Meeting Report

THE ROLE OF ORGANELLES IN THE CHEMICAL MODIFICATION OF THE PRIMARY TRANSLATION PRODUCTS OF SECRETORY PROTEINS

Peter N. CAMPBELL
Courtauld Institute of Biochemistry, The Middlesex Hospital Medical School, London, UK

and

Günter BLOBEL
The Rockefeller University, New York, USA

Received 12 November 1976

1. Introduction

A FEBS Advanced Course (No. 43) was held from September 21–23, 1976 at The Middlesex Hospital Medical School under this title. Since the Course was concerned with a new and original aspect of biochemistry it was suggested to us, as the joint organisers, that it might be helpful if we produced a survey of the main contributions during the Course and in particular provided a bibliography that would serve as an entrée to the field.

It has for long been realised that the polypeptides of secretory proteins, in transit from their site of synthesis on the ribosomes of the rough surfaced endoplasmic reticulum (ER), undergo changes to their structure as they pass through the smooth surfaced ER and the Golgi Complex. In particular they may be modified by phosphorylation and/or glycosylation and in some cases a peptide may be deleted, as in the case of insulin where a single chain protein is converted into one with two chains linked by disulphide bonds. There is much uncertainty concerning the precise intracellular site of such activities and of the particular enzymes involved.

Recent research has shown that the modifications of secretory proteins may be much more profound than those already indicated. Not only have an increasing number of proteins been shown to be produced as precursors, which are converted in the Golgi Complex, but it is becoming apparent that secretory proteins are characterized by their synthesis with a characteristic peptide at their N-terminus. This idea was originally put forward as a hypothesis by Blobel and Sabatini [1]. They suggested that secretory proteins would have a common sequence of amino acids near the N-terminus of the nascent chains and that the peptide, or a modification of it, would then be recognised by a factor mediating the binding to the membrane. It was Milstein et al. [2] who proposed, from experiments in which immunoglobulin light chain mRNA was translated in a reticulocyte lysate system, that light chains are initially synthesized as a precursor of slightly higher molecular weight, which is subsequently converted into the authentic product. From these experiments they proposed, independently of Blobel and Sabatini, that a short peptide at the N-terminus of a precursor protein would be a simple way to provide a signal to ensure that secretory proteins were synthesized on bound polysomes. As described later in this review further evidence for this hypothesis has been provided, particularly by Blobel and from results obtained from parathyroid hormone and insulin.

Much of the current work in this field depends on the fact that it is now possible to isolate a mRNA for a specific protein and for this to be translated in

Reprint requests to: Professor P. N. Campbell, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W1P 7PN, England

heterologous cell-free systems. Such an approach also leads to an understanding of the subunit size of large proteins and has demonstrated, in at least one case, that a giant protein is synthesized in one tissue and transported to another where it is cleaved into two biologically active proteins.

2. The basic secretory pathway

The plan of the Course was to describe first what may be called the basic secretory pathway (Palade) [3] and then to move on to a consideration of particular proteins.

G. Blobel of The Rockefeller Univ., New York started the Course by dealing with the initial event in the secretory pathway. The so-called 'Signal Hypothesis' (fig.1) has been elaborated in his laboratory [4] to provide a scheme for the translation of mRNAs for secretory proteins on membrane bound ribosomes and the transfer of the translation products across the microsomal membrane by the ribosome membrane junction. It is proposed that all mRNAs for secretory proteins code for a unique peptide – the signal peptide – which constitutes a metabolically short-lived amino terminal extension present only in nascent, incomplete chains. The signal peptide of the nascent chain emerging from a space within the large ribosomal subunit penetrates into the microsomal membrane thereby causing association of several ribosome receptor proteins of the ER membrane to form a proteinaceous tunnel in the membrane. The signal peptide induces association of these proteins to provide the conditions for a coordinated binding of the large ribosomal subunit of the translating ribosome to the ER membrane (linking the tunnel in the large ribosomal subunit with the newly formed tunnel in the membrane) and furthermore permits the passage of the signal peptide and continuous sequences of the nascent chain through the ER membrane into the intracisternal space. The signal peptide is removed before the chain is completed by a membrane-bound enzyme – referred to as 'signalase'. Following release of the nascent chain from the ribosome the latter is detached from the membrane eliminating the membrane tunnel and ready to translate any other mRNAs independent of whether the latter do or do not contain 'signal codons'.

The following evidence for the signal hypothesis has been accumulated in his laboratory. They have shown [5] that mRNAs for pancreatic secretory proteins are translated in an in vitro protein synthesizing system (which contains no membranes, that is, it contains no 'signalase' activity) into precursor molecules larger by 1–2000 mol. wt than authentic secretory proteins. Amino terminal sequence analysis revealed that these precursors contain an amino terminal extension of 16 amino acid residues with a striking sequence homology and consisting of a large percentage of hydrophobic residues. Translation of mRNAs for secretory proteins in the presence of

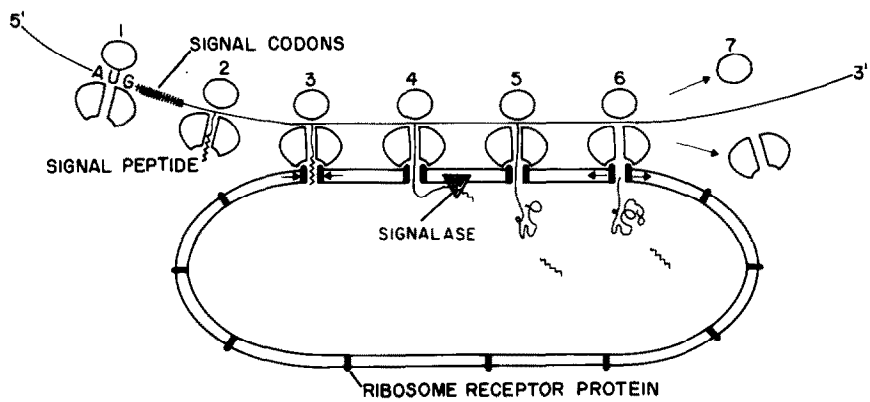


Fig.1. Blobel's Signal Hypothesis. A schematic representation of the translation of messenger RNA for secretory proteins on membrane bound ribosomes and the transfer of the translation products across the microsomal membrane.

