intensity of research in this direction makes it reasonable to assume that the use of the oocytes will continuously diversify in the future. As more investigators join in defining the intrinsic properties of oocytes, these cells will be further exploited to answer fundamental questions of cell biology.

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REFERENCES

1. Gurdon, J. B., Lane, C. D., Woodland, H. R., and Marbaix, G., Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells, *Nature (London)*, 233, 177, 1971.
2. Lane, C. D., Marbaix, G., and Gurdon, J. B., Rabbit haemoglobin synthesis in frog cells: the translation of reticulocyte 9S RNA in frog oocytes, *J. Mol. Biol.*, 61, 73, 1971.
3. Marbaix, G. and Lane, C. D., Rabbit haemoglobin synthesis in frog cells. II. Further characterization of the products of translation of reticulocyte 9S RNA, *J. Mol. Biol.*, 67, 517, 1972.
4. Graessmann, A. and Graessmann, M., Translation of mRNA injected into somatic cells, *Hoppe Zeylers Phys. Chem.*, 352, 527, 1971.
5. Gurdon, J. B., *The Control of Gene Expression in Animal Development*, Clarendon Press, Oxford, 1974.
6. Contreras, R., Cheroutre, H., and Fiers, W., A simple apparatus for injection of nanoliter quantities into *Xenopus laevis* oocytes, *Anal. Biochem.*, 113, 185, 1981.
7. Soreq, H., Bioassays of oocyte-produced brain enzymes, in *Molecular Biology Approach to the Neurosciences*, Soreq, H., Ed., John Wiley & Sons, New York, 1984, 205.
8. Asselbergs, F. A. M., Protein synthesis of heterologous messenger RNA in *Xenopus* oocytes, *Mol. Biol. Rep.*, 5, 199, 1979.
9. Kressman, A. and Birenstiel, M. L., *Transfer of Cell Constituents into Eukaryotic Cells*, Celis, J. E., Graessmann, A., and Loyter, A., Eds., NATO Advanced Study Institutes Series, Plenum Press, New York, 1980, 321.
10. Lane, C. D. and Knowland, J. S., The injection of RNA into living cells: the use of the frog oocytes for the assay of mRNA and the study of the control of gene expression, in *The Biochemistry of Animal Development*, Vol. 3, Weber, R. A., Ed., Academic Press, New York, 1975, 145.
11. Marbaix, G. and Huez, G., Expression of messenger RNAs injected into *Xenopus laevis* oocytes, in *Transfer of Cell Constituents into Eukaryotic Cells*, Vol. 31, Celis, J. E., Grassmann, A., and Loyter, A., Eds., NATO Advanced Study Institutes Series, Plenum Press, New York, 1980, 347.
12. Lane, C. D., Rabbit hemoglobin from frog eggs, *Sci. Am.*, 235, 60, 1976.
13. Lane, C. D., The fate of genes, messengers, and proteins introduced into Xenopus oocytes, in *Current Topics in Developmental Biology*, Vol. 18, Moscona, A. A. and Monroy, A., Eds., Academic Press, New York, 1983, 89.
14. Colman, A., Bahmra, S., and Valle, G., Post-translational modification of exogenous proteins in *Xenopus laevis* oocytes, *Trans. Biochem. Soc.*, in press.
15. Davidson, E. H., in *Gene Activity in Early Development*, Davidson, E. G., Ed., Academic Press, New York, 1976.
16. Gerhart, J.C., Biological regulating pattern formation in the amphibian egg and early embryo, in *Biological Regulation and Development*, Vol. 2, Goldberger, R. F., Ed., Plenum Press, New York, 133.
17. Dumont, J. N., Oogenesis in *Xenopus laevis* (Daudin). I. Stages of oocyte development in laboratory maintained animals, *J. Morphol.*, 136, 153, 1978.
18. Mohun, T. J., Lane, C. D., Colman, A., and Wylie, C., The secretion of proteins *in vitro* from *Xenopus* oocytes and their accessory cells: a biochemical and morphological study, *J. Embryol. Exp. Morphol.*, 61, 367, 1981.
19. Miskin, R. and Soreq, H., Microinjected *Xenopus* oocytes synthesize active human plasminogen activator, *Nucl. Acids Res.*, 9, 3355, 1981.
20. Opdenakker, G., Weening, H., Collen, D., Billian, A., and De Somer, P., Messenger RNA for human tissue plasminogen activator, *Eur. J. Biochem.*, 121, 269, 1982.
21. Miskin, A. and Soreq, H., Human and rat mRNAs are translated into urokinase-type and tissue plasminogen activators in microinjected *Xenopus* oocytes, in *Progress in Fibrinolysis*, Vol. 6, McLeod, C., Ed., Churchhill Livingston, Edinburgh, in press.
22. Soreq, H. and Miskin, R., Plasminogen activator in the developing rat cerebellum: biosynthesis and localization in granular neurons, *Dev. Brain Res.*, 11, 149, 1982.

23. Reynolds, F. H., Jr., Premkumar, E., and Pitha, P. M., Interferon activity produced by translation of human interferon messenger RNA in cell free ribosomal systems and in *Xenopus* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 4881, 1975.
24. Labarca, C. and Paigen, K., mRNA-directed synthesis of catalytically active mouse beta-glucuronidase in *Xenopus* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 4462, 1977.
25. Deacon, M. J. and Ebringer, A., Post translational modification of rat immunoglobulins synthesized in the *Xenopus* oocyte translation system, *Immunology*, 38, 137, 1979.
26. McKnight, S. L. and Gavis, E. R., Expression of the herpes thymidine kinase gene in *Xenopus laevis* oocyte: an assay for the study of deletion mutants constructed *in vitro*, *Nucl. Acid. Res.*, 8(24), 5931, 1980.
27. Cordingly, M. G. and Preston, C. M., Transcription and translation of the herpes simplex virus type 1 thymidine kinase gene after microinjection into *Xenopus laevis* oocytes, *J. Gen. Virol.*, 54(2), 409, 1981.
