MEETING REPORT

The plant secretory system: mechanisms, pathways
and applications in biotechnology
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Introduction

In March 1992 David Robinson and Karl Oparka organized a meeting on ‘Vesicle traffic and protein transport in plants and yeast’ which was held in Göttingen, Germany (Journal of Experimental Botany 44, Supplement). One of the positive spin-offs of this meeting was that a small group of the participants (Jürgen Denecke, Loïc Faye, Lynne Roberts, David Robinson, and Alessandro Vitale) decided to create a network within the Human Capital and Mobility Programme of the European Union. The network obtained funding from the EU for a second international meeting on the plant secretory pathway. Additional funding from the Nestlé Research and Development Centre and the Journal of Experimental Botany enabled this meeting to take place at the University of York, 2–5 July 1997. 100 participants made for some 30 invited oral presentations, 15 selected poster presentations, and 60 posters.

To set the tone, and to bring everybody up to date with developments in non-plant systems, the audience was appropriately impressed by three keynote lectures. Tony Pugsley (Pasteur Institute, Paris) described ‘The general secretory pathway in bacteria’ and pointed out that the bacterial periplasm is equivalent to the endoplasmic reticulum (ER) in many respects. It contains chaperones, which protect correctly folded proteins from degradation. Exit of properly folded polypeptides from the periplasm is controlled by a complex pore, the secreton, which is encoded by a large polycistronic transcript. Akihiko Nakano (RIKEN, Japan) reviewed ‘The yeast secretory pathway’ and illustrated how the genetic approaches used in the yeast field can be of use to plant researchers. Ari Helenius (Yale, USA) discussed ‘Protein folding in the endoplasmic reticulum’ in mammalian cells and yeasts, and summarized the latest work on the function(s) of calreticulin and calnexin. All three contributions showed combinations of approaches ranging from pure genetics to protein biochemistry, but as Natasha Raikhel (East Lansing, USA) in a closing address remarked, ‘we have come a long way in the past five years and plant research has become increasingly multidisciplinary’.

Essentially following the secretory pathway, the talks and posters centred around protein folding and quality control in the ER, post-translational modifications and how proteins are packaged into specific transport vesicles and targeted to the correct destination. Detailed descriptions of individual molecules, e.g. aquaporins, the auxin-binding protein, as well as the potential uses of the plant secretory system for industrially important products such as antibodies, constituted two further sessions. A look into the future was provided by presentations on new methodologies.

Storage proteins and a toxin as indicators of protein folding, assembly and quality control in the endomembrane system

In addition to their nutritional importance seed storage proteins have become important tools to study protein assembly, post-translational processing, and vacuolar protein targeting. The exciting phenomenon of mRNA target-
ing to subdomains of the ER was introduced by Tom Okita (Pullman, USA). Sorting of prolamine mRNA in wheat is independent of translation or the presence of the signal peptide. This suggests that the targeting information resides elsewhere on the mRNA. The next step will be to identify this region, which will then be the first report of a nucleic acid sorting signal.

A model system for protein folding and assembly is bean phaseolin, a storage protein which undergoes glycosylation and trimerization prior to exit from the ER. Results were presented by four different speakers. Alessandro Vitale (Milano, Italy) showed that the C-terminal alpha helical region of phaseolin constitutes one of the regions of interaction between monomers. This same region is also a determinant for association with the binding protein BiP, an ER chaperone. This suggests that the same regions involved in assembly also mediate ER-retention of unassembled subunits.

In vivo degradation of a mutated, assembly-defective form of bean phaseolin is slow and cannot be inhibited by treatment with Brefeldin A or heat shock. In addition, the glycan of the defective protein does not acquire Golgi modifications. Together, these data point to a quality control function for the plant ER, in which defective proteins are retained and targeted for degradation (Pedrazzini et al., 1997).

Eliot Herman (USDA, Maryland, USA) has explored the role of the lytic vacuole in the quality control process using genetically engineered high methionine-containing phaseolin which proved to be very unstable. Unlike the deletion mutant used by Vitale’s group, this protein is competent for trimer formation and is targeted via the Golgi apparatus to the vacuole, where it is degraded. As a second marker α-zein was used. This zein subunit is very unstable when expressed alone without other partner zeins. It also appears to be degraded in the vacuole, but by a process of autophagy of the PB. Therefore, the vacuole in plants can also act as a compartment for the degradation of defective proteins, reached either through the Golgi-mediated route or through Golgi-independent autophagy of ER-derived PB.

