CHAPTER 4

THE FATE OF GENES, MESSENGERS, AND PROTEINS INTRODUCED INTO *XENOPUS* OOCYTES

Charles D. Lane

LABORATORY OF DEVELOPMENTAL BIOCHEMISTRY
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
LONDON, ENGLAND

I. Introduction

The fully grown *Xenopus* oocyte, a giant cell over 1 mm in diameter, can be gripped on one side using watchmakers' forceps thereby enabling a glass needle, held by a semimicromanipulator, to be inserted from the other side. The glass micropipet is connected by oil-filled tubing to a micrometer screw. Thus fluid can be squirted into the living oocyte simply by turning the micrometer screw (Gurdon, 1974). Why over the past 10 years has this process been repeated in experiments described in more than 200 scientific papers? (For more detailed reviews see Asselbergs, 1979; Gurdon, 1974; Kressman and Birnstiel, 1980; Lane and Knowland, 1975; Marbaix and Huez, 1980; Wickens and Laskey, 1981; and for general reviews see de Robertis and Gurdon, 1979; de Robertis *et al.*, 1977a; Lane, 1976.) Why have experimentalists filled oocytes with DNA (see Section III), mRNA (see Section I), proteins (see Section II), and subcellular organelles (Gurdon, 1974; McKinnell, 1978)?

CURRENT TOPICS IN DEVELOPMENTAL BIOLOGY, VOL. 18

ISBN 0-12-153118-X

The introduction of a substance into a living cell yields information of two kinds: first, the properties of a component operating within the natural milieu can be revealed, and such studies complement experiments performed *in vitro*. The *Xenopus* oocyte is a cell specialized for the synthesis and storage of components used later in embryogenesis (Davidson, 1976) but, in addition, the complex architecture of the frog cell reflects the subcellular systems involved in the export and import of proteins (Dumont and Brummett, 1978; Mohun *et al.*, 1981). The oocyte is therefore a rich source of materials for use *in vitro*, as are the egg and early embryo. Thus cell-free systems active in transcription (Birkenmeier *et al.*, 1978; Wormington *et al.*, 1981), replication (Benbow *et al.*, 1977; Goldberg *et al.*, 1981; Laskey *et al.*, 1979; Richter *et al.*, 1981), chromatin assembly (Laskey *et al.*, 1977a,b), mitochondrial protein synthesis (Swanson, 1971), membrane transfer of newly made proteins (Ohlsson *et al.*, 1981) but, surprisingly, not cytosolic protein synthesis itself, are easily prepared. Consequently the oocyte, egg, and early embryo can be used to study the properties of macromolecules or subcellular organelles both by microinjection and by addition to a cell-free extract. The full benefits of such complementarity are achieved only when, as in the above examples, both the *in vivo* and *in vitro* systems employ a given cell type.

In the second place, an injected foreign substance can serve as a probe and can reveal the nature and specificity of the biochemical pathways, in particular the control systems, in operation within the living cell. Nonetheless, artifacts can arise because of course the injection of substances, especially in large quantities, perturbs the natural state. For example, chicken ovalbumin messenger injected into oocytes translates, to a certain extent, in the wrong subcellular site, giving rise to protein molecules which cannot cross the endoplasmic reticulum (Colman *et al.*, 1981a; Lane *et al.*, 1979). Such effects can be quite revealing, especially if one wishes to study the rate-limiting steps in a particular pathway and it is therefore surprising that microinjection and kinetic studies have rarely been combined. Given interest in metabolic fluxes, it is not unexpected that one such study concerns the control of glucose metabolism (Ureta, 1980; Ureta and Radojkovic, 1978, 1979). Admittedly, from the outset (Moar *et al.*, 1971) there was interest in the kinetic consequences of varying the supply of mRNA to the oocyte. The elegant study of Laskey *et al.* (1977a) demonstrated that the supply of messenger does not limit to any significant extent the overall rate of protein synthesis (see also Asselbergs *et al.*, 1979a; Lane, 1976; Lingrel and Woodland, 1974).

II. The Fate of Injected Messenger RNA

A. Translation

What happens therefore when messenger RNA is injected into an oocyte? In physical terms surprisingly little is known and there is no compelling evidence to support the widely accepted view that the mRNA distributes itself evenly throughout the oocyte. Nonetheless within minutes of injection (Gurdon *et al.*, 1971) some of the mRNA is translated: 7 hours later most stable messengers are fully established, although very large mRNAs such as those coding for vitellogenin or avian sarcoma virus take about twice as long to reach their maximum translational activity (Asselbergs *et al.*, 1979a; Berridge and Lane, 1976; Huez *et al.*, 1974). The rate-limiting step in messenger recruitment may well be diffusion. The generally accepted view is that heterologous messengers engage translational machinery within the oocyte that is neither species (Lane *et al.*, 1971) nor phylum (Kindas-Mugge *et al.*, 1974) specific: strictly speaking most of the mRNA injection experiments performed do not establish this point because crude mRNA preparations were used. It can always be argued that species-specific factors are required for translation and were formed from other heterologous mRNAs introduced into the frog cell. Furthermore, experiments with purified messengers from nonovarian tissues of foreign species do not even prove that the apparatus within the frog oocyte lacks cell type specificity, because the alien messenger might circumvent any translational restrictions associated with a particular differentiated state. Partially purified messengers from the specialized tissues of frogs have been translated in oocytes of the same amphibian species implying the existence of some machinery lacking cell type specificity (Berridge and Lane, 1976). It can still be argued that specific factors are required for the translation of specific messengers or specific classes of messenger, provided one assumes that the oocyte has a complete array of such factors. However, the large amounts of heterologous proteins made, the competition seen between different injected messengers (Asselbergs *et al.*, 1979a), and the competition between injected and endogenous mRNAs (Laskey *et al.*, 1977a) suggest that all messengers use at least some common machinery. Indeed it is generally assumed that all the engaged translational machinery is the same, although it will be interesting to see if the machinery involved in the translation of membrane-bound and free messengers is exactly equivalent. Whatever the underlying mechanism, it is clear that the frog oocyte can be programmed with a wide variety of eukaryotic mes-

sengers, including some from insects, mammals, birds, viruses (see Table I), fishes, and plants (see reviews listed in the introduction).