heterologous ribosome-stripped microsomal membranes led to ribosome attachment to the membrane, transfer of the nascent chain into the intravesicular space and removal of the signal peptide from the nascent chain. In contrast, globin mRNA (lacking signal codons) did not result in ribosome attachment or transfer into the intravesicular space [6] – as predicted in the signal hypothesis. They have also shown that the signal peptide is cleaved from the nascent chain before the chain is completed [4]. Furthermore a detachment factor has been isolated which detaches ribosomal monomers (not containing nascent polypeptide chains) from the membrane [7].

After an explanation of current views on the subsequent events in the secretory process by C. R. Hopkins (Univ. Liverpool, England), c. Poort (State Univ., Utrecht, The Netherlands) described briefly some experiments on the intracellular transport of puromycyl-peptides in rat pancreas. F. Melchers (Basel Institute for Immunology, Switzerland) then considered critically the glycosylation of immunoglobulins (Ig). First, however, he described the synthesis of the surface-membrane-bound form and the secreted form of IgM as it occurs in resting and stimulated, normal and malignant B-lymphocytes.

In the surface membrane of B-cells Ig binds haptenic determinants of antigens as a part of a complex of receptors involved in the regulation of growth and differentiation of Ig-secreting cells. Stimulation of the small, surface IgM receptor-containing B-cells, results in proliferation and maturation of clones of cells secreting the particular type of Ig which served as the receptor molecule on the originally triggered cell. One of the earliest changes observed in small B-cells after stimulation is the induction of active secretion. This reprogramming of IgM synthesis, from the synthesis of the membrane-bound to the secreted form, occurs also in the absence of DNA- and RNA-synthesis and may, therefore, initially be regulated at the translational level. During growth, after stimulation, B-cells appear to balance, within the cell cycle, between reactions leading to maturation (secretion) and reactions leading to growth (receptor synthesis) [8,9]. The assay for secretion depends on a new plaque assay with which all cells secreting Ig of a given type or class are detected [10].

He described the addition of carbohydrate to Ig as it passes from the polysomes to the rough ER to the smooth ER. It seems that, through dolichol phosphate intermediates, the 'core' polysaccharides containing mostly mannoses and *N*-acetyl-glucosamines are added to the polypeptides in one piece in the rough ER. Further lengthening of the carbohydrate chains by 'branch' monosaccharides, such as galactoses, fucoses and *N*-glycolyl-neuraminic acids occurs in steps in the smooth ER. Concerning the role of glycosylation in secretion one must take account of the fact that some Ig light chains are produced which have no carbohydrate and yet these are satisfactorily secreted. Melchers concluded, therefore, that the role of glycosylation in the secretion of proteins is unknown.

3. Endocrine Systems

3.1. *Pituitary*

The Course then turned to a discussion of the synthesis of specific secretory proteins considering first endocrine systems. For the pituitary, M. Wallis (Univ. Sussex, England) described current views on the biosynthesis of growth hormone (GH) and prolactin.

It was suggested some years ago that GH is synthesized as a precursor: this could explain the heterogeneity seen at the N-terminus of the bovine hormone [11]. Various studies suggested that high-molecular-weight complexes may merely be associations of GH with other proteins and/or nucleic acids [12]. Recently Sussman et al. [13] have translated the mRNA for rat GH in the wheat germ cell-free system and demonstrated the existence of a precursor of GH (pre-GH) with a mol. wt of about 24 000 (compared with about 20 000 for rat GH itself). Processing of the precursor in the pituitary cell seems to be very rapid. The relation between this pre-GH and the large-molecular-weight forms of the hormone detected previously remains unclear.

During the last year several groups have provided evidence for a biosynthetic precursor of rat prolactin (pre-prolactin) [14–16]. Again, translation of mRNA for the hormone in a wheat germ cell-free system has been employed. Pre-prolactin has a mol. wt of 24–28 000, compared to about 22 000 for prolactin itself.

3.2. *Placenta*

B. Peeters from the laboratory of W. Rombauts (Catholic Univ. Louvain, Belgium) considered the placenta and described the properties of a biologically active mRNA from human tissue. This RNA was extracted from polysomes, purified by chromatography on poly(U)–Sepharose and was shown to contain 6% poly(A) and to sediment at about 12 S. It was used to investigate radioactive amino acid incorporation after injection into oocytes of *Xenopus laevis* or incubation in a cell-free system from wheat or rye germs. Using a highly specific and sensitive immunoprecipitation reaction human placental lactogen (hPL) was identified as one of the total mRNA induced proteins. The synthesis of the hormone (3.3%) could still be detected after 6 days of incubation of oocytes, while in the wheat germ cell-free system hPL accounted for 15% as measured by immuno-precipitation.

Analysis by SDS–gel electrophoresis demonstrated that the in vitro synthesized product had the same mobility on a 10% gel as a ^{14}C-labelled acetylated hPL marker. However recent analysis of the products synthesized in the wheat germ cell-free system, using a 15% SDS–gel in Tris–glycine buffer, allowed a separation of the immunoprecipitate into three radioactive bands, one having the same mol. wt as the authentic hormone (22 300) and two precursor products (24 000–25 100) which were also observed by other groups [17–19]. The nature of the latter products is under investigation.

