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The Fate of Foreign Proteins Introduced into Xenopus Oocytes

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How interchangeable are the components of living cells? Results obtained with the Xenopus oocyte microinjection system have contributed to the belief that macromolecules and even organelles purified from one kind of cell will function when introduced into quite a different cell type. Thus messenger RNAs from mammals, birds, fishes, insects, viruses and plants direct the synthesis of heterologous proteins when injected into Xenopus oocytes. DNA injected into the nucleus of these giant cells can also program the production of foreign proteins. As well as providing a surrogate gene expression system, the oocyte, as the precursor to the totipotent egg cell, is of intrinsic interest. The stockpile of materials contained in the oocyte and used later in embryogenesis includes ribosomes, membrane systems, mitochondria and mRNAs, all of which are made internally. Moreover, there is also export and import of materials, and the complex architecture of the oocyte reflects these dynamic functions as well as those of synthesis and storage. The oocyte is therefore a rich source of components for use in vitro and, consequently, it is possible, with the same cell type, to study processes both in vivo and in vitro.

What happens to a foreign protein made in the frog oocyte? What does this reveal about the enzyme systems and sorting machinery of the oocyte, and how do the results obtained extend the usefulness of the system? The injection of calf lens-cell 14S mRNA provided the first evidence that enzymes within the oocyte are capable of correct modification of a protein characteristic of another cell type. Thus the N-terminal methionine of αA2 crystallin is N-acetylated whether the protein is formed in oocytes or in calf lens cells (Berns et al., PNAS *69,* 1606-1609, 1972). As reviewed by Asselbergs (Mol. Biol. Rep. *5,* 199-208, 1979), the oocyte is capable of a wide variety of secondary modifications, ranging from phosphorylation, hydroxylation and glycosylation to signal sequence removal, further cleavage of polypeptides, including viral precursors, and the assembly of multimeric proteins. In general, secondary modifications that take place in terminally differentiated cells can also occur in oocytes.

It is usually assumed that homologous oocyte enzymes, rather than heterologous enzymes formed by minor messenger species present in the injected RNA, are responsible for modification. The relatively few studies performed with purified messengers bear out this assumption. Why then are proteins modified in the manner expected of the highly specialized cell types used to prepare the donor RNA? Perhaps the enzymes responsible for secondary modification are present in oocytes, and possibly in all other cell types, and it is the nature of the substrate that determines the processing pathway. For example, the egg-yolk precursor proteins from insects and amphibians are processed differently in frog oocytes and, as far as can be seen, the pathways followed are similar to those of the donor cells. More specifically, Jilka et al. (BBRC *79,* 625-630, 1977) have shown that mouse kappa chains made in oocytes are glycosylated only if the donor RNA is extracted from plasmacytoma cells producing glycosylated kappa chains.

Nonetheless, the oocyte does provide some evidence of cell-type-specific enzymes. The frog cell fails to remove the pro sequence from proinsulin, and promelittin also remains unscathed. Beef thyroglobulin is probably not iodinated, whereas some products such as mouse βglucuronidase and guinea pig caseins have anomalous mobilities on gels. However, in general the oocyte processes polypeptides faithfully: one consequence of such fidelity is the production of biologically active proteins. Thus interferon mRNA is often assayed by measuring interferon titers in and around oocytes (Reynolds et al., PNAS *72,* 4881-4885, 1975). The occurrence of correct processing implies that foreign proteins reach the appropriate enzymes, many of which are believed to occupy specific subcellular locations.

How is the fate of newly made foreign proteins influenced by the complex subcellular architecture of the amphibian oocyte? Is there any interaction between foreign proteins and the sorting machinery of the frog cell? The mechanisms responsible for directing different proteins to different subcellular destinations are not well understood, and in fact the oocyte provides a useful in vivo system for analyzing the sorting problem. The nuclear compartment was the first to be examined (Gurdon, Proc. Royal Soc. *176,* 303-314, 1970). The nucleocytoplasmic distribution of a wide range of injected proteins has been studied (Bonner, JCB *64,* 421-430, 431-437, 1975). The results reveal the specific nuclear affinity of most nuclear proteins, as well as restrictions on entry into the nucleus for many but not all large proteins. The giant nucleus of the oocyte can be removed in its entirety and it can also be injected in situ; thus Feldherr and Ogburn (JCB *87,* 589-593, 1980) have shown, by pricking holes in the nucleus, that the entry

of specific oocyte proteins does not depend on there being an intact nuclear membrane.