Are there any messengers that are read with low efficiency or are not translated at all? It is clearly very difficult to answer such a question because failure to find a heterologous protein may result from the milieu in which the foreign macromolecule is deposited, or it may merely reflect limitations in the detection method. The foreign protein may be unstable or it may not be correctly modified and may then have an anomalous gel mobility (Labarca and Paigen, 1977; Lane *et al.*, 1979, Wunner *et al.*, 1980). Evidence to date suggests that all bona fide eukaryotic mRNAs function in oocytes and that other kinds of messenger are inactive. Thus synthetic polynucleotides (Woodland and Ayers, 1974), bacteriophage mRNA (Gurdon *et al.*, 1971; Marbaix and

TABLE I

VIRAL PROTEINS MADE IN *Xenopus* OOCYTES UNDER THE DIRECTION OF INJECTED RNA AND DNA

Viral RNA or DNA	Translation Products	Modifications	References
Encephalomyo carditis RNA	Viral poly-peptides	Proteolytic cleavage of precursor protein	Laskey *et al.* (1972, 1977a)
Rauscher leukemia RNA	Viral poly-peptides	Proteolytic cleavage of precursor protein, glycosylation of envelope protein	Van Zaane *et al.* (1977); Asselbergs *et al.* (1980); Salden *et al.* (1976a); Reynolds *et al.* (1978)
Avian myelo-blastosis RNA	Viral core proteins	Proteolytic cleavage of core precursor protein	Salden *et al.* (1976b); Ghysdael *et al.* (1977a; b)
Avian sarcoma RNA	Viral poly-peptides	Proteolytic cleavage of core precursor protein	Katz *et al.* (1979)
Bovine leukaemia RNA	Viral core proteins	Proteolytic cleavage	Ghysdael *et al.* (1977a, 1979)
Mouse mammary tumor RNA	Viral proteins	Proteolytic cleavage and phosphorylation of precursor protein	Nusse *et al.* (1978)
Moloney RNA	Viral proteins	—	Hesselink *et al.* (1981)
Rabies RNA	Viral proteins	—	Wunner *et al.* (1980)
Adenovirus RNA	Viral proteins	—	de Robertis *et al.* (1977b)
Reovirus RNAs	Viral poly-peptides	—	McCrae and Woodland (1981)

TABLE I (*Continued*)

Viral RNA or DNA	Translation Products	Modifications	References
Simian virus 40 and polyoma RNA	Viral polypeptides and tumor antigens	—	Lane *et al.* (1981a)
Alfa mosaic RNA	Viral proteins	—	Van Vloten-Doting *et al.* (1977); Rutgers *et al.* (1976); Rutgers (1977)
Barley mosaic RNA	Viral proteins	—	Rutgers *et al.* (1977)
Brome mosaic RNA	Viral proteins	—	Kondo *et al.* (1975)
Cucumber mosaic virus RNA	Viral proteins	—	Schwinghamer and Symons (1977)
Tobacco mosaic RNA	Viral proteins	Proteolytic cleavage	Knowland (1974)
Citrus exocortis RNA	None	—	Semancik *et al.* (1977)
Herpes DNA	Thymidine kinase	—	McKnight and Gavis (1980); Cordingley and Preston (1981)
Polyoma and simian virus 40 DNA	Viral proteins and tumor antigens	—	Rungger and Turler (1978); Rungger *et al.* (1979a); Lane *et al.* (1981a)

Huez, 1980), and mitochondrial mRNAs (Moorman *et al.*, 1977) all fail to produce detectable amounts of product. The results obtained by Moorman *et al.* (1977) are consistent with the finding that, within the mitochondria, the opal terminator codon UGA specifies tryptophan. It is difficult to reconcile these observations with those of Eggitt and Scragg (1975) who reported that yeast mRNAs injected into the cytosol produce normal mitochondrial proteins.

B. The Relationship between the Structure and Function of Injected Messengers

The behavior *in vivo* of macromolecules which have been modified *in vitro* is of considerable interest, although the effects observed are not necessarily of physiological significance. Prokaryotic messengers are uncapped and there is direct evidence (Paterson and Rosenberg, 1979) that such mRNAs are only translated efficiently in eukaryotic systems

if they are artificially capped. Removing or breaking open the cap structure of globin mRNA causes a dramatic (>95%) reduction in its ability to direct globin synthesis in oocytes (Lockard and Lane, 1978). The pioneering study by Furuichi *et al.* (1977) suggests that the physical stability of reovirus RNA in oocytes is reduced, albeit from a fairly low level, by cap removal. More recent studies (McCrae and Woodland, 1981) indicate that the capped species are in fact quite stable and that cap removal greatly destabilizes all 10 reovirus messengers. Thus direct and indirect evidence suggests that a prokaryotic mRNA could not be translated in oocytes without being capped. Nonetheless naturally occurring uncapped eukaryotic mRNAs such as satellite tobacco necrosis virus RNA may both persist and function in the frog cell.

Are there also stringent requirements for an intact 3′-poly(A) tail? An elegant series of experiments (Huez *et al.*, 1974, 1975, 1977a,b, 1978, 1981; Marbaix *et al.*, 1975, 1979) has exploited to its furthest the oocyte as an *in vivo* system for studying the relationship between structure and function. Rabbit globin mRNA lacking a poly(A) tail lacks both physical and function stability: initially it is translated, but within 24 hours over half the tailless mRNA is degraded. In contrast, normal globin mRNA translates for weeks in cultured oocytes and is at least as stable as the average endogenous messenger (Gurdon *et al.*, 1973). Stability can be restored by adding back the poly(A) tail (Huez *et al.*, 1975). There appears to be a critical tail length of about 30 adenylic residues (Nudel *et al.*, 1976). In general, messengers possessing tails are stable, but naturally occurring mRNAs lacking tails are not necessarily unstable, as shown by McCrae and Woodland (1981) for all 10 reovirus mRNAs. At first it seemed that interferon mRNA, which normally has a tail, was rather unstable in oocytes whether or not it was polyadenylated (Sehgal *et al.*, 1978). However, Marbaix and Huez (1980) argued that exported protein had not been carefully accounted for, thereby leading to a false comparison between the stabilities of normal and deadenylated interferon messengers. Soreq *et al.* (1981) refute this argument and provide evidence that poly(A) removal has little or no effect on the decay, which is biphasic ($t_{1/2}$ is 6–10 hours phase 1 and 30 hours phase 2), of fibroblast interferon messengers.

