confirmed by co-electrophoresis and fingerprinting (Millis et al., 1983). In addition to being an abundant protein, it is now clear that the properties of PDI are highly conserved over a wide taxonomic range. Thus although the enzyme has not been purified to homogeneity from a non-mammalian source, data on partially purified preparations from wheat and from embryonic chick tendon show close similarities in M, pl and in kinetic properties with the homogeneous enzymes from bovine and rat liver (Table 3).

In summary, protein disulphide-isomerase is an abundant and well-conserved protein whose catalytic properties, tissue distribution, subcellular location and developmental properties are all consistent with a role in the formation of native disulphide bonds in protein biosynthesis.

Bergman, L. W. & Kuehl, W. M. (1979b) J. Biol. Chem. 254, 8869-8876

Post-translational modification of exogenous proteins in Xenopus laevis oocytes

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Full-grown Xenopus oocytes are large cells (>1.2 mm diam.) and contain a considerable quantity of various macromolecules and organelles which form a maternal reserve for use during early embryonic development (Woodland, 1982). Three attributes have made the oocyte an attractive test system for studies on the transcription (Gurdon & Melton, 1981), replication (Harland & Laskey, 1980), assembly (Laskey et al., 1978), partition (De Robertis, 1983; Lane, 1983) and translation (Lane & Knowland, 1975; Asselbergs, 1979; Lane, 1983; Colman, 1984; Soreq, 1984) of injected macromolecules. This article will focus on the ability of oocytes to translate and then post-translationally modify proteins encoded on microinjected mRNA or cloned DNA. In 1971, John Gurdon and his colleagues (Gurdon et al., 1971) demonstrated that Xenopus oocytes would not only translate injected mRNA but would do so far more efficiently than cell-free translation systems. Since that time, over 80 different mRNAs have been successfully translated in the oocyte. However, for the majority of routine assays of mRNA, cell-free extracts remain the simplest and most economical translation systems. It is in the area of post-translational modification and processing that the oocyte offers special advantages. The processes of precursor cleavage, phosphorylation, glycosylation, segregation and secretion can all be correctly performed within the oocyte. Even biologically active, foreign proteins synthesized in oocytes can acquire their native activity despite the fact that this may involve complex subunit interactions.

In this review we will first document the various modifications which the oocyte has been reported to perform on exogenous proteins and, where possible, comment on the fidelity of some of these modifications as compared with the 'native' protein. It will become apparent that there are limitations in the ability of the oocyte to perform the correct post-translational modifications. How-

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ever, despite these limitations, there are powerful arguments for persevering with the oocyte system. These arguments will be illustrated in the context of some of the work carried out in our laboratory and those of other workers.

**Post-Translational Modifications in the Oocyte**

Table 1 shows the variety of post-translational modifications which can occur to foreign proteins in oocytes. For many of these proteins these modifications are consequent on the segregation of the protein into oocyte membranes where many of the modification enzymes are located. Some of the more general and striking of these modifications will now be examined in more detail.

**Removal of the Signal Peptide.** It is probable that all foreign membrane or secretory proteins which possess cleavable signal sequences lose these sequences during translocation through oocyte membranes. However, for technical and other reasons, a demonstration of such cleavage has only been made for a small number of proteins. Recently the lack of cell- or even species-specificity in the processing of the signal sequence has been highlighted by the demonstration of Koren et al. (1983) that a synthetic murine Ig signal peptide, injected into oocytes, competitively inhibited the synthesis of secretory and membrane proteins but not of cytoplasmic proteins.