An alternative route for the disposal of malfolded proteins in mammalian cells and yeasts is degradation by the proteasome after reverse translocation from the ER lumen to the cytosol (Kopito, 1997). Evidence for such a pathway in plants is now emerging (see below), but regardless of the mechanism, it remains unclear how the quality control machinery distinguishes defective proteins from folding and assembly intermediates which still have a chance to mature properly.

The role of glycans in protein folding was explored by two groups who also used phaseolin as a model. Aldo Ceriotti (Milano, Italy) established that fully glycosylated phaseolin monomers (which have two Asn-linked glycans) assemble into trimers more slowly than singly glycosylated monomers. In addition, he provided evidence that the ER lectins calreticulin or calnexin may regulate phaseolin trimerization via interaction with partially trimmed glycans containing only one glucose residue. These results indicate that glucose removal of high mannose glycans is the rate-limiting step in the assembly of phaseolin (Lupattelli et al., 1997). An even more extreme example was provided by Francesca Sparvoli (Milano, Italy) who showed that in Phaseolus lunatus cotyledons, the inhibitor of N-glycosylation tunicamycin blocks transport of the storage proteins to the vacuoles. This causes the formation of large aggregates in the ER containing phaseolin, phytohaemagglutinin, α-amylase inhibitor and also BiP.

In mammalian cells, virus proteins have been a major help in studying protein folding. Lorenzo Frigerio (Warwick, UK and Milano, Italy) has now provided fascinating evidence that plant toxins might also prove to be useful indicators of quality control in the plant ER. He addressed the problem as to how the toxin ricin can be manufactured by the plant ER without lethal effects to the plant itself, whereas application of the toxin to mammalian cells leads to cell death. Toxin entry into mammalian cells is thought to occur through retrograde transport via the Golgi to the ER followed by reverse translocation into the cytosol, essentially parasitizing a mechanism used for the disposal of malfolded proteins (see above). It is synthesized as one precursor peptide which is cleaved in the vacuole into A and B chains linked by a disulphide bridge. As a test system Frigerio used a protoplast-based transient expression system in which individual ricin chains were co-transfected with another gene, whose expression was used as a measure of cell viability. Ricin A-chains proved to be toxic when expressed in the ER or in the cytosol. However, when expressed together with its partner B-chain in the ER, the toxicity of the A-chain was reduced. Evidence was presented showing that the B-chain promotes heterodimer assembly and ER export, possibly preventing reverse translocation of the A chain to the cytosol.

Further work was presented on storage proteins from cereals. The ER-located protein bodies of maize endosperm are composed of different classes of zein prolamines, which have also been studied for more than a decade. The two important questions are: (1) How do the subunits assemble together? and (2) What are the signals responsible for their efficient ER retention and high stability? Suman Bagga (Tucson, USA) illustrated that transgenic tobacco plants synthesizing β-zein have protein bodies (PB) with a different morphology from that of PB seen in δ-zein producers. When the two proteins are co-expressed, δ-zein becomes more stable whereas β-zein is unaffected. PB made of β- and α-zein or of β-zein alone are very similar, indicating that the two proteins interact with each other and that β-zein has a stabilizing effect on the partner subunit (Bagga et al., 1997). These transgenic plants have increased levels of
BiP, suggesting that this chaperone is required for the synthesis of high levels of zeins. Dolores Ludevid (Barcelona, Spain) demonstrated that a mutated γ-zein, in which the repeat and ProX domains had been deleted, was secreted as an oligomer that sediments on sucrose gradients like the normal, ER-located PB, indicating that assembly into the large PB is not sufficient to determine ER retention. Clearly, storage proteins have attracted a great deal of attention, which is not surprising given their high nutritional importance. This area is likely to remain a focus of interest, thereby pushing it into the forefront of research on protein folding and chaperone action.