Have these oocyte injection experiments solved the mystery of the poly(A) tail? Clearly, removal of the tail destabilizes globin messenger and this structure is therefore an essential part of the molecule, as is the cap. Furthermore, the effect of tail removal is the same in HeLa cells as it is in oocytes (Huez *et al.*, 1981). Yet one does not necessarily expect parts of molecules to be stable in living cytoplasm. It is therefore significant that intact human histone messenger can be stabilized

by adding a poly(A) tail (Huez *et al.*, 1978). Poly(A) itself is stable in oocytes (Allende *et al.*, 1974) and one can argue that stability would be conferred by any polynucleotide resistant to exonucleases. Woodland and Wilt (1980a,b) have shown that injected sea urchin histone mRNAs are also unstable in both oocytes and early embryos, although in oocytes a small fraction of the injected mRNA is both stable and probably remains deadenylated. Hentschel *et al.* (1980) and Probst *et al.* (1979) report that histone mRNA made under the direction of injected sea urchin genes is quite stable. These intriguing observations reopen the whole question of an obligatory relationship between mRNA stability and polyadenylation, as do the findings that deadenylated mengovirus RNA (Revel and Groner, 1978) and interferon RNA (Soreq *et al.*, 1981) are stable in oocytes.

It should perhaps be emphasized that there has to date been no systematic and general study of mRNA stability in oocytes. The relationship between the amount of mRNA injected and mRNA half-life has not been investigated in detail, although Allende *et al.* (1974), using radioactive RNA presumed to be messenger, found that unengaged mRNA was rapidly degraded. Other authors, perhaps injecting smaller amounts of RNA, noted that the stability of deadenylated globin mRNA seemed inversely related to translational efficiency (Huez *et al.*, 1977a). McCrae and Woodland (1981) injected a mixture of similar amounts of 10 radioactive reovirus mRNA species and showed that mRNA stability was quite independent of translational efficiency. It is well known that injecting large amounts of messenger does not produce correspondingly large amounts of product, and, although it seems likely, there is no proof that some of this excess mRNA is degraded.

III. Posttranslational Events in *Xenopus* Oocytes

A. Secondary Modification of Foreign Proteins

The fate of translation products as well as that of messengers can be studied using the oocyte system: the destiny of newly made foreign proteins was examined initially from the standpoint of secondary modification. Thus Berns *et al.* (1972) noticed that the N-terminal methionine of αA2 crystallin was N-acetylated whether the protein was formed in oocytes or calf lens cells. Such an experiment does not prove that frog enzymes within the oocyte are capable of modifying heterologous proteins. Although partially purified (14 S) crystallin mRNA was used, one can still argue that the acetylating enzyme was made by some minor messenger species present in the injected RNA. However, recent experiments (Cutler *et al.*, 1981) with highly purified

TABLE II

FUNCTIONAL PROTEINS MADE IN FROG OOCYTES UNDER THE DIRECTION OF FOREIGN GENES AND MESSENGER RNAS

Source of messenger RNA injected into occytes	Translation product	Bioassay	Authors
Human fibroblast	Interferon	Inhibition of viral infection	Reynolds *et al.* (1975)
Mouse kidney	β-Glucuronidase	Enzyme activity	Labarca and Paigen (1977)
Pig colostrum	Immunoglobulin	Antigen binding	Kortbeek-Jacobs and Van der Donk (1978)
Xenopus liver	Vitellogenin	Uptake by oocytes	Lane, Champion, Colman, James, and Applebaum (unpublished)
Rat spleen	Immunoglobulin	Antigen binding	Deacon and Ebringer (1979)
Herpes virus (DNA)	Thymidine kinase	Enzyme assay	McKnight and Gavis (1980)
Rat liver	Cytochrome P-450	Enzyme assay (deethylase activity)	Ohlsson *et al.* (1981)
Torpedo electric organ	Acetylcholine receptor	α-Bungarotoxin binding	Sumikawa *et al.* (1981)

messenger have established that N-glycosylation and other modifications of chicken ovalbumin occur through the action of endogenous frog enzymes. The injected oocyte can, it appears, carry out a whole range (Asselbergs, 1979) of such enzymatic reactions (see Table II), ranging from phosphorylation, hydroxylation, glycosylation, and acetylation, to signal sequence removal and further cleavage of polypeptides including viral precursors (see Tables I, II, and III). The formation of disulfide bonds and the assembly of multimeric proteins also takes place. If, as seems likely, all these reactions are carried out by endogenous oocyte enzymes, why are these heterologous proteins modified in the manner expected of their parental cell types? Perhaps it is the nature of the substrate rather than the spectrum of enzymes that determines the processing pathway. Thus mouse kappa chains are glycosylated whether formed in oocytes or plasmacytoma cells, yet a mutant kappa chain remains unglycosylated (Jilka *et al.*, 1977) when made in either cell type. Similarly, newly made egg yolk precursor proteins from lo-

cust and frog are processed differently in *Xenopus* oocytes (see Fig. 1). The locust vitellogenin is processed extensively prior to export while the frog precursor is exported intact but is then imported, cleaved, and assembled into yolk platelets.

The formation of foreign proteins within oocytes does, however, provide some evidence albeit sparse of cell type specific enzymes. Certain newly made proteins such as mouse β-glucuronidase (Labarca and Paigen, 1977) and guinea pig caseins (Lane *et al.*, 1979) have anomalous gel mobilities. The oocyte lacks significant casein kinase activity and the proteins made from injected mammary gland mRNA cannot be labeled with ATP or phosphate. Thus caseins formed in oocytes appear underphosphorylated and indeed will only electrophorese with guinea pig milk caseins if the latter have been treated with phosphatase (Pas-