**Removal of other peptide sequences.** Many secretory and lysosomal proteins start the translocation process from the lumen of the endoplasmic reticulum to their final destination as precursor forms bearing an N-terminal peptide. In several cases, though not all (e.g. pancreatic proteases), this 'pro' sequences is removed intracellularly before exocytosis occurs. Oocytes have proved incapable of performing this type of cleavage. Carp pro-insulin (Rapoport, 1981) and honey bee promellitin (Lane et al., 1981a) are not processed correctly in oocytes. The precursor to castor bean ricin A and B chains is also not processed by oocytes to yield mature A and B chains although normally glycosylation takes place (see below). In all these examples it is likely that tissue-specific cleavage enzymes are necessary for

<table>
<thead>
<tr>
<th>Modification</th>
<th>Examples</th>
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<tbody>
<tr>
<td><strong>Modification of amino acid residues</strong></td>
<td></td>
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<tr>
<td>α NH₂-acetylation</td>
<td>Califens A2 crystallin (Berns et al., 1972)</td>
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<tr>
<td>ε NH₂-acetylation</td>
<td>Sea-urchin histones (Woodland &amp; Wilt, 1980)</td>
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<tr>
<td>Hydroxylation</td>
<td>Mouse fibroblast collagen (Lane &amp; Knowland, 1975)</td>
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<td>Phosphorylation</td>
<td>Trout testis protamine (Gedamu et al., 1978); synthetic peptides (Maller et al., 1978)</td>
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<td>Glycosylation</td>
<td>Chick ovalbumin (Colman et al., 1981); chick ovomucoid (Colman et al., 1981); mouse Igs (Jilka et al., 1979; Vallee et al., 1983); human chorionic proteins (Minn et al., 1980); castor-bean lectin (A. Colman &amp; J. M. Lord, unpublished work); rat prostatic binding protein (Mous et al., 1982); human α₁-antitrypsin</td>
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<tr>
<td><strong>2. Cleavage of polypeptides</strong></td>
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<tr>
<td>Signal-sequence removal</td>
<td>Mouse Ig light chains (Jilka et al., 1979; Vallee et al., 1981); bee prepromelittin (Lane et al., 1981a); uteroglobin (Beato &amp; Rungger, 1975); pre-pro-insulin (Rapport, 1981); pre α₂G-globulin (Mertens &amp; Verhoven, 1981); silk-moth chorion proteins (Bock et al., 1982); chick lysozyme (A. Colman, unpublished work); rat seminal vesicle proteins (Higgins et al., 1981)</td>
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<tr>
<td>Formation of new proteins by polypeptide cleavage</td>
<td>Various viral proteins (see Lane, 1983); locust vitellogenin (Lane et al., 1983); frog vitellogenin (Berridge &amp; Lane, 1976)*</td>
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<td><strong>3. Assembly of polypeptides</strong></td>
<td></td>
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<tr>
<td>Mode of assembly</td>
<td>Igs (various) (Deacon &amp; Erbring, 1979; Colman et al., 1982; Vallee et al., 1981, 1982); Rabbit uteroglobin (Beato &amp; Rungger, 1975)</td>
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<tr>
<td>S-S bond formation</td>
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<td>Non-covalent assembly</td>
<td>Calif α- and β-crystallins (Asselbergs et al., 1978); Torpedo acetylcholine receptor (Sumikawa et al., 1981); mouse β-glucuronidase (Labarca &amp; Paigen, 1977); human brain receptors (Gunderson et al., 1984); human histocompatibility antigens (Kinnon, 1984; Long et al., 1982)</td>
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<tr>
<td><strong>4. Segregation and secretion of polypeptides</strong></td>
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<tr>
<td>Segregation</td>
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<tr>
<td>Plant proteins ('') (Lane et al., 1981a); chick oviduct proteins (S) (Colman et al., 1981); mouse Igs (S) (Vallee et al., 1981, 1982, 1983); human α₁-antitrypsin (S) (Foreman et al., 1984); guineapig caseins (S) (Colman &amp; Morser, 1979); locust vitellin (S) (Lane et al., 1983); human and mouse interferons (S) (Colman et al., 1979; Lebleu et al., 1978); rat seminal vesicle proteins (S) (Higgins et al., 1981)</td>
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correct polypeptide maturation. It is interesting to note that the correct polypeptide cleavage of a variety of foreign viral proteins does occur in the oocyte cytosol (see Lane, 1983).