28. Ohlsson, I., Lane, D., and Guengerich, F., Synthesis and insertion, both *in vivo* and *in vitro*, of rat liver cytochrome P-450 and epoxide hydratase into *Xenopus laevis* membranes, *Eur. J. Biochem.*, 115, 367, 1981.
29. Soreq, H., Parvari, R., and Silman, I., Biosynthesis of acetylcholinesterase in rat brain and *Torpedo* electric organ is directed by scarce mRNA species, *Prog. Brain Res.*, 58, 107.
30. Soreq, H., Zevin-Sonkin, D., and Razon, N., Expression of cholinesterase gene(s) in human brain tissues: translational evidence for multiple mRNA species, *EMBO J.*, 3, 1371, 1984.
31. Meedel, T. H. and Whittaker, J. R., Development of translationally active mRNA for larval muscle acetylcholinesterase during ascidian embryogenesis, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 4761, 1983.
32. Sumikawa, K., Houghton, M., Emtage, J. S., Richards, B. M., and Barnard, E. A., *Xenopus* oocytes injected with mRNA from a human B-cell line: active multi-subunit ACh receptor assembled by translation of heterologous mRNA in *Xenopus* oocytes, *Nature (London)*, 292, 862, 1981.
33. Farber, N. M. and Zanjani, E. D., Translation of messenger RNA from human kidneys into biologically active erythropoietin following microinjection into *Xenopus* oocytes, *J. Lab. Clin. Med.*, 102, 681, 1983.
34. Burmeister, M., Studies on the Biosynthesis of Epidermal Growth Factor in Microinjected *Xenopus* Oocytes, M. Sc. thesis, Weizmann Institute of Science, Rehovot, Israel, 1983.
35. Shulman, L. and Revel. M., Interferon dependent induction of messenger RNA activity for 2′-5′ oligo-isoadenylate synthetase, *Nature (London)*, 288, 98, 1980.
36. St. Laurent, G., Yoshie, O., Floyd-Smith, G., Samanta, H., and Sehgal, P. B., Interferon action: two (2′-5′) (A)n synthetases specified by distinct mRNAs in Ehrlich ascites tumor cells treated with interferon, *Cell*, p. 95 1983.
37. Preston, C. M. and Cordingley, M. G., mRNA and DNA-directed synthesis of Herpes Simplex virus-coded exonuclease in *Xenopus laevis* oocytes, *J. Virol.*, 43, 386, 1982.
38. Soreq, H., Parvari, R., and Silman, I., Biosynthesis and secretion of catalytically active acetylcholinesterase in *Xenopus* oocytes microinjected with mRNA from rat brain and from *Torpedo* electric organ, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 830, 1982.
39. Gundersen, C. B. and Miledi, R., Acetylcholinesterase activity of *Xenopus laevis* oocytes.
40. Parvari, R., Silman, I., and Soreq, H., Ontogenetic and agranulation-induced alterations in cholinesterases and in cholinesterase mRNA in the rodent cerebellum, in *Cholinesterases—Fundamental and Applied Aspects*, Brzin, M., Ed., W. De Gruyter, Berlin, 1984, 219.
41. Richter, J. D., Evers, D. C., and Smith, L. D., The recruitment of membrane-bound mRNAs for translation in microinjected oocytes, *J. Biol. Chem.*, 258, 2614, 1983.
42. Jilka, R. L., Famillетti, P., and Pestka, S., Synthesis and processing of the mouse MOPC-321 K chain in *Xenopus laevis* oocytes, *Arch. Biochem. Biophys.*, 192, 290, 1979.
43. Van Der Donk, J. A. and Van Dam, R. H., The use of *Xenopus* egg cells to assay the mRNA of single cells, *Nature (London)*, 271, 479, 1978.
44. Valle, G., Besley, J., and Colman, A., Synthesis and secretion of mouse immunoglobulin chains from *Xenopus laevis* oocytes, *Nature (London)*, 291, 5813, 1981.
45. Deacon, N. J. and Ebringer, A., Fucose incorporation into oocyte-synthesized rat immunoglobulins, *FEBS Lett.*, 79, 191, 1979.
46. Long, E. O., Gross, N., Wake, C. T., Mach, J. P., Carrel, S., Accolla, R., and Mach, B., Translation tion and assembly of HLA-DR antigens in *Xenopus* oocytes injected with mRNA from a human B-cell line, *EMBO J.*, 1, 649, 1982.
47. Mous, J., Peeters, B., and Rombauts, W., Synthesis of rat prostatic binding protein in *Xenopus* oocytes and in wheat germ, *Biochem. Biophys. Res. Commun.*, 79, 1111, 1977.
48. Mous, J., Peeters, B., Rombauts, W., and Heyns, W., Synthesis and glycosylation of rat prostatic binding protein in *Xenopus laevis* oocytes, *FEBS Lett.*, 103, 81, 1979.

49. Mous, J. M., Peeters, B. L., Heyns, W. J., and Rombauts, W. A., Assembly, glycosylation and secretion of the oligomeric rat prostatic binding protein in *Xenopus* oocytes, *J. Biol. Chem.*, 257, 11822, 1982.
50. Barnard, E. A., Miledi, R., and Sumikawa, K., Translation of exogenous messenger RNA coding for nicotinic acetylcholine receptors produces functional receptors in *Xenopus* oocytes, *Proc. R. Soc. London*, B215, 241, 1982.
51. Raj, N. B. K. and Pitha, P. M., Relationship between interferon production and interferon messenger RNA synthesis in human fibroblasts, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 1483, 1977.
52. Lebleu, B., Hubert, E., Content, J., DeWit, L., Brande, I. A., and De Clercq, E., Translation of mouse interferon mRNA in *Xenopus laevis* oocytes and in rabbit reticulocyte lysates, *Biochem. Biophys. Res. Commun.*, 82, 665, 1978.