TABLE III

SECONDARY MODIFICATION AND SEGREGATION OF FOREIGN PROTEINS MADE IN *Xenopus* OOCYTES

Modification in parental cell type	Protein	References (selected examples)[a,b]
N-Acetylation	Calf lens αA2 crystallin	Berns *et al.* (1972)
Hydroxylation	Mouse fibroblast collagen	Lane and Knowland (1975)
Glycosylation	Mouse plasmacytoma immunoglobulin	Jilka *et al.* (1977)
	Thyroid stimulating hormone	Kourides and Weintraub (1979); Kourides *et al.* (1979)
	Rat prostatic binding protein	Mous *et al.* (1979)
	Human chorionic gonadotrophin	Mous *et al.* (1980)
	Chicken ovalbumin	Colman *et al.* (1981b)
	Rat immunoglobulin	Deacon and Ebringer (1977, 1979)
Signal sequence removal	Mouse plasmacytoma immunoglobulin light chain	Mach *et al.* (1973)
	Mouse (MOPC 321) kappa chain	Jilka *et al.* (1979)
	Honey bee venom gland promelittin	Lane *et al.* (1981b)
Phosphorylation	Trout testis protamine	Gedamu *et al.* (1978)
Cleavage of polyprotein	*Xenopus* liver vitellogenin	Berridge and Lane (1976)
	Viral polyproteins	See Table I
S–S bond formation	Rabbit uteroglobulin	Beato and Rungger (1975)
	Immunoglobulins (Various)	Deacon and Ebringer (1977, 1979) Valle *et al.* (1981)

(*continued*)

TABLE III (*Continued*)

Modification in parental cell type	Protein	References (selected examples)[a,b]
Noncovalent assembly of protein subunits	Calf α- and β-crystallins	Asselbergs *et al.* (1978, 1979b)
	Mouse kidney β-glucuronidase	Labarca and Paigen (1977)
Noncovalent metalloporphyrin addition	Acetylcholine receptor	Sumikawa *et al.* (1981[2])
	Rabbit hemoglobin (tetramer)	Lane (unpublished)
Protein export	Guinea pig milk proteins	Colman and Morser (1979)
Protein sequestration within vesicles	*Xenopus* liver albumin	Zehavi-Willner and Lane (1977)
	Mouse immunoglobulins	Winberry *et al.* (1980)
Insertion of integral membrane protein	Rat liver epoxide hydratase	Ohlsson *et al.* (1981)
Assembly into yolk platelets	*Xenopus* liver phosvitin and lipovitellin	Berridge and Lane (1976)
Assembly into ribosomal subunits	*E. coli* and *Artemia salina* acidic proteins	Kalthoff and Richter (1979)
Assembly into protein bodies	Maize storage proteins	Hurkman *et al.* (1981)
Entry into nuclei	*Xenopus* nuclear proteins	de Robertis *et al.* (1978)

[a] For a more complete set of references see Lane and Knowland (1975), Lane *et al.* (1981a), Colman *et al.* (1981a), and especially Asselbergs (1979). An attempt has been made to cite references which add to the extensive list compiled by the latter author.

[b] Acetylcholine receptors are, surprisingly, also present in uninjected oocytes (Kusano *et al.*, 1977).

cal, Boulton, Lane, and Craig, unpublished). Thyroglobulin is probably not iodinated (Vassart *et al.*, 1975) and β-crystallin fails to assemble correctly in oocytes (Asselbergs *et al.*, 1979a,b).

The frog cell also fails to remove the pro sequence from insulin (Rapoport, 1981), and promelittin, which is not even exported to any significant extent, also remains intact. Promelittin made in oocytes behaves anomalously and has therefore been analyzed in some detail (Kindas-Mugge *et al.*, 1974; Lane *et al.*, 1981b). The N-terminus of the molecule is "frayed" as is the promelittin of the venom gland cell: yet in the oocyte further removal of dipeptides is so slow that melittin formation cannot even be detected. At the C-terminus, the oocyte product fails to undergo transamidation to form the characteristic glutamine amide moiety. Thermodynamic calculations (Von Heijne, 1980) and the results of subcellular fractionation experiments suggest that the hydrophobic promelitting molecule becomes marooned within intracellular membranes of the oocyte. As a rule, however, foreign proteins are