N-Glycosylation of foreign proteins. Many secretory and membrane proteins are glycosylated. The most common form of glycosylation involves the addition (core glycosylation) of preformed oligosaccharide units to the amide side-chain of asparagines which are located within the polypeptide chain in the sequence context Asn-X-Thr/Ser; not all such asparagines are glycosylated. During translocation through the secretory apparatus the side-chain is modified (peripheral glycosylation) by the removal of specific sugars (mainly mannose residues) and their replacement with new sugars (e.g. sialic acid, fucose etc.). Only proteins which in their native state are N-glycosylated become glycosylated in oocytes, and this glycosylation is site-specific as demonstrated by work on the glycosylation of wild-type and mutant Ig chains (Jilkja et al., 1979). An apparent exception to this was discovered in our laboratory when a mutant, non-secretory MOPC 315 A chain was found to be glycosylated in oocytes (Valle et al., 1983) in contrast to earlier observations on the protein synthesized by myeloma cells (Mossmann & Williamson, 1980). However, this proved to be a kinetic phenomenon; when myeloma cells were incubated in radioactive media for the prolonged periods we usually use for oocytes (>16h), we found that up to 50% of the mature mutant A chains become glycosylated (Valle et al., 1983). Unusually, this glycosylation was exclusively post-translational in nature.

Since the composition of the oligosaccharide chain can vary from tissue to tissue, even within the same animal, it is to be expected (though not yet demonstrated), that the composition of the chain added by the oocyte will differ from the 'native' chain. However, it is now clear that the subsequent trimming and replacement steps are also different in oocytes; in both human chorionic gonadotrophin (Moss et al., 1980) and rat prostatic binding protein (Moss et al., 1982), mannose trimming is either absent or incomplete. Such aberrant trimming could explain our results concerning the Endo H sensitivity of murine IgG heavy chains in oocytes (Colman et al., 1982). Endo H resistance is normally acquired by glycoproteins on the removal of mannose residues in the Golgi. As a result of this 'trimming' heavy chains secreted from myeloma cells, though not oocytes, are therefore resistant to Endo H; those secreted by oocytes remain sensitive. These results with Ig heavy chain have been confirmed recently by Kinnon (1984); however, this worker also found that human histocompatibility antigens synthesized in the same oocyte did become resistant to Endo H. The interpretation of these results remains unclear.

Phosphorylation. Both trout-tetis testis protamine (Gedamu et al., 1978) and injected synthetic peptides (Maller et al., 1978) can become phosphorylated in oocytes. However, guinea-pig caseins produced in oocytes cannot be labelled with ATP or phosphates and the oocyte products will only co-migrate with authentic caseins after treatment of the latter with phosphatase (Lane, 1983). As discussed earlier it is probable that tissue-specific enzymes are required for some, though not all, phosphorylation events.

Assayed by mRNAs of multiple proteins in oocytes. The ability of oocytes not only to translate injected mRNAs, but to assemble the products into functional proteins, remains the most spectacular and useful post-translational process provided by these cells. Several examples are shown in Table 1. Perhaps the most striking of the demonstrations that injection of Torpedo electric organ mRNA leads to the synthesis and assembly of all four subunits of the nicotinic acetylcholine receptor into a functional receptor located in oocyte plasma membranes (Sumikawa et al., 1981; Miledi et al., 1982), whilst injection of human brain mRNA leads to the formation of active sodium channels and receptors to serotonin and kainate in the oocyte membranes (Gunderson et al., 1984). However, the appearance of oligomeric complexes does not per se indicate that the complex or its constituent parts are identical to their 'native' counterparts. Thus the mouse b-glucuronidase made in oocytes is active, yet it shows a different mobility on non-denaturing gels to the authentic mouse lysosomal enzyme (Labarca & Paigen, 1977). A further example is the failure of the calf b-crystallin subunits to assemble correctly (Asselbergs et al., 1978).