3.3. *Pancreas*

For the third endocrine system attention turned to the pancreas, a historically important source of concepts regarding the organization of the secretory process. Steiner (Univ. Chicago, USA) described recent studies on precursors of islet hormones. Insulin has long been known to be formed from a precursor, proinsulin, in which the two chains are linked by a peptide [20]. The primary structure of the linking peptide from many species shows a wide variation and it is probably not biologically active [20,21]. Many other small peptide hormones are now known to be derived from similar larger precursor forms and to be processed to their active forms via specific intracellular cleavage at sites bearing pairs of basic residues [22]. The processing of most pro-proteins occurs intracellularly as the newly synthesized secretory product passes near or through the Golgi region into condensing vacuoles or newly formed secretory granules [20]. In the case of insulin and other islet hormones such as glucagon, the cells of origin contain only a small percentage of intact precursor or intermediate forms, and this is reflected in the secretion normally of only very small amounts of these materials into the blood stream. However, precursor forms may tend to accumulate in the blood due to their relatively slow metabolism [23]. The kidney often is a major site for their removal from the blood; thus high levels of proinsulin and of 9000 mol. wt glucagon are found in the plasma of patients with renal disease [24].

Recent work in several laboratories also indicates that such pro-proteins may contain more than one active sequence, e.g., β-lipotropin, a 90 residue pituitary peptide, contains both β-MSH and C fragment or β-endorphin, a peptide having strong opiate activity, linked together through a dibasic cleavage site [25,26]. Steiner proposed that the major role of such precursors may be to overcome length constraints in biosynthesis. Thus, at least 70 residues may be required to span the distance from the site of synthesis between the ribosomal subunits across the membrane to the interior of the cisternae of the rough ER. Exceptions to this simple rule are pro-albumin and proparathyroid hormone which both exceed this minimum size criterion. Interestingly, these pro-proteins both have similar highly basic hexapeptide extensions at their N-terminal suggesting either a role in intracellular transport or in feedback control of transcription or translation by the pro-peptide.

Duguid et al. [27] have recently isolated and partially characterized the mRNA for insulin and Chan et al. [28] have translated this mRNA in a cell-free system derived from wheat germ. The mRNA was about 9.3 S (mol. wt = 210 000) and had a 'cap', i.e., methylated 5′-terminus $m^7G^{5'}$ $ppp^{5'}N$ [26]. In agreement with reports from several laboratories [29,30] the product of translation is larger than pro-insulin by a mol. wt of about 2–3000. Chan et al. have shown that this preproinsulin differs from proinsulin in having an N-terminal extension of approximately 23 amino acids [28]. The pre-peptide sequence is particularly rich in hydrophobic residues such as leucine, and the distribution of these is similar but

not identical in comparing the bovine and rat preproinsulins [Chan, Lomedico and Steiner, unpublished data].

S. Howell (Univ. Sussex, England) described recent work on the intracellular storage of insulin. Following its biosynthesis in the rough surfaced ER, proinsulin is transported to the Golgi Complex in membrane-limited vesicles by an energy dependent process [31,32]. Enzymic conversion of proinsulin to insulin is initiated in the Golgi Complex and completed during formation of the storage granules. The mature granules are known to contain an electron-opaque core which may be composed of insulin in a crystalline form, and in addition zinc (in some species), connecting peptide, and biogenic amines [32]. Preliminary electron microscope X-ray micro-analyses of the ion content of the granules in frozen sections show the presence of sodium, phosphorus, sulphur, chlorine, potassium and calcium. This calcium content appears to be relatively stable and, unlike the labile mitochondrial pool of this ion, seems unlikely to be involved in the minute by minute regulation of rates of insulin secretion [33].

3.4. *Thyroid*

The thyroid gland was the next endocrine system considered. G. Vassart (Univ. Libre de Bruxelles, Belgium), spoke about the synthesis and degradation of thyroglobulin. Thyroglobulin is the major product of thyroid protein synthesis. It represents both a precursor and a storage form of the thyroid hormones. The number and the size of the protomers of this huge protein (mol. wt 660 000, sedimentation coefficient 19 S) has been the matter of a long-lasting controversy. Molecular weights ranging from 20–200 000 have been reported. Recently, it has been suggested that contamination of thyroglobulin preparations by proteolytic enzymes could explain in part these discrepancies. However, even in proteolytic-free thyroglobulin at least three kinds of subunits have been isolated (mol. wt 300 000, 200 000, 100 000) the relative proportion of which is related to the iodine content of the molecule. These results could be explained either by iodine related post-translational cleavages of a unique 300 000 mol. wt precursor, or by the existence of three different subunits, the synthesis of which would be regulated by the availability of iodine.

The aim of their work has been to characterize the true thyroglobulin precursor(s) from the heterologous translation of its (their) mRNA(s). The results may be summarised as follows:

(1) Thyroglobulin is synthesized on a population of very large (> 50) membrane bound polysomes.

(2) These polysomes contain a poly(A)-rich RNA of sedimentation coefficient about 33 S.

(3) The 33 S poly(A)–RNA is translated in the *Xenopus* oocyte to give a 300 000 mol. wt peptide immunologically related to thyroglobulin.

(4) No RNA of other size could promote the synthesis of immuno-reactive thyroglobulin.

(5) The 300 000 mol. wt peptide polymerizes spontaneously into 19 S thyroglobulin.

(6) Tryptic peptide analysis reveals chemical identity between the 300 000 mol. wt peptide and authentic 19 S thyroglobulin.

From these results, Vassart concluded that thyroglobulin is synthesized from the translation of a 33 S mRNA into two 300 000 mol. wt subunits. All subunits of other size would thus originate from post-translational cleavages of this precursor. An influence of iodination on these cleavages is suggested but remains to be demonstrated [34,35].