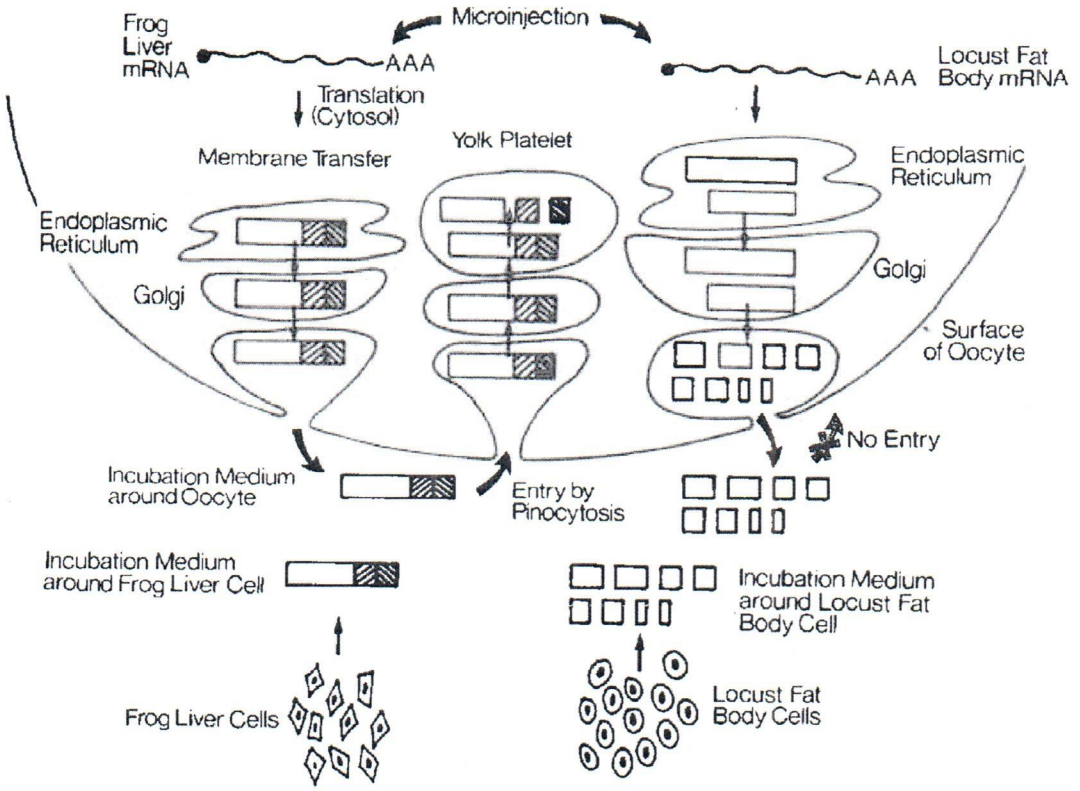

FIG. 1. The fate of *Xenopus* and locust vitellogenins made in *Xenopus* oocytes: an export–import-processing model. Microinjection of locust fat body or *Xenopus* liver messenger leads to the synthesis and sequestration within membranes of high-molecular-weight vitellogenin species. A single band of about M_r 210,000 is seen with frog mRNA while in oocytes programmed with locust messenger a doublet, shown by peptide mapping to be the products of two genes (Chen, 1980), is found in the range M_r 220,000–250,000. There is no direct evidence that the yolk precursors then progress through the Golgi and secretory vesicles, although such structures are present in oocytes: there is however direct evidence that some at least of the vitellogenin is exported, the locust protein probably being processed just prior to secretion. The pinocytotic uptake pathway shown is well established (see review by Wallace, 1978). The occurrence of intact frog vitellogenin in washed yolk platelets is consistent with the internally generated precursor adopting the pathway followed by exogenous frog vitellogenin. The four major locust polypeptides exported from oocytes programmed with fat body RNA migrate electrophoretically with the four major locust egg yolk proteins (vitellins). Processing of the minor locust polypeptides has not been investigated thoroughly, although minor species are seen in the expected molecular weight range. Thus as judged by SDS–gel electrophoresis, the egg yolk precursor processing pathways of the frog oocyte are characteristic of the cell used to prepare the injected RNA. The evidence that locust vitellogenins do not rapidly enter frog oocytes is provided by gel analysis of subcellular fractions, but entry followed by rapid degradation has not been ruled out. The above model is consistent with the effect on the conversion of internally generated *Xenopus* vitellogenin of a variety of externally applied agents. Thus mRNA directed yolk platelet formation is inhibited by antivitellogenin antibodies, excess unlabeled vitellogenin, other (uninjected) oocytes, and various drugs (tunicamycin, colchicine, and cytocholasin). There is evidence to suggest that at least the antibodies and the whole oocytes act externally. However it must be stressed that such observations do not prove the export–import-processing model nor do they exclude the existence or purely internal pathways of messenger directed platelet protein formation (Lane, Champion, Colman, James, and Applebaum, unpublished).

processed faithfully and are, therefore, biologically active (see Table III). Interferon mRNA is routinely assayed by microinjection (Reynolds *et al.*, 1975) and human fibroblast interferon exported from oocytes retains its cell type specificity (Morser and Colman, unpublished). Catalytically active thymidine kinase is formed in oocytes under the direction of DNA introduced directly into the nucleus, an elegant demonstration (Cordingley and Preston, 1981; McKnight and Gavis, 1980) that, if the appropriate naked DNA sequence is presented all subsequent steps in the gene expression pathway take place automatically (see reviews by de Robertis and Gurdon, 1979; Lane, 1976). 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.

B. The Fate of Foreign Proteins and Transfer RNAs in Oocytes

The mechanisms responsible for directing specific proteins to specific subcellular destinations are not well understood, and the *Xenopus* oocyte microinjection system provides an *in vivo* approach to the so ing problem. The direct injection of proteins permits analysis of the restrictions governing entry into the nucleus (Bonner, 1975a,b, 1980; Gurdon, 1970). Most nuclear proteins have a specific affinity for the nucleus of the oocyte (de Robertis *et al.*, 1978). Rather surprisingly, disrupting the nuclear membrane with a microinjection needle fails to abolish the selectivity of the uptake process (Feldherr and Ogburn, 1980).

The fate of nascent chains is often different from that of completed polypeptides and it is frequently more revealing to study the interaction with the protein sorting machinery of macromolecules formed *in situ*. In the first such experiment, liver mRNA was found to program the synthesis of vitellogenin which was subsequently converted to lipovitellin and phosvitin, the latter products being assembled into yolk platelets (Berridge and Lane, 1976). Injected vitellogenin was merely degraded and it seemed that the purified protein could only act as a precursor when presented to the surface of the oocyte (Wallace, 1978). Yet the oocyte was regarded as a closed system. The first light to be shed on this paradox came from further experiments combining mRNA injection with subcellular fractionation. Newly made albumin, milk proteins, and vitellogenin were found sequestered within membranous vesicles which could be isolated by sucrose gradient centrifugation (Zehavi-Willner and Lane, 1977). Then Lebleu *et al.* (1978) noticed that when oocytes were programmed with RNA from virus-infected mouse cells, interferon could be detected by bioassay in the

surrounding medium. Proof that the oocyte is indeed a secretory cell was provided by the elegant work of Colman and Morser (1979), in which leakage artifacts were ruled out by coinjecting mRNAs coding for globin and milk proteins, only the latter being exported. The *Xenopus* oocyte can be used as a surrogate secretory system (Lane *et al.*, 1981a) and will export proteins made by messengers from plants, insects, birds, and mammals, the exception being honey been venom gland RNA which directs the synthesis but not the export, at least in detectable amounts, of melittin or promelittin (Lane *et al.*, 1981b).

The paradoxical results obtained with vitellogenin mRNA could now be explained by an export–import-processing model (see Fig. 1). The addition to the surrounding medium of antivitellogenin antibodies blocks mRNA-directed yolk platelet formation, as predicted by the model. Moreover, if defolliculated oocytes are used (the procedure seems to decrease recapture of secreted vitellogenin) yolk platelet proteins made under the direction of injected mRNA accumulate within the platelets of uninjected oocytes present in the same incubation vessel (Lane, Champion, Colman, James, and Appelbaum, unpublished).