Segregation and secretion of proteins

Zehavi-Willner & Lane (1977) first demonstrated that the newly synthesized secretory proteins Xenopus liver albumin and guinea-pig caseins, became segregated within membranous vesicles which could be isolated from homogenized oocytes by sucrose gradient centrifugation. Subsequently we demonstrated that the caseins were secreted from oocytes (Colman & Morser, 1979). Many foreign membranes and secretory proteins have now been shown to become correctly located after their synthesis in oocytes, as indeed do nuclear proteins (Table 1). In fact the fidelity of this aspect of the post-translation processing by oocytes appears extremely high, with several foreign non-secretory mutant proteins the exhibiting a similar phenotype when tested in oocytes (Table 1). One exception to this generalization is bee venom promellitin which is not significantly exported from oocytes (Lane et al., 1981a). In this case the incomplete processing and hydrophobicity of the molecule may impede its translocation.

Specialized uses of the modification capacity of the oocyte

The question arises as to why, given potential imperfections in the processing mechanisms, this aspect of oocyte metabolism is so regularly exploited? There are several answers. First as evidenced by work on the interleukins (Reynolds et al., 1975), interleukins (Taniguchi et al., 1982), tissue-specific plasminogen activators (Miskin & Soreq, 1982), b-glucuronidase (Labarca & Paigen, 1977), and other biologically active proteins (Table 1), exquisitely sensitive bioassays have made detection of the encoding mRNAs possible, thereby facilitating the cloning of the corresponding complementary DNAs. Secondly, the relationships between post-translational modification and the structure and function of a protein or protein complex cannot always be conveniently studied in the native tissue. Problems of this nature can sometimes be circumvented by use of the oocyte, and an example will be cited below. Thirdly, technological advances have made mRNA enrichment a routine procedure even to the point of generating homogeneous populations of single species of mRNA. The use of such preparations can (a) boost production levels of the desired protein, (b) pinpoint or eliminate mRNA heterogeneity as the cause of protein heterogeneity (e.g. interferons (Cavalieri et al., 1977)), (c) allow the post-translational processing of a specific protein to be examined in the absence of other tissue-specific proteins (e.g. ovalbumin (Cutler et al., 1981)) and (d) allow the synthesis in oocytes of novel combinations of different proteins either from one or more cell types (see below). Finally, the use of the oocyte as a coupled transcription and translation assay allows the introduction of mutations into studied proteins; the intracellular fate of such proteins can be monitored (see below).

Studies on Igs

The MOPC 21 plasmacytoma cell line NS1 synthesizes but does not secrete a k-light-chain Ig (Cotton et al., 1973). A similar phenotype was exhibited when NSI
mRNA was injected into oocytes (Valle et al., 1981). When purified Ig heavy-chain mRNA was co-injected with NS1 mRNA or even injected 24h later, the marooned light chains were assembled into a tetramer (IgG) with the heavy chains and secreted (Colman et al., 1982). Heavy-chain mRNA injection alone led to the formation of non-secretory covalent dimers (Valle et al., 1981). For this latter demonstration, the use of the oocyte was necessitated by the absence of any cell lines producing heavy chain alone, probably due to the lethality of such a phenotype (Kohler, 1980).

The use of purified heavy and light-chain mRNA has also allowed the participation of other myeloma-specified factors in the assembly and secretory process to be assessed. Thus tetrameric IgG was secreted from oocytes co-injected with pure heavy and light chains (Colman et al., 1982), thereby demonstrating that the presence of another myeloma protein, J protein, was not necessary for these events; J chain is essential for the secretion of IgM molecules. Recently similar 'reconstitution' studies have been used by Kinnon (1984) to demonstrate that the presence of human $\beta_2$-microglobulin was essential for the assembly and

![Fig. 1. Segregation and secretion of glycosylated and unglycosylated chick ovalbumin and human $\alpha_1$-antitrypsin](image)