53. Cavalieri, R. L., Havell, E. A., Vilcek, J., and Pestka, S., Synthesis of human interferon by *Xenopus laevis* oocytes: two structural genes for interferons in human cells, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3287, 1977.
54. Sehgal, P. B., Lyles, D. S., and Tamm, I., Super induction of human fibroblast interferon production: further evidence for increased stability of interferon messenger RNA, *Virology*, 89, 186, 1978.
55. Weissenbach, J., Chernajovsky, Y., Zeevi, M., Shulman, L., Soreq, H., Nir, U., Wallach, D., Perricaudet, M., Tiollais, P., and Revel, M., Two interferon mRNAs in human fibroblasts: *in vitro* translation and *Escherichia coli* cloning studies, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 7152, 1980.
56. Sehgal, P. B. and Sagar, A. D., *Heterogeneity of Poly Inosinic-Acid-Poly Cytidylic-Acid Induced Human Fibroblast Interferon Messenger RNA Species*, The Rockefeller University, New York, 1980, 95.
57. Sloma, A., McCandliss, R., and Pestka, S., Translation of human interferon mRNAs in *Xenopus laevis* oocytes, *Methods Enzymol.*, p. 68, 1981.
58. Wallace, D. M., Hitchcock, M. J. M., Reber, S. B., and Berger, S. L., Translation of human immune interferon messenger RNA in *Xenopus laevis* oocytes, *Biochem. Biophys. Res. Commun.*, 100(2), 865, 1981.
59. Sehgal, P. B., Sagar, A. D., and Brande, I. A., Further heterogeneity of human interferon mRNA species, *Science*, 214, 803, 1981.
60. Seghal, P. B., May, L. T., LaForge, K. S., and Inouye, M., Unusually long mRNA species coding for human alpha and beta interferons, *Proc. Natl. Acad. Sci. U.S.A .*, 79, 6932, 1982.
61. Devos, R., Cheroutre, H., Taya, Y., and Fiers, W., Isolation and characterization of IfN-gamma mRNA derived from mitogen-induced human splenocytes, *J . Interferon Res.*, 2, 409, 1982.
62. Sarkar, F. H., Sussman-Berger, P., and Pickering, L. A., Mouse interferon messenger RNA: characterization of the *Xenopus laevis* oocyte translation product, *Arch. Biochem. Biophys.*, 217, 273, 1982.
63. Contreras, R., Cheroutre, H., Degrave, W., and Fiers, W., Simple, efficient *in vitro* synthesis of capped RNA useful for direct expression of cloned eukaryotic genes, *Nucl. Acids Res.*, 10, 6353, 1982.
64. Shuttleworth, J., Morser, J., and Burke, D. C., Expression of interferon alpha and interferon beta genes in human lymphoblastoid (Namalwa) cells, *Eur. J. Biochem.*, in press.
65. Lin, Y., Stadler, B. M., and Rabin, H., Synthesis of biologically active interleukin 2 by *Xenopus* oocytes in response to poly(A)-RNA from a gibbon T-cell line, *J. Biol. Chem.*, 257(4), 1587, 1982.
66. Pestka, S., The human interferons from protein purification and sequence to cloning and expression in bacteria: before, between and beyond, *Arch. Biochem. Biophys.*, 221, 1, 1983.
67. Burmeister, M., Avivi, A., Schlessinger, J., and Soreq, H., Production of EGF-containing polypeptides in *Xenopus* oocytes microinjected with submaxillary gland mRNA, *EMBO J.*, 3, 1499, 1984.
68. Rapoport, T. A., Prehn, S ., and Tsamaloukas, A., Demonstration of specific receptors on the rough endoplasmic reticulum for the signal sequence of carp preproinsulin, *Biol. Implic. Protein Nucl. Acid Interact.*, p. 215, 1980.
69. Rapoport, T. A., Thiele, B. J., Prehn, S., Marbaix, G., Cleuter, Y., Hubert, E., and Huez, G., Synthesis of carp proinsulin in *Xenopus* oocytes, *Eur. J. Biochem.*, 87, 229, 1978.
70. Koren, R., Burstein, Y., and Soreq, H., Synthetic leader peptide modulates secretion of proteins from microinjected *Xenopus* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 80, 7205, 1983.
71. Laskey, R. A., Mills, A. D., Gurdon, J. B., and Partington, G. A., Protein synthesis in oocytes of *Xenopus laevis* is not regulated by the supply of messenger RNA, *Cell*, 11, 345, 1977.
72. Asselbergs, F. A. M., Van Venrooij, W. J., and Bloemendal, H., Messenger RNA competition in living *Xenopus* oocytes, *Eur. J. Biochem.*, 94, 249, 1979.
73. Richter, J. D. and Smith, L. D., Differential capacity for translation and lack of competition between mRNAs that segregate to free and membrane-bound polysomes, *Cell*, 27, 183, 1981.
74. Nudel, U., Soreq, H., Littauer, U. Z., Marbaix, G., Huez, G., Leclercq, M., Hubert, E., and Chantrenne, H., Globin mRNA species containing poly(A) segments of different lengths: their functional stability in *Xenopus* oocytes, *Eur. J. Biochem.*, 64, 115, 1976.

75. Allende, C. C., Allende, J. E., and Firtel, R. A., The degradation of ribonucleic acids injected into *Xenopus laevis* oocytes, *Cell*, 4, 189, 1974.
76. Huez, G., Marbaix, G., Gallwitz, D., Weinberg, E., Devos R., Hubert, E., and Cleuter, Y., Functional stabilization of HeLa cell histone messenger RNAs injected into *Xenopus* oocytes by 3′-OH polyadenylation, *Nature (London)*, 271, 572, 1978.
77. Soreq, H. and Miskin, R., Secreted proteins in the medium of microinjected *Xenopus* oocytes are degraded by oocyte proteases, *FEBS Lett.*, 128(2), 305, 1981.
78. Furuichi, Y., Lafiandra, A., and Shatkin, A. L., 5′-Terminal structure and mRNA stability, *Nature (London)*, 266, 235, 1977.