What are the requirements for the secretion of foreign proteins from oocytes? Not surprisingly, topological segregation is a prerequisite for export: miscompartmentalized ovalbumin whether formed *in situ* (and lacking glycosyl residues) or introduced by injection, remains trapped within the cytosol while unglycosylated ovalbumin, made by tunicamycin treatment of oocytes, is secreted, as is the correctly modified eggwhite protein (Colman *et al.*, 1981a). The export of heterologous proteins is inhibited by colchicine. Cytocholasin acts synergistically suggesting that microtubules stabilized by microfilaments are required for the release of foreign proteins from oocytes (Colman *et al.*, 1981b). Certain aspects of the secretory process depend on the kind of protein being exported and indeed the oocyte system is well suited to the study of such interactions. Valle *et al.* (1981) have demonstrated that H and L immunoglobulin chains are exported only in a stoichiometric (1 : 1) ratio: there is an absolute requirement for light chains. Interaction at the protein subunit level can take place even when separated heavy and light chain mRNAs are injected into different poles of the oocyte. Thus either the mRNAs or their products diffuse thereby permitting subunit assembly.

Does the oocyte normally use the functional secretory system revealed by mRNA microinjection experiments? Mohun *et al.* (1981) answered this question by removing the layers of cells, including the tightly adhering follicle cells, which normally surround oocytes and then assaying the external medium for exported proteins. Two-

dimensional gel electrophoresis revealed several polypeptides at least two of which could also be identified within a membrane vesicle fraction isolated from oocyte homogenates. These vesicles were known to be part of the secretory pathway. Further experiments showed that the surrounding layers of cells as well as the oocyte itself secrete proteins. Folliculated oocytes export proteases (Soreq and Miskin, 1981) whose activity can be abolished by adding a mixture of inhibitors.

Heterologous secretory proteins can be degraded inside as well as outside the oocyte, the extent of the loss varying with the nature of the protein and the time spent in the secretory pathway. Colman *et al.* (1981b) proposed a "conveyor belt" model, having noted that the rate of loss, allowing for both degradation and secretion, is constant for a given protein. Thus each species appears to move along the secretory pathway at a characteristic rate and is either exported or destroyed. Further evidence is required to substantiate such a model. It is nonetheless clear that different proteins, for example lysozyme and ovalbumin, are secreted at fundamentally different rates. Ovalbumin made under the direction of highly purified messenger maintains its characteristic rate of export, which suggests that the kinetics, at least in frog cells, are not influenced by any oviduct specific factors (Cutler *et al.*, 1981).

Microinjection experiments involving other subcellular compartments are rare, despite the promise of the oocyte system for studying the molecular traffic signals that govern the entry of proteins into mitochondria, lysosomes, storage granules, and membranes themselves. For example, heterologous mitochondria have been introduced into (Abramova, 1979) and survive in frog cytoplasm (Pinon *et al.*, 1975), and the oocyte is also packed with mitochondria of its own. Moreover, functional lysosomal enzymes can be made by injecting mouse cell mRNA (Labarca and Paigen, 1977). Yet there are no significant experiments on either of these two organelles. As regards storage proteins, two studies exist: first (Lane *et al.*, 1981a), it was shown that barley seed storage proteins are not exported, in contrast to other plant proteins made at the same time. Second, in a study (Hurkman *et al.*, 1979, 1981; Larkins *et al.*, 1980) which includes an improved method of subcellular fractionation, it was revealed that maize storage proteins are retained in the oocyte within structures that resemble plant protein bodies. Presumably such structures are induced by the storage proteins themselves being encoded directly by the injected mRNA. As regards membrane proteins, the synthesis, but not the fate, of the plasmalemmal glycoprotein 5′-nucleotidase was studied by Bergeron *et al.* (1975). Ohlsson *et al.* (1981) demonstrated the insertion

of rat cytochrome P-450 and functional epoxide hydratase into intracellular membranes of the oocyte. The insertion process was then studied in further detail using an *in vitro* system containing *Xenopus* membranes. The frog membrane preparation provides a convenient alternative to, for example, systems which use endoplasmic reticulum from dog pancreas.

Microinjection is one obvious means of studying protein degradation *in vivo,* an important objective given the lack of suitable *in vitro* systems. Taking into account the specialized role played by storage, experiments were required to prove that the oocyte contains an active degradatory system. Studies in which protein export is disregarded (Wallace and Hollinger, 1979) do not prove the point. However, the lack of stability of primary translation products bearing detachable signal sequences provides such evidence, and has led to speculation (Lane *et al.,* 1979) that the oocyte contains proteases which correct errors of compartmentation. The concept of such error-correcting machinery can be generalized to include degradation within the cytosol of DNA (Wyllie *et al.,* 1977, 1978) or of RNA containing introns. Many injected proteins are destroyed with surprising rapidity, that is, in a matter of minutes, presumably by a nonlysosomal cytosolic enzyme system akin to that discovered in mammalian cells by Bigelow *et al.* (1981). Injected proteins can also serve as probes, for example, to prove the presence (presumably within the cytosol) of a particular protein kinase (Maller *et al.,* 1978; Masaracchia *et al.,* 1979); more generally, the introduction of specific antibodies into living oocytes is potentially very rewarding as an experimental approach. Nuclear injection of antihistone (Scheer *et al.,* 1979) and antiactin antibodies (Rungger *et al.,* 1979b) has been reported, and studies involving the introduction of antibodies raised against putative gene regulatory elements are eagerly awaited.

The fate of foreign transfer RNAs in oocytes is somewhat predictable: they appear quite stable (Allende *et al.,* 1974) and are functional (Gatica *et al.,* 1975, 1979; Gatica and Allende, 1977). Enzymes within the frog cell will catalyze the addition of 3′-terminal C-A nucleotides to tRNA species lacking these two residues (Solari *et al.,* 1977). The oocyte can therefore be used as a functional assay, for example, in the detection of eukaryotic suppressor tRNAs (Bienz *et al.,* 1980) or of impaired tRNAs produced, for example, by ethionine treatment of rats (Ginzburg *et al.,* 1979). Joshi *et al.* (1978) have demonstrated the aminoacylation and processing of turnip yellow mosaic virus RNA in oocytes, thereby confirming results obtained *in vitro* suggesting that the viral RNA is a surrogate tRNA. Specific tRNAs can also serve as probes and in the elegant experiments of Bienz *et al.* (1981) injection of

suppressor tRNAs revealed that all three termination codons can be used by a given cell type, the *Xenopus* oocyte. Yeast mitochondrial tryptophanyl transfer RNA_{Trp} also functions as a suppressor when introduced into the cytoplasm of the frog cell (Grosjean *et al.*, 1981) provided that (Martin *et al.*, 1981), if the tRNA is not already activated, some *E. coli* acylating enzyme is coinjected. Such studies complement those on the microinjection of tRNAs, including suppressor tRNAs (Capecchi *et al.*, 1980), into somatic cells (see review by Celis *et al.*, 1980).