ER resident proteins in plants

A lot of effort has been spent on the cloning and characterization of genes encoding ER resident proteins in plants over the last five years. The best characterized genes are BiP, protein disulphide isomerase (PDI), endoplasmic, and calreticulin/calnexin. At this conference, the first functional studies on these were presented, which form a starting point for future work.

Nathalie Leborgne (York, UK) introduced a novel model system to study ER stress and the function of ER chaperones such as BiP. Treatment with tunicamycin markedly decreased the synthesis of α-amylase (a secretory protein), but not that of GUS (a cytosolic protein) when these were expressed transiently in tobacco protoplasts. As α-amylase is not a glycoprotein and tunicamycin cannot have a direct effect on its synthesis, it was suggested that tunicamycin causes a depletion of free BiP in the ER with negative effects on the co-translational translocation of secretory proteins. Co-expression of BiP strongly alleviated the tunicamycin effect, thus providing a system to monitor BiP activity and to study which regions of BiP are important for correct function. Plants overexpressing BiP showed a down-regulation of the endogenous genes, as seen by a lower basal mRNA level and a lack of induction by tunicamycin, demonstrating that sufficient BiP is present to cope even with stress conditions. These transgenic plants will be of great value in the further elucidation of BiP functions.

Further results on the ER resident protein calreticulin were presented by Jürgen Denecke (York, UK). Calreticulin has two glycans which normally are of the high-mannose type, but acquire a complex structure when cells are treated with Brefeldin A. If retention of calreticulin in the ER occurs via recycling from the Golgi apparatus, this process does not seem to involve the medial and trans cisternae. BiP and calreticulin form abundant complexes in vivo. These complexes contain a large portion of the total BiP (possibly up to 50%). Evidence was presented suggesting that BiP is not associated with calreticulin molecules which are in the process of folding or misfolded. It was therefore proposed that association with calreticulin regulates the availability of free BiP.

Marja Makarow (Helsinki, Finland) presented a novel chaperone of the HSP70 family in the ER of yeast, with about 50% similarity to BiP, and contains a classical ER retention motif. This protein plays a role in the recovery of proteins that are denatured in the ER when cells are subjected to heat-shock. Recovery occurs with great efficiency after the stress is released and the proteins ultimately re-acquire, with different kinetics, transport competence. ATP, but not de novo protein synthesis, is required for the recovery. This refolding chaperone may be part of a fundamental survival machinery for this unicellular organism, but may also have counterparts in higher eukaryotes (Saris et al., 1997).

In a session devoted to the actual products of the endomembrane system, Richard Napier (Wellesbourne, UK) recapitulated all the current knowledge on the auxin binding protein (ABP1), which has kept several laboratories busy for the last decade. ABP has an ER retention signal and a single glycan. Less than 2% of the glycan has a Golgi modified structure, which is consistent with the tiny amount of ABP found on the cell surface (Henderson et al., 1997). The pH optimum for auxin binding to ABP is 5, which could be expected for the apoplast but not for the ER. It still remains to be shown why a protein whose action is required at the cell surface exhibits an ER retention motif.

Glycosylation in the endomembrane system

A complete session was devoted to the glycosylation events in the plant endomembrane system to illustrate their importance/problems when plants are to be used as ‘green’ factories. Patrice Lerouge (Rouen, France) reported that IgGs also have high mannose glycans when expressed in tobacco, whereas normally they have only complex glycans, and that typical plant glycans, i.e., containing fucose and xylose can replace glycans with sialic acid without loss of biological activity. Loïc Faye (Rouen, France) elaborated on the more general aspects of glycosylation and pointed out that a general model for glycan function cannot be established. Whereas glycans are crucial for the activity and transport of some proteins (i.e. carrot invertase), work from Arabidopsis mutants indicates that the acquisition of a complex glycan is not necessary for plant survival and reproduction. The same protein can be glycosylated in different ways when produced by different plants. A large number of plant glycoproteins have a terminal ‘Lewis a’ epitope (Galβ(1,3)[Fucα(1,4)]GlcNAc). These structures are typical of the cell-surface antigens of red blood cells. Interestingly, in plant cells many of these are localized to the plasma membrane suggesting that they may play a role in cell–cell recognition, as indeed they do in animal cells (Fitchette-Lainé et al., 1998). Complex glycans of the laccase type are found only in extracellular glycopro-
teins and not in vacuolar glycoproteins, an observation which gave rise to renewed interest in the role of glycans in protein transport.