Oocytes were injected with either chick ovalbumin mRNA or human $\alpha_1$-antitrypsin mRNAs (Pi MM or Pi ZZ) and cultured for 24h in media containing $[^{35}S]$methionine in the presence or absence of tunicamycin (see Colman et al., 1981). Media (S) and homogenized oocytes (O) were immunoprecipitated with anti-ovalbumin antibody (a and b) or anti-$\alpha_1$-antitrypsin antibody (c and d). In (a) homogenized oocytes have been further fractionated into membraneous vesicle (M) and cytosol (C) fractions on sucrose gradients (see Valle et al., 1983). In (a) the glycosylated and miscompartmented ovalbumins are indicated. In (c) and (d) bands 1, 2, 3 refer to unglycosylated, partially glycosylated and fully glycosylated $\alpha_1$-antitrypsin (see Foreman et al., 1984). The presence of some fully glycosylated $\alpha_1$-antitrypsin in the media around Pi MM mRNA-injected oocytes in (d) indicates that the tunicamycin treatment was not completely effective.
Studies on ovalbumin

Chick ovalbumin is unique amongst eukaryotic secretory proteins in that it lacks a cleavable signal sequence (Palmieri et al., 1978). Injection of ovalbumin mRNA into oocytes leads to the synthesis of both glycosylated and unglycosylated ovalbumin (Fig. 1a and Colman et al., 1981). Only the glycosylated forms which segregate with oocyte membranes are secreted. The unglycosylated form remains in the oocyte cytosol and is not secreted. The use of tunicamycin, an inhibitor of N-glycosylation, has shown that the absence of the oligosaccharide side chains has no effect on secretion (Colman et al., 1981 and see Fig. 1b). In contrast, the unglycosylated form of normal α1-antitrypsin (Pi MM), a serum protease inhibitor with considerable sequence homology to chick ovalbumin (Leicht et al., 1982), is only poorly secreted when N-glycosylation is inhibited (Fig. 1d and Foreman et al., 1984). A mutant α1-antitrypsin (Pi ZZ) where a single amino acid change reduces efficient peripheral glycosylation and secretion in the liver (Carrell et al., 1982), also shows a similar phenotype in oocytes (Fig. 1c). In the absence of glycosylation the unglycosylated mutant protein is not secreted at all (Fig. 1d). These results indicate that complete glycosylation is imperative to the efficient secretion of α1-antitrypsin but not ovalbumin. Presumably, the amino acid substitution present in the Pi ZZ protein greatly inhibits the peripheral glycosylation events, which appear even in the Pi MM protein to be important for secretion. It is salient to point out that for technical and ethical reasons the experiments on α1-antitrypsin could not be carried out on human liver tissue.

Finally, the injection into oocyte nuclei of ovalbumin genes in the form of cloned complementary DNA linked to a viral promoter (Colman et al., 1983) allows the expression and segregation of mutant ovalbumins to be tested. Fig. 2 shows one such experiment where the loss of amino acids 20–145 in ovalbumin, leads to the failure of the protein to enter the oocyte membranes. Table 2 tabulates a series of results which indicate that the ovalbumin signal sequence, whilst being located within the first 140 amino acids of the molecule, must nevertheless be located after amino acid 20, since mutant ovalbumins lacking this N-terminal sequence still enter oocyte membranes.

Conclusions

The oocyte will continue to be used simply as an assay for particular mRNAs, especially those encoding biologically active proteins or as a means to study the biosynthesis of proteins normally made in experimentally intractable tissues. It will also have more general uses: a detailed understanding of any cellular process requires the dissection of that process into individual components and its reconstitution from those components. The work cited earlier on receptor synthesis and function in oocytes vividly illustrates this point. However, this reductionist approach has the problem that the dynamic aspects of inter-related processes are inevitably disrupted during the fractionation. The oocyte offers a compromise: the complete biogenesis of a foreign protein, i.e. from DNA to finished protein, can be affected with reasonable fidelity; experimental perturbation of any stage of the expression of the protein can then be examined for its effect on succeeding events in the processing pathway.