79. Asselbergs, F. A. M., Koopmans, M., Van Venrooij, W. J. , and Bloemendal, H., Post translational assembly of lens alpha crystallin in the reticulocyte lysate and in *Xenopus laevis* oocytes, *Eur. J. Biochem.*, 91, 65, 1978.
80. Chan, L., Kohler, P. O., and O'Malley, B. W., Translation of ovalbumin mRNA in *Xenopus laevis* oocytes, *J. Clin. Invest.*, 57, 576, 1976.
81. Bienz, M., Kubli, E., Kohli, J., de Henan, S., and Grosjean, H., Nonsense suppression in eukaryotes: the use of the *Xenopus* oocyte as an *in vivo* assay system, *Nucl. Acids Res.*, 8, 5169, 1980.
82. Bienz, M., Kubli, E., Kohli, J., de Henan, S., Huez, G., Marbaix, G., and Grosjean, H., Usage of the termination codons in a single eukaryotic cell, the *Xenopus laevis* oocyte, *Nucl. Acid Res.*, 9, 3835, 1981.
83. Foulton, A. B., How crowded is the cytoplasm?, *Cell*, 30, 345, 1982.
84. Beato, M. and Rungger, D., Translation of the messenger RNA for rabbit uteroglobin in *Xenopus* oocytes, *FEBS Lett.*, 59, 305, 1975.
85. Brachet, J., Nucleo cytoplasmic interactions in cell differentiation, in *Molecular Biology of Nucleocytoplasmic Relationships*, Vol. 14, Elsevier, Amsterdam, 1975, 189.
86. Wickens, M. P. and Gurdon, J. B., Post-transcriptional processing of Simian virus 40 late transcripts in injected frog oocytes, *J. Mol. Biol.*, 163, 1, 1983.
87. Probst, E., Kressman, A., and Birenstiel, M. L., Expression of sea urchin histone genes in the oocyte of *Xenopus laevis*, *J. Mol. Biol.*, 135, 709, 1979.
88. Woodland, H. R., Gurdon, J. B., and Lingrel, J. B., The translation of mammalina globin mRNA injected into fertilized eggs of *Xenopus laevis*. II. The distribution of globin synthesis in different tissues, *Dev. Biol.*, 39, 134, 1974.
89. Froehlich, J. P., Browder, L. W., and Schultz, G.A., Translation and distribution of rabbit globin mRNA in separated cell types of *Xenopus laevis* gastrulae, *Dev. Biol.*, 56, 356, 1977.
90. De Robertis, E. M., Lienhard, S., and Parisot, R. F., Intracellular transport of microinjected 5S and small nuclear RNAs, *Nature (London)*, 295, 572, 1982.
91. Gedamu, L., Dixon, G. H., and Gurdon, J. B., Studies of the injection of poly (A)⁺protamine mRNA into *Xenopus laevis* oocytes, *Exp. Cell Res.*, 117, 325, 1978.
92. Larkins, B. A., Pedersen, K., Handa, A. K., and Smith, L. D., Synthesis and processing of maize storage proteins in *Xenopus laevis* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 6448, 1979.
93. Gurdon, J. B., Lingrel, J. B., and Marbaix, G., Message stability in injected frog oocytes: long life of mammalian alpha and beta globin messages, *JMB*, 80, 539, 1973.
94. Huez, G., Marbaix, G., Hubert, E., Cleuter, Y., Leclercq, M., Chantrenne, H., Devos, R., Soreq, H., Nudel, U., and Littauer, U. Z., Readenylation of polyadenylate-free globin mRNA restores its stability *in vivo*, *Eur. J. Biochem.*, 59, 589, 1975.
95. Maniatis, G. M., Ramirez, F., Cann, A., Marks, P. A., and Bank, A., Translation and stability of human globin mRNA in *Xenopus* oocytes, *J. Clin. Invest.*, 58, 1419, 1976.
96. Deshpande, A. K., Chatterjee, B., and Roy, A. K., Translation and stability of rat liver messenger RNA for alpha 2 u globulin in *Xenopus* oocytes. The role of terminal poly(A), *J. Biol. Chem.*, 254, 8937, 1979.
97. Marbaix, G., Huez , G., Burny, A., Cleuter, Y., Hubert, E., Leclercq, M., Chantrenne, H., Soreq, H., Nudel, U., and Littauer, U, Z., Absence of polyadenylate segment in globin mRNA accelerates its degradation in *Xenopus* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 72, 3065, 1975.
98. Huez, G., Marbaix, G., Burny, A., Hubert, E., Leclercq, M., Cleuter, Y., Chantrenne, H., Soreq, H., and Littauer, U. Z., Degradation of deadenylated rabbit alpha-globin mRNA in *Xenopus* oocytes is associated with its translation, *Nature (London)*, 266, 472, 1977.
99. Huez, G., Bruck, C., and Cleuter, Y., Translational stability of native and deadenylated rabbit globin mRNA injected into HeLa cells, *Proc. Natl. Acad. Sci. U.S.A.*, 78, 908, 1981.
100. Woodland, H. R. and Wilt, F. H., The functional stability of sea urchin histone mRNA injected into oocytes of *Xenopus laevis*, *Dev. Biol.*, 75, 199, 1980.
101. Sehgal, P. B., Soreq, H., and Tamm, I., Does 3′-terminal poly(A) stabilize human fibroblast interferon mRNA in oocytes of *Xenopus laevis*?, *Proc. Natl. Acad. Sci. U.S.A.*, 75, 5030, 1978.
102. Soreq, H., Sagar, H., and Sehgal, P. B., Translational activity and functional stability of human fibroblast beta$_1$ and beta$_2$ interferon mRNA species lacking 3′-terminal sequences. *Proc. Natl . Acad. Sci. U.S.A.*, 78, 1741, 1981.

103. Littauer, U. Z. and Soreq, H., The regulatory function of poly(A) and adjacent 3' sequences in translated RNA, *Prog. Nucl. Acid Res. Mol. Biol.*, 27, 53, 1982.