A large number of glycosylation reactions occur in the cisternae of the Golgi apparatus, which consequently requires that nucleotide sugars be imported from the cytosol where they are synthesized. Ariel Orellana (Santiago, Chile) reported that GDP-fucose is taken up by pea stem Golgi. He proposed that the rapid breakdown of the ensuing UDP is the driving force for polysaccharide biosynthesis. Orellana also obtained evidence for the presence of a 67 kDa phosphoglucomutase on the cytosolic surface of the Golgi membrane. The protein can be phosphorylated by UDP-Glc and he suggested that it is involved in the synthesis of this nucleotide sugar. The group from Santiago also reported data on the xyloglucan fucosyl transferase that adds fucose, from GDP-Fuc, to xyloglucans. Proteolysis experiments indicate that this activity is located in the lumen of Golgi cisternae and Triton X–114 phase partitioning indicates that the enzyme is an integral membrane protein.

Protein targeting in the endomembrane system, new work done on cargo molecules

Belinda Phillipson (York, UK) reported on the contribution of the membrane spanning domains in determining the final localization of type I and II membrane spanning proteins. The transmembrane domain and the cytosolic tail of the ER resident calnexin (a type I protein) fused to the secreted form of PAT leads to ER localization of the fusion protein in the correct orientation. Deletion of the cytosolic tail led to lower protein levels but did not result in detectable accumulation in the vacuoles nor stable degradation intermediates. Treatment with Brefeldin A did not result in a stabilization of the truncated molecule, indicating that degradation does not necessarily occur in a later compartment. When a type II transmembrane domain (from sec12) was fused to PAT the protein was enriched in microsomes, was very stable, but again absent from the tonoplast.

Interest in vacuolar protein trafficking has grown again due to the recognition that individual plant cells may contain more than one vacuole. Unlike lysosomal acid hydrolases in mammalian cells, which are segregated using a universal targeting signal, sorting of vacuolar proteins occurs on the basis of the recognition of signals occurring either within the molecule or at the N- or C-termini. Some signals are processed and removed once the proprotein reaches its destination, some remain part of the protein. Ken Matsuoka and Kenzo Nakamura (Nagoya, Japan) demonstrated with sporamin, that some sorting signals can even be moved from one location to another without loss of targeting efficiency. Clearly the high variability of vacuolar targeting signals supports the discovery of more than one type of vacuole in plant cells.

Gerhard Saalbach (Gatersleben, Germany) presented evidence that the last four amino acids (IAGF) of the brazil nut 2S storage albumin are necessary for vacuolar targeting of the 2S albumin, but when fused to invertase, they are not sufficient for vacuolar targeting (Saalbach et al., 1996). Kenzo Nakamura (Nagoya, Japan) discussed the role of redox conditions in the targeting of the sweet potato storage protein sporamin to the vacuole. The reducing agent dithiothreitol applied at 0.2 to 20 mM leads to mis-sorting of sporamin precursors to the cell surface. This is not due to an effect on sporamin disulphide bonds because a mutant in which the four cysteines have been eliminated by mutagenesis, and which is normally targeted to the vacuole, was also secreted in the presence of dithiothreitol. Therefore, the sporamin vacuolar targeting machinery is sensitive to reducing agents.

Jean-Marc Neuhaus (Neuchâtel, Switzerland) introduced a new reporter protein to study vacuolar targeting: rat β-glucuronidase (RGUS), which is a lysosomal protein. Surprisingly, its C-terminal propeptide (15aa) targets the protein to the plant vacuole. When this propeptide is deleted, the truncated protein is secreted and suitable for transplanting plant C-terminal sorting signals. Neuhaus also generated a series of green fluorescent protein fusions for cytosolic, vacuolar, ER, and secretory proteins. The vacuolar form (which has the targeting signal of chitinase) was localized to a neutral vacuolar compartment and not to the lytic one, identified by the accumulation of neutral red. This indicates that the targeting signal of chitinase directs proteins to the neutral vacuoles. The discovery is of significant importance as it was generally assumed that tobacco protoplasts have a single large central vacuole.