IV. The Introduction of Foreign Genes into Oocytes

DNA was first injected into eggs and oocytes of *Xenopus* in 1969 (Gurdon *et al.*, 1969; Gurdon and Speight, 1969), but the aim of this pioneering investigation was the study of gene replication. As reviewed by Harland and Laskey (1980), Laskey *et al.* (1979), and Laskey and Harland (1981) the above approach has born fruit. Transcription of microinjected templates was first investigated by Knowland (1971). Colman (1975) introduced synthetic, mammalian, and viral templates into eggs and oocytes: in these important experiments high voltage paper electrophoresis established beyond doubt that transcription of the heterologous DNA had occurred. However, the results were variable and a second breakthrough was required before the system could be used to study the control of transcription. Thus Gurdon *et al.* (1976a,b) found that nuclei could be deposited within the nucleus of the oocyte and once there swelled (Gurdon, 1976) and became transcriptionally very active. The nucleus of the oocyte is so large that direct injection of substances is really very easy, yet the prospect had daunted earlier investigators. Purified DNA molecules were soon injected (Mertz and Gurdon, 1977), the initial studies focusing on SV40 transcription. Genes transcribed by polymerase I were then shown to be active, whether derived from cloned ribosomal genes (Brown and Gurdon, 1977, 1978) or from purified ribosomal DNA (Gurdon and Brown, 1977, 1978). Individual transcription complexes formed by the injected DNA can be seen by electron microscopy (Trendelenburg *et al.*, 1978, 1980; Trendelenburg and Gurdon, 1978). In another important series of experiments Kressman *et al.* (1977, 1978, 1979) developed an even simpler method of injecting DNA (in this case tRNA genes) into nuclei. Oocytes were lightly centrifuged so as to bring the nucleus to the surface of the pigmented pole, where it could be seen as a translucent body lying just below the cell surface. Now the target was visible, it was easy to insert the micropipet to exactly the right distance. Using this

method, the Swiss group have also analyzed in detail the relationship between sequence and function within the sea urchin histone gene repeating unit (Grosschedl and Birnstiel, 1980a,b; Hentschel *et al.*, 1980; Probst *et al.*, 1979). In this context, gene function includes the production of authentic histone proteins (Etkin and Maxson, 1980; de Robertis and Mertz, 1977). Such genes are of course transcribed by polymerase II and it only required further studies on heterologous tRNA genes, namely, those from yeast (de Robertis and Olson, 1979) and from nematodes (Cortese *et al.*, 1978), to demonstrate that the oocyte system could be used to investigate transcription by all three classes of polymerase. The experiments on tRNA formation have also been very detailed and have shed light on sites of modification and processing, including splicing, as well as on transcription itself (Cortese *et al.*, 1980; Hofstetter *et al.*, 1981; Melton *et al.*, 1980; Melton and Cortese, 1979; Telford *et al.*, 1979). It is clear that the enzymes of the frog cell can recognize the splicing signals present on a variety of heterologous transcripts (Rungger and Turler, 1978). For example, ovalbumin is formed by injected ovalbumin genes, although splicing efficiency may well be lower than in the parental cell type (Wickens *et al.*, 1980; see also Ladner *et al.*, 1979). The splicing enzymes are located within the nucleus, but not apparently within the nuclear membrane (de Robertis *et al.*, 1981).

Nuclear injection of centrifuged oocytes is described with clarity in the review by Kressman and Birnstiel (1980). Other oocyte microinjection systems have been described (Contreras *et al.*, 1981; Hengst, 1977), that of Hitchcock and Friedman (1980) being semiautomatic, but it should be emphasized that nuclear injection is not a difficult technique (see apparatus described by Stephens *et al.*, 1981). Consequently there is a rapidly growing literature on transcriptional studies in oocytes as can be seen from the review by Wickens and Laskey (1981). It is difficult even using centrifuged oocytes to ensure that DNA is always introduced into the nucleus and never into the cytoplasm. Fortunately, for most experiments this does not matter. If covalently closed, supercoiled DNA is injected into the cytoplasm the polymer is first relaxed, then nicked, then linearized, and then cleaved by an endonuclease. Similar polymers introduced into the nucleus are also relaxed but then form new supercoiled structures and finally, in the case of SV40 DNA, minichromosomes. Wyllie *et al.* (1977, 1978) concluded their important study on the fate of injected DNA by showing that linear species are degraded within the oocyte nucleus, presumably by an exonuclease, during which time however some transcripts are formed.

Why do oocytes transcribe injected genes? Why are the normal control mechanisms seemingly overridden? Nearly all DNA microinjection experiments reported have involved the infusion of vast numbers of genes, and so it is possible that the normal control mechanisms operate at physiological gene dosages. It is also conceivable that heterologous genes circumvent the control systems, and there are few examples of the injection of *Xenopus* genes, especially those coding for proteins. However, it is also possible that, if the right nucleotide sequences are present, naked DNA is always transcriptionally active. For example, Miller and Melton (1981) could detect the activity of a *Xenopus* 5 S RNA pseudogene which in *Xenopus* oocytes is normally dormant, or at least whose activity cannot be detected. Must the DNA be in the correct form, that is to say assembled into a chromosome, if the cytoplasm of the oocyte is to impose a specific pattern of gene expression on material introduced by microinjection? In an elegant series of experiments the Cambridge School (de Robertis *et al.*, 1977a,b,c; de Robertis and Gurdon, 1977, 1979; Gurdon, 1977) has shown that injected nuclei respond selectively to transcriptional control elements present in the cytosol of the oocyte. For example, kidney cell nuclei from newts express proteins characteristic of newt oocytes when injected into *Xenopus* oocytes, while kidney cell functions are repressed. The transcriptional control mechanisms within the *Xenopus* oocyte therefore lack species specificity, but cell type specificity manifests itself clearly.