A somewhat contrary view was taken by Ph. de Nayer (ICP, Univ. Louvain, Belgium). He described experiments in which bovine thyroid RNA species were separated by cellulose chromatography and/or by sucrose density gradient ultracentrifugation. Identification of mRNA was performed by incubating aliquots of RNA thus fractionated in a cell-free system of Krebs II ascites cell lysate. Stimulation of specific protein synthesis was obtained with RNA fractions with approximate S-values of 40, 30, 24 and 16. Experiments were performed to characterize these mRNAs, and determine their mol. wt. This was done using gel electrophoresis of RNA isolated from thyroid slices previously incubated with the labelled precursors, [^{3}H]uridine or [^{3}H]adenosine. In total RNA, as well as in poly(A) rich RNA samples, the radioactivity was located in 4 bands. The electrophoretic mobility of these bands corresponded to the mobility of RNA molecules with an apparent mol. wt of 3.1×10^6, 2.2×10^6, 1.2×10^6 and 0.6×10^6. The 3 largest RNA could contain the information for peptides of about 3×10^5, 2×10^5 and 1×10^5 mol. wt. The estimates of the

encoded proteins are close to the values recently reported by Haeberli et al. [36] for the 3 polypeptide chains of guinea-pig thyroglobulin.

The presence of several mRNA species raises the question of a possible regulatory role of these mRNAs in thyroglobulin synthesis and assembly. In this regard it is interesting to mention preliminary observations indicating that the addition of TRH or KI (10^{-6} M) to the cell cultures alters the distribution of the radioactivity among the 4 bands of RNA [37].

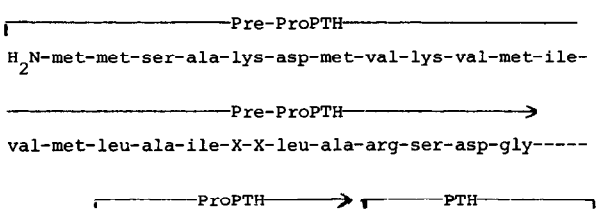
3.5. *Parathyroid*

Attention then turned to the parathyroid gland and J. F. Habener and J. T. Potts, Jr. (both of Mass. General Hospital, Boston, USA) described their work on parathyroid hormone (PTH). Studies of PTH biosynthesis in vitro indicate that the predominant cellular hormonal product, PTH, a single-chain polypeptide of 84 amino acids, arises from a larger precursor via two successive proteolytic cleavages:

$$\text{Pre-ProPTH(115 aa's)} \xrightarrow{1-2\ \text{min}} \text{ProPTH}$$

$$\text{(90 aa's)} \xrightarrow{15\ \text{min}} \text{PTH (84 aa's).}$$

Pre-Proparathyroid hormone (Pre-ProPTH) is the major protein synthesized by translation of parathyroid mRNA in a cell-free system derived from wheat germ and appears to be the initial protein coded for by the gene for PTH. Analysis by a sensitive, micro-sequencing procedure of the amino acid sequence of radioactive Pre-ProPTH from the wheat germ system gives the following structure:



Appropriate identification of each radioactive amino acid at its known position within the sequence of ProPTH was used as a criterion for the correct identification of the amino acid in the sequence of Pre-ProPTH. The N-terminal methionine is incorporated into Pre-ProPTH selectively by initiator methionyl tRNA. The C-terminal tryptic tetrapeptides of Pre-ProPTH, ProPTH and PTH are identical when analysed by two-dimensional chromatoelectrophoresis. Translation of parathyroid mRNA in a Krebs ascites cell-free system containing reticular membranes reveals that the major product synthesized is ProPTH and that a substantial fraction of the Pre-ProPTH synthesized lacks both N-terminal methionines. Kinetics pulse and pulse-chase labelling studies using intact slices of parathyroid glands show that small amounts of radioactive Pre-ProPTH are detectable and reach a maximum within 2 min, ProPTH appears by 1 min and PTH does not appear until 15 min.

The data in toto suggest a role for the precursors in the sequential segregation and translocation of the hormonal polypeptides within subcellular organelles. They propose that the hydrophobic 'pre' sequence is a 'signal sequence'. The delay of 15 min, before tryptic-like proteolytic conversion of ProPTH to PTH occurs, corresponds to the time required for the transport of the newly-synthesized polypeptide to the Golgi Complex and suggests that the 'pro' sequence may be involved in the transport of the prohormone within the cisternal space (from RER to Golgi) [38–40].

Potts described the problems of microsequencing of the amino acids in the signal peptide. It is of course only possible to determine the primary structure of these peptides by the use of radioactive amino acids during their biosynthesis in a cell-free system; conventional techniques for sequence analysis cannot be used because only picogram quantities of precursor peptide are present in tissues. Under good conditions and by careful interpretation of the results the method is capable of providing an analysis which is as satisfactory as the more macro methods [41].

4. Exocrine Systems

4.1. *Mammary gland*

From the endocrine systems the Course moved to an exocrine organ, namely the mammary gland and

P. N. Campbell (Courtauld Institute, London, England) spoke of the biosynthesis and secretion of milk proteins.

The bulk of the proteins in the milk are synthesized in the mammary gland under hormonal control. The milk proteins, synthesized in the gland, can be separated into caseins and the whey protein α-lactalbumin (α-LA). In addition, the whey of ruminants contains a further protein β-lactoglobulin (β-LG). α-LA plays a role in the synthesis of lactose and interacts with galactosyl-transferase which is a marker enzyme in the Golgi Complex [42]. There are three main groups at present studying the biosynthesis of milk proteins. Rosen at Baylor, Houston, uses rats, Houdebine and Gaye at the C.N.R.S., France, use ewes and rabbits and Campbell and Craig at the Courtauld Institute, London, use guinea-pigs.

In guinea-pigs it has been shown that for α-LA the primary translation product is a precursor with 7–10 additional amino acids at the N-terminus [43]. Other groups have not so far detected a similar precursor in their systems.