If one is to investigate the transfer across membranes of nascent chains as well as completed polypeptides, it is essential to inject both messenger RNAs and proteins. Study of the intracellular fate of proteins synthesized under the direction of injected messenger began with a paradox (Berridge et al., Cell *8,* 283–297, 1976): the injection of vitellogenin messenger RNA (but not vitellogenin protein) led to the formation of yolk proteins assembled, apparently correctly, within platelets; yet the normal pathway was known to involve pinocytotic uptake by oocytes of vitellogenin from the bloodstream. The first clue came from the finding that mRNAs coding for secretory proteins transferred their products across membranes within the oocyte. Thus newly made albumin, milk proteins and vitellogenin itself were recovered within a membrane-vesicle fraction (Zehavi-Willner et al., Cell *11,* 683–693, 1977). It required the important observation by Lebleu et al. (BBRC *82,* 665–673, 1978) and Colman and Morser (Cell *17,* 517–526, 1979) that interferon could be detected outside oocytes injected with RNA from virus-infected cells, and the proof that export did not result from leakage, to establish that the oocyte is not a closed system: clearly the frog cell possesses a functional secretory pathway. Subsequent work has shown that secretory mRNAs from plants and animals will in almost every instance direct the synthesis and export of proteins (Colman and Morser, loc. cit.). Vitellogenin is no exception and there is now evidence that the precursor protein does not accumulate in the surrounding medium but instead is imported, organized into platelets and processed, thereby resolving the paradox.

Does the oocyte actually use the secretory apparatus it so clearly possesses? Mohun et al. (J. Embryol. Exp. Morph. 61, 367–383, 1981) have demonstrated the export of Xenopus proteins from uninjected, defolliculated oocytes, and have also established that, while the follicular layers are themselves secretory, they are not required for secretion from oocytes of either heterologous or homologous proteins. Pulse-chase experiments show that Xenopus oocytes export Xenopus proteins very slowly, while many foreign proteins, such as interferon, are secreted in a matter of hours. Other foreign species take days to complete their passage out of the frog cell. It seems that different proteins are exported at intrinsically different rates. Other attributes of the pathway have been studied by Colman et al. (Eur. J. Biochem. *113,* 339–348, 1981), who have shown that correct intracellular location is a prerequisite for secretion and that, for ovalbumin at least, glycosylation is of little importance. Thus miscompartmentalized unglycosylated ovalbumin, made from injected chicken oviduct RNA, remains trapped in the cytosol; yet sequestered unglycosylated ovalbumin, produced by tunicamycin treatment of RNA-injected oocytes, is exported. The occurrence and lack of stability within the cytosol of primary translation products bearing detachable signal sequences has led to speculation that the oocyte contains proteases that correct errors of compartmentation (Lane et al., Eur. J. Biochem. *101,* 485–495, 1979). Thus, as suggested by Wallace and Hollinger (Exp. Cell Res. *119,* 227–287, 1979), the oocyte provides a convenient experimental system for the study of in vivo protein degradation.

Surprisingly little attention has been paid to other subcellular compartments, although the oocyte would also seem to be a good in vivo system for investigating the molecular traffic signals that determine entry into lysosomes, mitochondria and membranes themselves. However, it is known that heterologous mitochondria continue to function in frog cytoplasm (Pinon, in Molecular Biology of Nucleocytoplasmic Relationships, Puiseux-Dao, ed. [Amsterdam: Elsevier Scientific], 1975), and that oocytes will make functional mouse lysosomal enzymes (Labarca and Paigen, PNAS *74,* 4462–4465, 1977) and will also insert newly synthesized rat rough endoplasmic reticulum enzymes into frog intracellular membranes in functional form (Ohlsson et al., Eur. J. Biochem., in press). Insertion of heterologous proteins into Xenopus membranes has been studied both in vivo and in vitro. Nonetheless, while the oocyte appears to be an excellent system for analyzing posttranslational events in general and the sorting problem in particular, all the major advances have stemmed from in vitro studies with other tissues. Results obtained with oocytes have merely confirmed existing theories, such as the signal hypothesis, or have emphasized the interchangeability of constituent parts between cell types. In the future, one can envisage using the oocyte to analyze the relationship between sequence and subcellular localization. Sequence manipulation at the DNA level constitutes a feasible approach (Grosschedl and Birnstiel, PNAS *77,* 1432–1436, 1980) given, for example, that ovalbumin and functional thymidine kinase can be formed from cloned DNAs injected into the oocyte nucleus. The promise of the oocyte as a system for analyzing posttranslational events is generating much excitement, yet at present this promise remains largely unfulfilled.