How can the oocyte system be used to dissect out the components involved in such developmental regulation? One approach is to remove substances from nuclei until the latter begin to lack responsiveness to cytoplasmic signals, and then to find out which factors will restore the regulated state. Korn and Gurdon (1981) have done just this and have "activated" by salt extraction 5 S RNA genes within nuclei. It will be interesting to see if the repressible state can then be restored by adding back the extracted proteins. This type of experiment is, one hopes, the precursor to more detailed *in vivo* studies on developmental regulation. One should not, however, underestimate the usefulness of *in vitro* systems, including those derived from oocytes. It will be interesting to see what role the intriguing transcriptional control element, discovered (Engelke *et al.*, 1980; Pelham *et al.*, 1981; Pelham and Brown, 1980) during *in vitro* studies on 5 S RNA transcription, plays in modulating the activity of 5 S genes introduced into whole oocytes.

V. The Use of the Oocyte as a Surrogate Gene Expression System

Will the oocyte continue to be a useful experimental system? The work of the last decade has established the frog cell as a medium for

surrogate gene expression. It is clear that machinery lacking either cell type or species specificity exists within the oocyte and that all the steps from transcription of the gene to the fate of the processed protein can therefore be studied. Nonetheless, there are many other surrogate systems. Informational macromolecules can be inserted into the cells of eukaryotes by direct addition (Wigler *et al.*, 1978) or by means of viral vectors (Berg, 1981), or via liposomes (Gregoriadis, 1980), or using red cell ghosts (Kriegler and Livingstone, 1977; Loyter *et al.*, 1975; Schlegel and Rechsteiner, 1978). Furthermore in addition to frog oocytes, other cell types, including cells of normal size, can be microinjected (Graessmann and Graessmann, 1971; Stacey and Allfrey, 1976), although inserting substances into the nuclei of such cells is difficult. Oocyte microinjection is not confined to *Xenopus* (Borovkov, 1975; Brachet *et al.*, 1973; May and Glenn, 1974) nor even to amphibia: indeed Brinster *et al.*, (1980, 1981a,b) using mouse oocytes have repeated many of the basic studies on transcription and translation of heterologous macromolecules, and Gordon *et al.* (1980) as well as Wagner *et al.* (1981) and Harbers *et al.* (1981) provide evidence of transformation by injected DNA. It is relatively easy to inject defined amounts into giant cells, for example the *Xenopus* oocyte, but other large cells, such as those of protozoa (Knowles *et al.*, 1978) or algae (Cairns *et al.*, 1978) are also available.

The oocyte offers a particularly convenient general system in which to test known amounts of substances within either the nuclear or cytoplasmic compartments. As such it should continue to enjoy a certain popularity. Doubtless the relationship between the structure and the fate or function of macromolecules will be explored further using oocytes. For example, sequence manipulation at the DNA level could be correlated with topological segregation of a given protein. In theory, microinjection combined with subcellular fractionation can be used to shed light on the function of an ill-characterized translation product or of an unknown gene or messenger. The oocyte is clearly an excellent system for studying posttranslational events, but progress with cell-free systems is so rapid that, except for those processes involving complex structures, the frog cell system may not in the end prove very useful, unless one wishes to study kinetics or control mechanisms.

The use of whole cell systems for the assay of messenger RNA can be justified only in terms of special requirements, such as the need to measure a biologically active end product, or the availability of only minute amounts of mRNA, or, because of problems *in vitro* with, for example, a very large messenger. Nonetheless, when it comes to the control of protein synthesis the system still has potential. The interac-

tion of exogenous mRNAs with putative control elements, either exogenous (Giglioni *et al.*, 1973) or endogenous, can be investigated as can regulation of the endogenous mRNAs, many of which are at any given time unengaged and are possibly masked (Davidson, 1976). The engaged messenger fraction is found both attached to the endoplasmic reticulum and free in the cytosol, and the rules governing this allocation can also be studied by oocyte microinjection.

The mechanisms involved in transcription and processing are also amenable to analysis by oocyte injection, but once again *in vivo* work is likely to center on the control of these processes. It seems likely that the focus will be on developmental regulation, because many of the basic questions concerning promoters, splicing signals, and so forth can now be studied *in vitro*. Furthermore, interest in the mechanism behind variable gene expression leads to interest in the oocyte per se: hopefully, many studies will focus on the nature of the oocyte as the precursor to the totipotent egg cell. Microinjection experiments can of course be performed with eggs, and the potential of the *Xenopus* egg as a surrogate gene expression system should not be neglected. Stable macromolecules injected into eggs end up in differentiated cells, hence the interest in the system. Thus mammalian globin messengers persist (Gurdon *et al.*, 1974) and are translationally active within the tissues of both early (Froehlich *et al.*, 1977) and late frog embryos, and there is good evidence that even differentiated muscle cells can make rabbit globin (Woodland *et al.*, 1979). Injected frog muscle actin mRNA can be translated by early embryos while endogenous α-actin synthesis cannot be detected until the gastrula stage (Sturgess *et al.*, 1980). During early development, injected rabbit globin mRNA but not poly(A) is unequally distributed between different cell types (Froehlich *et al.*, 1977). Injected histone messenger has a short half-life (Woodland and Wilt, 1980b). Studies on interspecific hybrids have revealed that the maternal stockpile of histone mRNA is also turned over quite rapidly, and by the gastrula stage is largely replaced by newly synthesized transcripts (Woodland, 1980; Woodland *et al.*, 1979).

Globin genes introduced directly into the egg seem both to persist and to override the control mechanisms which so clearly repress the endogenous globin genes: heterologous transcripts can be detected, at least up to the gastrula stage. The injected foreign genes also seem to be replicated (Bendig, 1981). Integration has not as yet been studied, but the possibility that developmental regulation can be investigated by injecting genes into frogs eggs is causing considerable excitement. Thus the egg and oocyte of *Xenopus* are of intrinsic interest to the embryologist investigating amphibian development but these giant

cells with their stockpiles of components can also be used for both *in vivo* and *in vitro* studies on genes from other organisms and other tissues.

ACKNOWLEDGMENTS

The ideas and assistance of J. Champion and R. Harris are gratefully acknowledged, as are the comments of Dr. R. Mohun, Dr. R. Craig, Dr. J. Morser, Dr. A. Boulton, Dr. J. Pascal, and Dr. A. Colman are thanked for giving access to results prior to publication.

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