104. Huez, G., Cleuter, Y., Bruck, C., Van-Volten-Doting, L., Goldbach, R., and Verduin, B., Translational stability of plant viral RNAs microinjected into living cells: influence of a 3'-poly(A) segment, *Eur. J. Biochem.*, 130, 205, 1983.
105. Revel, M. and Groner, Y., Post-transcriptional and translational controls of gene expression in eukaryotes, *Ann. Rev. Biochem.*, 47, 1079, 1978.
106. Lockard, R. E. and Lane, C. D., Requirements for 7-methyl guanosine in translation of globin mRNA *in vivo*, *Nucl. Acids Res.*, 5, 3237, 1978.
107. McCrae, M. A. and Woodland, H. R., Stability of non-polyadenylated viral mRNAs injected into frog oocytes, *Eur. J. Biochem.*, 116, 467, 1981.
108. Mangiarotti, G., Ceccarelli, A., and Lodish, H. F., Cyclic AMP stabilizes a class of developmentally regulated *Dictyostelium discoideum* mRNAs, *Nature (London)*, 301, 616, 1983.
109. Asselbergs, F. A. M., Peters, W. H. M., Van-Venrooij, W. J., and Bloemendal, H., Cap analogues do not inhibit mRNA translation in *Xenopus laevis* oocytes, *FEBS Lett.*, 94, 195, 1978.
110. Richter, J. D., Wasserman, W. J., and Smith, L. D., The mechanism for increased protein synthesis during *Xenopus* oocyte maturation, *Dev. Biol.*, 89, 159, 1982.
111. Lingrel, J. B. and Woodland, H. R., Initiation does not limit the rate of globin synthesis in message-injected *Xenopus* oocytes, *Eur. J. Biochem.*, 47, 47, 1974.
112. Martin, R. P., Sibler, A.-P., Dirheimer, G., Dettenau, S., and Grosjean, H., Yeast mitochondrial $tRNA^{Trp}$ injected with *E. coli* activating enzyme into *Xenopus* oocytes suppresses UGA termination, *Nature (London)*, 293, 235, 1981.
113. Wallace, R. A., Denfel, R. A., and Misulovin, Z., Protein incorporation by isolated amphibian oocytes. VI. Comparison of autologous and xenogeneic vitellogenins, *Comp. Biochem. Physiol.*, 65B, 151, 1980.
114. Opresko, L., Wiley, H. S., and Wallace, R. A., Differential postendocytotic compartmentation in *Xenopus* oocytes is mediated by a specifically bound ligand, *Cell*, 22, 47 , 1980.
115. Opresko, L., Wiley, H. S., and Wallace, R. A., Proteins iodinated by the chloramine-T method appear to be degraded at an abnormally rapid rate after endocytosis, *Proc. Natl. Acad. Sci. U.S.A.*, 77, 1556, 1980.
116. Lane, C. D., Champion, J., Colman, A., James, T. C., and Applebaum, S. W., The fate of *Xenopus* and locust vitellogenins made in *Xenopus* oocytes: an export-import processing model, *Eur. J. Biochem.*, 130, 529, 1983.
117. Mertens, B. and Verhoeven, G., Synthesis and processing of pre-alpha-2U-globulin in different translational systems, *FEBS Lett.*, 133, 209, 1981.
118. Bock, S. C., Tiemeir, D. C., Mester, K., Wu, M., and Goldsmith, M. R., Hybridization-selected translation of *Bombix mori* high-cysteine chorion proteins in *Xenopus laevis* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 1032, 1982.
119. Salden, M., Asselbergs, F. A. M., and Bloemendal, H., Translation of oncogenic virus RNA in *Xenopus laevis* oocytes, *Nature (London)*, 259, 696, 1976.
120. Ghysdael, J., Hubert, E., Travnicek, M., Bolognesi, D. P., Burny, A., Cleuter, Y., Huez, G., Kettmann, R., Marbaix, G., Portetelle, D., and Chantrenne, H., Frog oocytes synthesize and completely process the precursor polypeptide to virion structural proteins after microinjection of avian myeloblastosis virus RNA, *Proc. Natl. Acad. Sci. U.S.A.*, 74, 3230, 1977.
121. Salden, M. H. L., Selten-Versteegen, A. M., and Bloemendal, H., Translation of Rauscher murine leukemia viral RNA: a model for the function of a virus-specific messenger, *Biochem. Biophys. Res. Commun.*, 72, 610, 1976.
122. Ghysdael, J., Kettmann, R., and Burny, A., Translation of bovine leukemia virus virion RNA in heterologous protein synthesizing systems, *J. Virol.*, 29, 1987, 1979.
123. Laskey, R. A., Gurdon, J. B., and Crawford, L. V., Translation of encephalomyocarditis viral RNA in oocytes of *Xenopus laevis, Proc. Natl. Acad. Sci. U.S.A.*, 69, 3665, 1972.
124. Nusse, R., Asselbergs, F. A. M., Salden, M. H. L., Michalides, R. J. A. M., and Bloemendal, H., Translation of mouse mammary tumor virus RNA: precursor polypeptides are phosphorylated during processing, *Virology*, 91, 106, 1978.
125. Katz, R. A., Maniatis, E. M., and Guntaka, R. V., Translation of avian sarcoma virus RNA in *Xenopus laevis* oocytes, *Biochem. Biophys. Res. Commun.*, 86, 447, 1979.
126. Rottier, P. J. M., Spann, W. J. M., Horzinek, M. C., and Van der Zeijst, B. A. M., Translation of three mouse hepatitis virus strain A59 subgenomic RNAs in *Xenopus laevis* oocytes, *J. Virol.*, 38, 20, 1981.
127. Asselbergs, F. A. M., Salden, M. H. L., and Bloemendal, H., Kinetics of synthesis and processing of precursor polypeptides of murine leukemia virus in frog oocytes following microinjection of viral RNA, *Eur. J. Biochem.*, 109, 395, 1980.