For casein no-one has yet conclusively demonstrated that the various proteins are synthesized in the form of a precursor with an N-terminal extension. When mRNAs from lactating mammary glands of either rat or guinea-pig were translated in wheat germ or ascites tumour cell systems and the products isolated by a double antibody technique were run on SDS–polyacrylamide gels then the synthesized products had a slightly higher mobility than the authentic caseins from milk. In view of the fact that caseins are glycosylated and phosphorylated and these modifications may influence their behaviour in gels it remains possible that a slightly larger casein precursor will yet be detected. No such precursor has been found in extracts from ewe mammary glands [44]. There is also a possibility that the final caseins are derived from a single macro-molecule since the mRNAs are larger than predicted.

mRNA for milk protein may be isolated from the polysomes of lactating glands in all the species previously mentioned [45]. If membrane-bound and free polysomes are isolated from ewe mammary glands then Gaye and Denamur [46] showed that the bound polysomes were the site of synthesis of β-LG. They have claimed an absence of mRNA for casein in free polysomes from the same tissue. Harrison et al. [47] have shown that both the bound and free polysomes in lactating glands from guinea-pig possess mRNA for casein. This would be in accord with the signal hypothesis as presumably chain initiation must involve free polysomes.

4.2. *Bee-Venom*

G. Kreil (Institute of Molecular Biology, Salzburg, Austria) reminded the Course that interesting studies can be done with eukaryotes other than mammals and he described work on the biosynthesis of melittin in the honeybee. Melittin, a peptide comprised of 26 amino acids, is the main constituent of honeybee venom. This venom is produced by a Y-shaped, tubular gland located in the abdomen of the insect. After feeding labelled amino acids to bees, a radioactive precursor of melittin could be detected in this gland. This precursor, called promelittin, contains 8–9 extra residues at the N-terminus [48]. In worker bees, venom production starts only slowly after the emergence of the insect. In contrast the venom gland of queen bees operates at full capacity at emergence [49].

From venom glands of young queen bees, the mRNA for promelittin has been isolated and characterized [50]. Injection of this mRNA into frog oocytes leads to the formation of a product closely resembling promelittin [51]. In contrast, translation of this mRNA in cell-free systems, derived from mammalian cells [52] or from wheat germ, yields a larger product which has been called protomelittin and which can now, in accordance with similar precursors, be termed pre-promelittin. Structural investigations of this substance gave the following results:

(1) At the C-terminus it contains an extra glycine residue; while the venom components terminate with gln–amide, pre-promelittin has a C-terminal gln–glyCOOH sequence.

(2) At the N-terminus, pre-promelittin starts with a sequence of at least 33 residues, of which about the first 20 are largely hydrophobic. This part contains tyr, phe and met residues which are not present in promelittin.

The biosynthesis of the toxic peptide melittin thus involves at least three posttranslational reactions:

(1) Conversion of pre-promelittin to promelittin;

(2) formation of the correct C-terminal amide with concomitant loss of one glycine residue;

(3) activation of promelittin to melittin.

5. Fibroblasts and the synthesis of collagen

The synthesis of collagen by the fibroblasts is probably the major protein synthetic activity of the body in early life in terms of quantitative output. M. E. Grant (Univ. Manchester, England) described work on the biosynthesis of collagen. In all the mammalian tissues studied collagen molecules seem to be essentially the same in that they are semi-rigid, rod-shaped molecules comprising three polypeptides (α chains) in a triple-helical conformation. Recent studies indicate, however, that from tissue to tissue, and even within the same tissue, collagen molecules can differ in their primary structure and at least four genetically distinct types (I–IV) are known. All four types are synthesized in precursor forms, procollagens, comprising poly-peptides (pro-α chains) which are approx. 50% of non-helical peptide extensions at both the amino- and carboxyl-terminal ends [53].

The amino acid sequence coded by procollagen mRNA must undergo at least 7 post-translational enzymic modifications before the protein is functional in the extracellular space. Considerable advances have been made in our understanding of these unique processes which include hydroxylation of prolyl and lysyl residues [54], glycosylation of hydroxylysyl residues [55], scission of the extension peptides [56] and eventual stabilisation of the extracellular fibrils by intermolecular cross-links [57]. The significance of these enzymic events and the role of the extension peptides in the assembly and secretion of the pro-collagen molecule was discussed (see also [58]).

There followed a talk by R. Harwood (Univ. Wales, Cardiff) on the role of free and membrane-bound ribosomes in the biosynthesis of procollagen [58,59].

6. The hepatocyte

6.1. *Vitellogenin*

On the final morning the Course turned to the hepatocyte and considered two proteins synthesized in the liver, namely vitellogenin and serum albumin. Vitellogenin is particularly interesting since here we have a precursor protein that is subsequently cleaved into two biologically active proteins in an organ other than that in which it was synthesized. M. Gruber (Univ. Groningen, The Netherlands) described progress in our understanding of this interesting phenomenon, particularly in the chick which is the system he has studied.

The yolk of oviparous vertebrates, like birds, contains two yolk-specific proteins, lipovitellin and phosvitin which together form the majority of the mass of yolk proteins. Phosvitin is a phosphoprotein with the highest phosphorus content of all known phosphoproteins (10% P) – it also contains some carbohydrate. Lipovitellin contains some phosphate groups. The two proteins are deposited in yolk in a one to one molar ratio of the subunit peptide chain (chicken lipovitellin peptide chain about 135 000 mol. wt phosvitin peptide chain about 25 000 mol. wt).

The liver is the origin of these two proteins. Phosvitin and lipovitellin are not synthesized separately, but as parts of one large molecule, vitellogenin. Vitellogenin is secreted by the liver and split into phosvitin and lipovitellin in the course of, or after, uptake by the ovary. Consequently, phosvitin and lipovitellin are present in the yolk granules in a molecular ratio of one to one.

The existence of vitellogenin was only recently discovered because it is very susceptible to traces of proteolytic enzymes. The vitellogenin peptide chain of more than 1500 amino acids is synthesized in the chicken liver on large polysomes (consisting of 30–40 ribosomes) which are bound to the membranes of the ER. This synthesis requires less than 4 min; the finished protein is secreted only 10–15 min after completion of translation. Phosphorylation is a late event occurring within the last 5–6 min before secretion [60–63].