128. Rapoport, R. A., Intracellular compartmentation and secretion of carp proinsulin synthesized in *Xenopus* oocytes, *Eur. J. Biochem.*, 115, 665, 1981.
129. Kindas-Mugge, I., Lane, C. D., and Kreil, G., Insect protein synthesis in frog cells: the translation of honey-bee promellitin messenger RNA in *Xenopus* oocytes, *J. Mol. Biol.*, 87, 451, 1974.
130. Lane, C. D., Champion, J., Haiml, Z., and Kreil, G., The sequestration, processing and retention of honey bee promelittin made in amphibian oocytes, *Eur. J. Biochem.*, 113, 273, 1981.
131. Mous, J., Peeters, B ., and Rombauts, W., Synthesis and core glycosylation of the alpha subunit of human chorionic gonadotropin in *Xenopus* oocytes, *FEBS Lett.*, 122, 105, 1980.
132. Cutler, D., Lane , D. C., and Colman, A., Highly purified mRNa induces the production of N-glycosylated chicken albumin in the oocytes, suggesting that glycosylation occurs through the action of endogenous frog enzymes, *J. Mol. Biol.*, 153, 917, 1981 .
133. Maller, J. L., Kemp, B. E., and Krebs, E. G., *In vivo* phosphorylation of a synthetic peptide substrate of cyclic AMP-dependent protein kinase, *Proc. Natl. Acad. Sci. U.S.A.* , 75, 248, 1978.
134. Colman, A., Besley, J., and Valle, G., Interactions of mouse immunoglobulin chains within *Xenopus* oocytes, fate of secretory proteins trapped in oocytes of *Xenopus laevis* by disruption of the cytoskeleton by imbalanced subunit synthesis, *J. Mol. Biol.*, 160, 459, 1982.
135. Kruppa, J., Darmer, D., Kalthoff, H., and Richter, D., The phophorylation of ribosomal protein S6 from progesterone-stimulated *Xenopus laevis* oocytes: kinetic studies and phosphopeptide analysis, *Eur. J. Biochem.*, 129, 537, 1983.
136. Valle, G., Besley, J., Williamson, A. R., Mosmann, T. R., and Colman, A., Post-translational fate of variant MOPC 315 Kappa chains in *Xenopus* oocytes and mouse myeloma cells, *Eur. J. Biochem.*, 132, 131, 1983.
137. Taniguchi, M., Tokuhisa, T., Kanno, M., Yaoita, Y., Shimizu, A., and Honjo, T., Reconstitution of antigen-specific suppressor activity with translation products of mRNA, *Nature (London)*, 298, 172, 1982.
138. Taniguchi, M., Functional roles of two polypeptide chains that compose an antigen-specific suppressor T cell factor, in *IR Genes, Past, Present and Future*, Pierce, C. W., Cullen, S. E., Kapp, J. A., Schwartz, B. D., and Shreffler, D. C., Eds., Humana Press, New Jersey, 1983.
139. Zehavi-Willner, T. and Lane, C. D., Subcellular compartmentation of albumin and globin made in oocytes under the direction of messenger RNA, *Cell*, 11, 683, 1977.
140. Colman, A. and Morser, J., Export of proteins from oocytes of *Xenopus laevis*, *Cell*, 17, 517, 1979.
141. Lane, C. D., Shannon, S., and Craig, R., Sequestration and turnover of guinea-pig milk proteins and chicken ovalbumin in *Xenopus* oocytes, *Eur. J. Biochem.*, 101, 485, 1979.
142. Teraoka, H., Arita, H., Tamaki, M., Fujii, Y., and Tanaka, K., Nature of albumin exported from the frog oocyte following microinjection of mouse liver mRNA, *J. Biochem.*, 91, 933, 1982.
143. Lane, C. D., Colman, A., Mohun, T., Morser, J., Champion, J., Kourides, I., Craig, R., Higgins, S., James, T. C., Applebaum, S. W., Ohlsson, R. I., Pancha, E., Houghton, M., Mathews, J., and Miflim, B. J., The *Xenopus* oocyte as a surrogate secretory system: the specificity of protein export, *Eur. J. Biochem.*, 111, 225, 1980 .
144. Colman, A., Lane, C. D., Craig, R., Boulton, A., Mohun, T., and Morser, J., The influence of topology and glycosylation on the fate of heterologous secretory proteins made in *Xenopus* oocytes, *Eur. J. Biochem.*, 113, 339, 1981.
145. Colman, A., Morser, J., Lane, C. D., Besley, J., Wylie, C., and Valle, G., *J. Cell Biol.*, 91, 770, 1981.
146. Hurkman, W. J., Smith, L. D., Richter, J., and Larkins, B. A., Subcellular compartmentalization of maize storage proteins in *Xenopus* oocytes injected with Zein mRNA, *J. Cell. Biol.*, 89, 292, 1981.
147. Larkins, B. A., Pederson, K., Handa, A. K., Hurkman, W. J., and Smith, L. D., Synthesis and processing of maize storage proteins in *Xenopus laevis* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 76, 6448, 1980.
148. De Herdt, E., Marbaix, G., Tencer, R., and Slegers, H., Subcellular distribution in *Xenopus laevis* oocytes of a microinjected poly(A)-binding protein from *Artemia salina* gastrulae, *Eur. J. Biochem.*, 132, 623, 1983.
149. Krohne, G. and Franke, W. W., A major soluble acidic protein located in nuclei of diverse vertebrate species, *Exp. Cell. Res.*, 129, 167, 1980.
150. Williams, M. A., Kleinschmidt, J. A., Krohne, G., and Franke, W. W., Argyrophilic nuclear and nucleolar proteins of *Xenopus laevis* oocytes identified by gel electrophoresis, *Exp. Cell. Res.*, 137, 341, 1982.
151. Krohne, G., Stick, R., Kleinschmidt, J. A., Moll, R., Franke, W. W., and Hansen, P., Immunological localization of a major karyoskeletal protein in nucleoli of oocytes and somatic cells of *Xenopus laevis*, *J. Cell. Biol.*, 94, 749, 1982.
152. Golden, L., Schafer, J., and Rosbash, M., Accumulation of individual PA⁺RNAs during oogenesis of *Xenopus laevis*, *Cell*, 22, 835, 1980.

153. Wakahara, M., Accumulation, spatial distribution and partial characterization of poly(A)⁺RNA in the developing oocytes of *Xenopus laevis*, *J. Embryol. Exp. Morphol.*, 66, 127, 1981.
154. Capco, D. G. and Jeffery, W. R., Transient localizations of messenger RNA in *Xenopus laevis* oocytes, *Dev. Biol.*, 89, 1, 1982.
155. Shiokawa, K., Mobilization of maternal mRNA in amphibian eggs with special reference to the possible role of membraneous supramolecular structures, *FEBS Lett.*, 151, 179, 1983.
156. Muller, W. E. G., Bernd, A., and Schroder, H. C., Modulation of poly(A)⁺ mRNA-metabolizing and transporting systems under special consideration of microtuble protein and actin, *Mol. Cell. Biochem.*, 53, 197, 1983.
157. Richter, J. D. and Smith, L. D., Developmentally regulated RNA binding proteins during oogenesis in *Xenopus laevis*, *J. Biol. Chem.*, 258, 4864, 1983.
158. Anderson, D. M., Richter, J. D., Chamberlin, M. E., Price, D. H., Britten, R. J., Smith, L. D., and Davidson, E. H., Sequence organization of the poly(A) RNA synthesized and accumulated in lampbrush chromosome stage *Xenopus laevis* oocytes, *J. Mol. Biol.*, 155, 281, 1982.
159. Richter, J. D., Anderson, D. M., Davidson, E. H., and Smith, L. D., Interspersed poly(A) RNAs of amphibian oocytes are not translatable, *J. Mol. Biol.*, in press.
160. Picard, B., Le Maire, M., Wegnez, M., and Denis, H., Biochemical research on oogenesis: composition of the 42S storage particles of *Xenopus laevis* oocytes, *Eur. J. Biochem.*, 109, 359, 1980.
161. Dixon, L. K. and Ford, P. J., Persistence of nonribosome bound 5S RNA in full-grown oocytes of *Xenopus laevis*, *Dev. Biol.*, 91, 474, 1982.
162. Korn, L. J., Gurdon, J. B., and Price, J., Oocyte extracts reactivate developmentally inert *Xenopus* 5S genes in somatic nuclei, *Nature (London)*, 300, 354, 1982.
163. Wakefield, L., Ackerman, E., and Gurdon, J. B., The activation of RNA synthesis by somatic nuclei injected into amphibian oocytes, *Dev. Biol.*, 95, 468, 1983.
164. Weisbrod, S., Wickens, M. P., Whytock, S., and Gurdon, J. B., Active chromatin of oocytes injected with somatic cell nuclei or cloned DNA, *Dev. Biol.*, 94, 216, 1982.
165. LaMarca, M. J., Srobel-Fidler, M. C., Smith, L. D., and Keem, K., Hormonal effects of RNA synthesis by stage 6 oocytes of *Xenopus laevis*, *Dev. Biol.*, 47, 384, 1975.
166. Mulner, O. and Ozon, R., The roles of follicular envelopes in the initiation of *Xenopus* oocyte maturation, *Gen. Comp. Endocrinol.*, 44, 335, 1981.
167. Hollinger, T. G. and Alvarez, I. M., Trifluoperazine-induced meiotic maturation in *Xenopus laevis*, *J. Exp. Zool.*, 224, 461, 1982.
168. Hanocq-Quertier, J. and Baltus, E., Induction of meiosis of *Xenopus laevis* oocytes by Mianserine, *Gamete Res.*, 4, 49, 1981.
169. Deshpande, A. K. and Koide, S. S., *In vitro* induction of germinal vesicle breakdown in *Xenopus laevis* oocytes by mellitin, *Differentiation*, 21, 127, 1982.
170. Sadler, S. E. and Maller, J. L., Identification of a steroid receptor on the surface of *Xenopus laevis* oocytes by photoaffinity labeling, *J. Biol. Chem.*, 257, 355, 1982.
171. Tso , J., Thibier, C., Mulner, O., and Ozon, R., Microinjected progesterone reinitiates meiotic maturation of *Xenopus laevis* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 5552, 1982.
172. Schorderet-Slatkine, S., Schorderet, M., and Baulieu, E. E., Cyclic AMP-mediated control of meiosis: effects of progesterone, cholera toxin, and membrane-active drugs in *Xenopus laevis* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 850, 1982.
173. Jordana, X., Allende, C. C., and Allende, J. E., Guanine nucleotides are required for progesterone inhibition of amphibian oocyte adenylate cyclase, *Biochem. Int.*, 3, 527, 1981.
174. Cartaud, A. and Ozon, R., *Xenopus laevis* oocyte calmodulin in the process of meiotic maturation, *J. Biol. Chem.*, 255, 9404, 1980.
175. Miot, F. and Erneux, C., Characterization of the soluble cyclic nucleotide phophodiesterases in *Xenopus laevis* oocytes: evidence for a calmodulin-dependent enzyme, *Biochem. Biophys. Acta*, 701, 253, 1982.
176. Tucciarone, L. M. and Lanclos, K. D., Effect of calmodulin inhibitor, Stelazine, on the endocytosis of vitellogenin and transglutaminase activity in *Xenopus laevis* oocytes, *Mol. Cell Biochem.*, 45, 159, 1982.
177. Webb, A. C. and Smith, L. D., Accumulation of mitochondrial DNA during oogenesis in *Xenopus laevis*, *Dev. Biol.*, 56, 219, 1977.
178. Belle, R., Boyer, J., and Ozon, R., Carbon dioxide reversibly inhibits meiosis of *Xenopus laevis* oocyte and the appearance of the maturation promoting factor, *Dev. Biol.*, 90, 315, 1982.