The Groningen group has isolated the mRNA for chicken vitellogenin. Its translation in a heterologous cell-free system gives rise to a protein with a slightly higher mobility on SDS–polyacrylamide gels than the authentic protein. This higher mobility is probably due to the lack of phosphorylation (and glycosylation) of the in vitro product, a situation comparable to casein, mentioned above. A small signal peptide in the in vitro product will thus not be reflected by

mobility in SDS. Translation of chicken vitellogenin mRNA in *Xenopus* oocytes leads to a product apparently identical with the chicken plasma vitellogenin. The oocyte thus also phosphorylates chicken vitellogenin. Probably, glycosylation occurs, too.

S. R. Farmer (N.I.M.R., London, England) briefly described some of his experiments on the primary and secondary induction of response to oestrogen in *Xenopus* liver in vivo [64].

A quite different system which involves the control of hormones on ovarian development in mosquitoes was then described by M. S. Fuchs (Univ. Notre Dame, Indiana, USA).

After adult eclosion, oocyte maturation in the anautogenous mosquito *Aedes aegypti* is characterized by a previtellogenic period during which slight growth and differentiation occurs. Further development ceases after about 48 h and at this time the ovary has reached its resting-stage. Such resting-stage ovaries will remain in this arrested condition indefinitely if the adult female is not allowed to blood-feed. New development leading to oocyte maturity proceeds only after blood engorgement whereupon yolk synthesis and its subsequent deposition ensues.

Females deprived of juvenile hormone (JH), shortly after eclosion (by allatectomizing them), do not exhibit resting stage ovaries and moreover apparently do not synthesize yolk protein [65].

Vitellogenesis in *A. aegypti* is normally regulated by the steroid moulting hormone (BH), which is produced by the ovary after blood-feeding. The major yolk proteins in many insects, including *A. aegypti,* are synthesized by the fat body and are transported via the haemolymph to the ovary. It is now clear that a primary function of MH (specifically β-ecdysone) in this species is to activate the fat body to synthesize these yolk proteins [66,67].

It has been postulated that blood-feeding results in the release of a 'brain hormone' which in turn is steroidiogenic with respect to the ovary. Thus, in mosquitoes a cycle is envisaged which is remarkably similar, both anatomically and biochemically, to the pituitary–ovary–liver–ovary cycle found in many vertebrates.

With regard to the action of JH and MH in yolk protein synthesis by the fat body, his evidence was consistent with the view that MH induces specific mRNA synthesis and MH then modulates the translation of these pre-formed messengers [68–70].

6.2. *Serum albumin*

J. D. Judah (Univ. College Hosp. Med. Sch., London, England) described current work on the biosynthesis of serum albumin in rat liver. Antibodies to rat serum albumin recognize at least two species when applied to detergent extracts of rat liver. One of them is more basic than serum albumin and the other is serum albumin itself. The proteins can easily be separated from each other by electrofocusing on pH gradients or by ion-exchange chromatography, but not by gel filtration. The basic component accounts for approximately 90% of the label in the antibody precipitate after a pulse of labelled amino acid, both in vivo and in vitro. It has been identified as a proalbumin, which is converted to serum albumin by limited proteolysis by trypsin or by cathepsin B. Proalbumin contains a hexapeptide with the sequence arg–gly–val–phe–arg–arg– attached to the N-terminal glu of rat serum albumin.

Kinetic experiments and balance studies provide firm evidence that proalbumin is the source of secreted serum albumin. If cycloheximide is used to block protein synthesis in vivo or in perfused livers, the proalbumin levels fall to account exactly both in rate and amount for the albumin exported.

Microtubule poisons such as colchicine inhibit the secretion of albumin. The rate of conversion of proalbumin in the liver is reduced to the same extent. No accumulation of proalbumin is seen, since the rate of biosynthesis falls to accommodate the diminished rates of conversion and secretion. [71–77].

7. Concluding remarks

In summarizing the main points of the meeting J. R. Tata (N.I.M.R., London, England) drew attention to the following.

(1) The concepts of the role of the organelles in the synthesis and secretion of proteins are based soundly on the early work of Palade [3] and his group at The Rockefeller University.

(2) There are many important enzymes associated with the ER. Not only does glycosylation and

phosphorylation take place on the membranes but pro-proteins have to be converted to the final product and now we realise that signal peptides have to be removed in the conversion of pre-pro-proteins to pro-proteins.

(3) There are some interesting similarities between the pro-peptides of albumin and parathyroid hormone but this does not lead to a conclusion concerning their function. The signal peptides appear to be rich in hydrophobic amino acid residues and their primary sequence is rapidly being determined. The work of Burstein et al. on the peptides of the light chain of IgG is particularly relevant and it is interesting that the signal peptides of the various light chains differ in structure [78,79].

(4) The signal hypothesis as formulated by Blobel has helped to clarify the work now being done on the biosynthesis of many different proteins. Although the development of mixed heterologous systems have helped to test the validity of the hypothesis many details remain to be proven.

(5) The recently published modification of the reticulocyte lysate system described by Pelham and Jackson [80], in which the endogenous mRNA is removed by ribonuclease, should provide a powerful new technique.

(6) There is great merit in studying as many different systems for the synthesis of specific proteins as possible. These various systems allow one to focus attention on the particular requirements of different cells, such as the need for thyroglobulin to conserve iodine. In the case of vitellogenin there is the opportunity to study reversible induction of protein synthesis.

(7) It is obvious that as yet we know little about the precise enzymology of glycosylation and other conversions although in this respect the role of dolichol phosphate adds a fascinating new dimension.

(8) Future work will no doubt centre on the need for polysome–membrane interaction in order to effect the removal of the signal peptide, and to the extent which the biosynthesis of intracellular proteins really differs from that of secretory proteins. Thus many non-secretory cells possess membrane bound ribosomes and it certainly remains possible that the proteins of the membranous organelles of the cell, which are made in the cytoplasm, are first made on the ribosomes bound to the ER as are secretory proteins.