179. Callen, J.-C., Tourte, M., Dennebouy, N., and Mounolou, J. -C., Mitochondrial development in oocytes of *Xenopus laevis*, *Biol. Cell.*, 38, 13, 1980.
180. Decroly, M., Goldfinger, M., and Six-Tondeur, N., Biochemical characterization of lysosomes in unfertilized eggs of *Xenopus laevis*, *Biochem. Biophys. Acta*, 587, 567, 1979.
181. Brachet, J. and Malpoix, P., Macromolecular synthesis and nucleocytoplasmic interactions in early development, *Adv. Morphogen.*, 9, 263, 1971.

182. Shih, R. J., O'Connor, C. M., Keem, K., and Smith, L. D., Kinetic analysis of amino acid pools and protein synthesis in amphibian oocytes and embryos, *Dev. Biol.*, 66, 172, 1978.
183. Younglai, E., Godeau, F., and Baulieu, E. E., Modifications in oocyte protein synthesis induced by progesterone in *Xenopus laevis*, *FEBS Lett.*, 127, 233 , 1981.
184. Younglai, E. V., Godeau, R., Mester, J., and Baulieu, E. E., Increased ornithine decarboxylase activity during meiotic maturation in *Xenopus laevis* oocytes, *Biochem. Biophys. Res. Commun.*, 96, 1274, 1980.
185. Nielson, P. J., Thomas, G., and Maller, J. L., Increased phosphorylation of ribosomal protein S6 during meiotic maturation of *Xenopus* oocytes, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 2937, 1982.
186. Rosbash, M., A comparison of *Xenopus laevis* oocyte and embryo mRNA, *Dev. Biol.*, 87, 319, 1981.
187. Wasserman, W. J., Richter, J. D., and Smith, L. D., Protein synthesis during maturation promoting factor and progesterone-induced maturation in *Xenopus* oocytes, *Dev. Biol.*, 89, 152, 1982.
188. Richter, J. D., Wasserman, W. J., and Smith, L. D., The mechanism for increased protein synthesis during *Xenopus* oocyte maturation, *Dev. Biol.*, 89, 159, 1982.
189. King, J. A. and Millar, R. P., Hypothalamic luteinizing hormone-releasing hormone content in relation to the seasonal reproductive cycle of *Xenopus laevis*, *Gen. Comp. Endocrinol.*, 39, 309, 1979.
190. Eusebi, F., Mangia, F., and Alfei, L., Acetylcholine elicited responses in primary and secondary mammalian oocytes disappear after fertilization, *Nature (London)*, 277, 651, 1979.
191. Bienz, M. and Gurdon, J. B., The heat-shock response in *Xenopus* oocytes is controlled at the translational level, *Cell*, 27, 811, 1982.
192. Miledi, R., A calcium-dependent transient outward current in *Xenopus laevis* oocytes, *Proc. R. Soc. London*, B215, 491, 1982.
193. Belle, R., Marot, J., and Ozon, R., Nature of progesterone action on amino acid uptake by isolated full grown oocytes of *Xenopus laevis*, *Biochim. Biophys. Acta*, 419, 342, 1976.
194. Jung, D. and Richter, H.-P., Changes of alanine-sodium cotransport during maturation of *Xenopus laevis* oocytes, *Cell Biol. Intern. Rep.*, 7, 697, 1983.
195. Kusano, K., Miledi, R., and Stinnakre, J., Acetylcholine receptors in the oocyte membrane, *Nature (London)*, 270, 739, 1977.
196. Dascal, N. and Landau, E. M., Types of muscarinic response in *Xenopus* oocytes, *Life Sci.*, 27, 1423, 1980.
197. Kado, R. T., Marcher, K., and Ozon, R., Electrical membrane properties of the *Xenopus laevis* oocyte during progesterone-induced meiotic maturation, *Dev. Biol.*, 84, 471, 1981.
198. Grainger, J. L., Winkler, M. M., Shen, S. S., and Steinhardt, R. A., Intracellular pH controls protein synthesis rate in the sea urchin egg and early embryo, *Dev. Biol.*, 68, 396, 1979.
199. Lee, S. C. and Steinhardt, R. A., pH changes associated with meiotic maturation in oocytes of *Xenopus laevis*, *Dev. Biol.*, 85, 358, 1981.
200. Mach, B., Faust, C. F., Vassali, P., and Rungger, D., Different size of the products of the 14S light chain mRNA translated *in vitro* and in amphibian oocytes, *Mol. Biol. Rep.*, 1, 3, 1973.
201. Band, C., Kado, R. T., and Marcher, K., Sodium channels induced by depolarization of the *Xenopus laevis* oocyte, *Proc. Natl. Acad. Sci. U.S.A.*, 79, 3188, 1982.
202. Valle, G., Jones, E. A., and Colman, A., Anti-ovalbumin monoclonal antibodies interact with their antigen in internal membranes of *Xenopus* oocytes, *Nature (London)*, 300, 71, 1982.
203. Bussuner, R., Huth, A., Manteuffel, R., and Rapoport, T. A., Secretion of plant storage globulin polypeptides by *Xenopus laevis* oocytes, *Eur. J. Biochem.*, 133, 321, 1983.
204. Stacy, D. W. and Alfrey, V. G., Microinjection studies of duck globin messenger RNA translation in human and avian cells, *Cell*, 9, 725, 1976.
205. Wang, L. H. and Stacey, D. W., Participation of subgenomic retroviral mRNAs in recombination, *J. Virol.*, 41, 919, 1982.
206. Jones, N. C., Richter, J. D., Weeks, D. L., and Smith, L. D., Regulation of adenovirus transcription by EIA in microinjected *Xenopus* oocytes, *Mol. Cell. Biol.*, in press.
207. Lane, C. D., The fate of foreign proteins introduced into *Xenopus* oocytes, *Cell*, 24, 281, 1981.
208. Soreq, H. and Miskin, unpublished observation.
209. Soreq, H., unpublished observation.