Acknowledgements

As authors we would like to acknowledge the assistance and advice of all those who spoke at the meeting. We have tried to represent fairly the various views by using wherever possible the original text of the participants but the final result is, of course, our responsibility.

References

[1] Blobel, G. and Sabatini, D. D. (1971) in: Biomembranes (Manson, L. A. ed) Vol. 2, 193–195, Plenum Pub. Corp. New York.
[2] Milstein, C., Brownlee, G. G., Harrison, T. M. and Mathews, M. B. (1972) Nature New Biol. 239, 117–120.
[3] Palade, G. (1975) Science 189, 347–358.
[4] Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. 67, 835–851.
[5] Devillers-Thiery, A., Kindt, T., Scheele, G. and Blobel, G. (1975) Proc. Natl. Acad. Sci. USA 72, 5016–5020.
[6] Blobel, G. and Dobberstein, B. (1975) J. Cell Biol. 67, 852–862.
[7] Blobel, G. (1976) Biochem. Biophys. Res. Commun. 68, 1–7.
[8] Melchers, F. (1973) in: Membrane-mediated information. 'Function and Biosynthesis of Membrane Lipids and Glycoproteins' (Kent, P. W. ed) 2, 39–56, in: Medical and Technical Publishing Co. Ltd., Lancaster, England.
[9] Melchers, F. and Andersson, J. (1974) Adv. Cytopharmacol. 2, 225–235.
[10] Gronowicz, E., Coutinho, A. and Melchers, F. (1976) Eur. J. Immunol.
[11] Wallis, M. and Davies, R. V. (1976) in: 'Growth Hormone and Related Peptides' Proc. 3rd Int. Symp. on Growth Hormone (Pecile, A. and Müller, E. E.) pp. 1–13, Excerpta Medica, Amsterdam.
[12] Stachura, M. E. and Frohman, L. A. (1975) Science 187, 447–449.

[13] Sussman, P. M., Tushinski, R. J. and Bancroft, F. C. (1976) Proc. Natl. Acad. Sci. USA 73, 29–33.
[14] Maurer, R. A., Stone, R. and Gorski, J. (1976) J. Biol. Chem. 251, 2801–2807.
[15] Dannies, P. and Taskjian, A. H. (1976) Biochem. Biophys. Res. Commun. 70, 1180–1189.
[16] Evans, G. A. and Rosenfeld, M. G. (1976) J. Biol. Chem. 251, 2842–2847.
[17] Boime, I., Boguslawski, S. and Caine, J. (1975) Biochem. Biophys. Res. Commun. 62, 103–109.
[18] Cox, G. J., Weintraub, B. D., Rosen, S. W. and Maxwell, E. S. (1976) J. Biol. Chem. 251, 1723–1730.
[19] Peeters, B., Mous, J., Van Belleghem, H. and Rombauts, W. (1976) unpublished results.
[20] Steiner, D. F., Kemmler, W., Clark, J. L., Oyer, P. E. and Rubenstein, A. H. (1972) in: Handbook of Physiology-Endocrinology I (Steiner, D. F. and Freinkel, N. eds) Chapter 10, p. 175, Williams and Wilkins, Baltimore.
[21] Lernmark, A., Chan, S. J., Choy, R., Nathans, A., Carroll, R., Tager, H. S., Rubenstein, A. H., Swift, H. H. and Steiner, D. F. (1976) in: 'Polypeptide Hormones: Molecular and Cellular Aspects' CIBA Foundation Symposium 41, 7–30, Elsevier/Excerpta Medica, North-Holland, Amsterdam.
[22] Steiner, D. F., Kemmler, W., Tager, H. S., Rubenstein, A. H., Lernmark, A. and Zühlke, H. (1975) in: Proteases and Biological Control, pp. 531–549, Cold Spring Harbor Laboratory.
[23] Kuku, S. F., Jaspan, J. B., Emmanouel, D. S., Zeidler, A., Katz, A. I. and Rubenstein, A. H. (1976) J. Clin. Invest 58, 742–750.
[24] Horwitz, D. L., Starr, J. I., Mako, M. E., Blackard, W. G. and Rubenstein, A. H. (1976) J. Clin. Invest. 55, 1278–1283.
[25] Goldstein, A. (1976) Science 193, 1081–1086.
[26] Ling, N. and Guillemin, R. (1976) Proc. Natl. Acad. Sci. USA 73, 3308–3310.
[27] Duguid, J. R., Steiner, D. F. and Chick, W. L. (1976) Proc. Natl. Acad. Sci. USA 73, 3539–3543.
[28] Chan, S. J., Keim, P. and Steiner, D. F. (1976) Proc. Natl. Acad. Sci. USA 73, 1964–1968.
[29] Lomedico, P. T. and Saunders, G. F. (1976) Nucleic Acids Res. 3, 381–391.
[30] Permutt, M. A., Biesbroeck, J., Chyn, R., Boime, I., Szczesna, E. and McWilliams, D. (1976) in: 'Polypeptide Hormones: Molecular and Cellular Aspects' CIBA Foundation Symposium 41, 97–116, Elsevier/Excerpta Medica, North-Holland, Amsterdam.
[31] Howell, S. L. (1974) Advances in Cytopharmacology 2, 319–327.
[32] Steiner, D. F., Kemmler, W., Tager, H. S. and Rubenstein, A. H. (1974) Advances in Cytopharmacology 2, 195–205.
[33] Howell, S. L., Montague, W. and Tyhurst, M. (1975) J. Cell Sci. 19, 395–409.
[34] Vassart, G. and Dumont, J. E. (1973) Eur. J. Biochem. 32, 322–330.
[35] Vassart, G., Refetoff, S., Brocas, H., Dinsart, C. and Dumont, J. E. (1975) Proc. Natl. Acad. Sci. USA 72, 3839–3843.
[36] Haeberli, A., Salvatore, G., Edelhoch, H. and Rall, J. E. (1975) J. Biol. Chem. 250, 7836–7841.
[37] De Nayer, Ph., Caucheteux, D., Rowie, E. and Winand, R. (1976) Acta Endocr. (København) 82, Suppl. 204, 69.
[38] Habener, J. F., Kemper, B., Potts, J. T., Jr. and Rich, A. (1975) Biochem. Biophys. Res. Commun. 67, 1114–1121.
[39] Kemper, B., Habener, J. F., Ernst, M. D., Potts, J. T., Jr. and Rich, A. (1976) Biochemistry 15, 15–19.
[40] Habener, J. F., Potts, J. T., Jr. and Rich, A. (1976) J. Biol. Chem. 251, 3893–3899.
[41] Kemper, B., Habener, J. F., Potts, J. T., Jr. and Rich, A. (1976) Biochemistry 15, 20–25.
[42] Brew, K. and Powell, J. T. (1976) Fed. Proc. 35, 1892–1898.
[43] Craig, R. K., Brown, P. A., Harrison, O. S., McIlreavy, D. and Campbell, P. N. (1976) Biochem. J. 160, 57–74.
[44] Gaye, P. and Houdebine, L. M. (1975) Nucleic Acids Res. 2, 707–722.
[45] Rosen, J. M., Woo, S. L. C. and Comstock, J. P. (1975) Biochemistry 14, 2895–2903.
[46] Gaye, P. and Denamur, R. (1970) Biochem. Biophys. Res. Commun. 41, 266–272.
[47] Harrison, O. S., Craig, R. K. and Campbell, P. N. (1976) Biochem. Soc. Trans. 4, 340–341.
[48] Kreil, G. (1973) Eur. J. Biochem. 33, 558–566.
[49] Bachmayer, H., Kreil, G. and Suchanek, G. (1972) J. Insect. Physiol. 18, 1515–1521.
[50] Kindas-Mugge, I., Frasel, L. and Diggelmann, H. (1976) J. Mol. Biol. 105, 177–181.
[51] Kindas-Mugge, I., Lane, C. D. and Kreil, G. (1974) J. Mol. Biol. 87, 451–462.
[52] Suchanek, G., Kindas-Mugge, I., Kreil, G. and Schreier, M. (1975) Eur. J. Biochem. 60, 309–315.
[53] Fessler, L. I., Morris, N. P. and Fessler, J. H. (1975) Proc. Natl. Acad. Sci. USA 72, 4905–4909.
[54] Cardinale, G. L. and Udenfriend, S. (1974) Adv. Enzymol. 41, 245–300.
[55] Harwood, R., Grant, M. E. and Jackson, D. S. (1975) Biochem. J. 152, 291–302.
[56] Davidson, J. M., McEneany, L. S. G. and Bornstein, P. (1975) Biochemistry 14, 5188–5194.
[57] Bailey, A. J., Robins, S. P. and Balian, G. (1974) Nature (London) 251, 105–109.
[58] Grant, M. E. and Jackson, D. S. (1976) Essays in Biochem. 12, 77–114.
[59] Harwood, R., Durrant, B., Grant, M. E. and Jackson, D. S. (1975) Biochem. Soc. Trans. 3, 914–915.
[60] Bergink, E. W., Wallace, R. A., Van den Berg, J. A., Bos, E. S., Gruber, M. and Ab, G. (1974) American Zoologist 14, 1177–1193.

[61] Clemens, M. J. (1974) Prog. Biophysics Mol. Biol. 28, 69–108.
[62] Gruber, M., Bos, E. S. and Ab, G. (1976) Mol. Cell. Endocrinol. 5, 41–50.
[63] Schirm, J., Gruber, M. and Ab, G. (1973) FEBS Lett. 30, 167–169.
[64] Berridge, M. V., Farmer, S. R., Green, C. D., Henshaw, E. C. and Tata, J. R. (1976) Eur. J. Biochem. 62, 161–171.
[65] Lea, A. O. (1973) Gen. Comp. Endocrinol. Suppl. 3, 602–608.
[66] Schlaeger, D. A., Fuchs, M. S. and Kang, S. H. (1974) J. Cell Biol. 61, 454–465.
[67] Hagedorn, H. H., O'Connor, J. D., Fuchs, M. S., Sage, B., Schlaeger, D. A. and Bohm, M. (1975) Proc. Natl. Acad. Sci. USA 72, 3255–3259.
[68] Fong, W. F. and Fuchs, M. S. (1976) Mol. Cell. Endocrinol. 4, 341–351.
[69] Fong, W. F. and Fuchs, M. S. (1976) Insect Biochem. 6, 123–130.
[70] Fuchs, M. S. and Fong, W. F. (1976) J. Insect Physiol. 22, 465–471.
[71] Campbell, P. N. (1975) FEBS Lett. 54, 119–121.
[72] Dorling, P. R., Quinn, P. S. and Judah, J. D. (1975) Biochem. J. 152, 341–348.
[73] Quinn, P. S., Gamble, M. and Judah, J. D. (1975) Biochem. J. 146, 389–393.
[74] Russell, J. H. and Geller, D. M. (1975) J. Biol. Chem. 250, 3409–3413.
[75] Schreiber, G., Rotermund, H. M., Maeno, H., Weigand, K. and Lesch, R. (1969) Eur. J. Biochem. 10, 355–361.
[76] Smith, R. E. and Van Frank, R. M. (1975) in: Lysosomes in Biology and Pathology (Dingle, J. T. and Dean, R. T. eds) 4, 193–250, North-Holland, Amsterdam.
[77] Judah, J. D., Gamble, M. and Steadman, J. H. (1973) Biochem. J. 134, 1083–1091.
[78] Burstein, Y. and Schechter, I. (1976) Biochem. J. 157, 145–151.
[79] Burstein, Y., Kantor, F. and Schechter, I. (1976) Proc. Natl. Acad. Sci. USA 73, 2604–2608.
[80] Pelham, H. R. B. and Jackson, R. J. (1976) Eur. J. Biochem. 67, 247–256.