Kirs Törnäkangas (Helsinki, Finland) elaborated on the vacuolar sorting signal of barley aspartic proteinase. This is a vacuolar protein with an N-terminal sequence that contains an AsnProLeuArg (NPLR) motif, closely resembling the NPIR motif which is a vacuolar targeting determinant for aeurain and sporamin. However, the results of experiments made using mutated aspartic proteinase or different fusions of aspartic proteinase fragments with PAT as a reporter show that NPLR is neither sufficient nor necessary for vacuolar targeting. The plant enzyme has a plant specific insert (PSI) that distinguishes it from mammalian or microbial counterparts. Deletion of PSI causes secretion of the protein and a 20 fold increase in its accumulation in transgenic plants. The PSI contains an N-linked glycan, but inactivation of the glycosylation site did not affect vacuolar targeting and dramatically decreased the level at which it accumulated. Therefore, the PSI is necessary for the vacuolar targeting of the enzyme, and the stability of the enzyme is increased by its mis-localization to the cell wall and decreased by the absence of the glycan.
Clearly, the list of vacuolar sorting signals in plants is growing and the purification and characterization of different types of vacuoles constitutes an important challenge for the future.

**On the way to a vacuolar sorting machinery**

Until now, very little work has been done on the transacting factors in protein sorting such as receptors, vesicle coats and regulatory molecules to control vesicle budding, uncoating and fusion. At this meeting, the first progress in this direction was reported.

David Robinson (Göttingen, Germany) described the immunological identification of β-adaptin (108 kDa) in isolated plant clathrin coated vesicles (CCV), as well sec21p (part of COP I) and sec23p (part of COP II) homologues in cauliflower cytosol. In a combined effort, Jürgen Denecke’s and David Robinson’s groups have provided the first tangible evidence for a Golgi-ER recycling vesicle. An ER/Golgi fraction was prepared from leaves of tobacco plants transformed with a barley α-amylase construct (providing a measurable cargo molecule) with and without an ER retention motif (HDEL) tagged to its C-terminus. By incubating membranes containing α-amylase-HDEL in the presence of GTPγS, 50 nm vesicles were produced, which equilibrated on sucrose density gradients at around 48% sucrose. This fraction contained α-amylase activity and antigens recognized by Atsec21p antibodies, providing evidence for the in vitro induction of a COP-I type of vesicle. This method should lead to a routine vesicle budding assay which can be used to study the composition of transport vesicles.

Natasha Raikhel (East Lansing, USA) has identified a novel post-Golgi compartment using antibodies against the putative pre-vacuolar t-SNARE AtPEP12p (da Silva Conceição et al., 1997). The structures are tubulo-vesicles of around 100 nm in diameter, and could possibly be equivalent to the late endosomes of mammalian cells. The group has also cloned a putative plant Golgi v-SNARE, AtVti1p. Both AtPEP12p and AtVti1p can restore correct vacuolar targeting of carboxypeptidase Y in yeast deletion mutants.

Leonard Bevers (Norman, Oklahoma, USA) reviewed the identification of the first candidate of a vacuolar targeting receptor in plants, BP-80. This transmembrane protein, originally detected in CCV from pea cotyledons, binds to the N-terminal VTP of the thiol protease aleurain. Results on BP-80 gave rise to a very exciting poster session and discussions throughout the conference. As Gerhard Saalbach (Gatersleben, Germany) reported, BP-80 binds to the sorting signals of sporamin and brazil nut 2S albumin as well, which are structurally quite different. However, there are distinct BP-80 isoforms in Arabidopsis and it is not known whether 2S albumin is recognized by the same isoform as the one binding to sporamin and aleurain. Giselbert Hinz (Göttingen, Germany) showed that BP-80 is absent from the so-called dense vesicles, which are responsible for the transport of storage proteins in pea cotyledons. A poster from Natasha Raikhel’s group presented evidence that one of Arabidopsis BP-80 homologues is concentrated in CCV. In addition, BP-80 is present in a subcellular fraction from Arabidopsis, which appears to be the same as that which is characterized by the presence of the AtPEP12p mentioned above (Ahmed et al., 1997). David Robinson and Giselbert Hinz showed that in pea cotyledons this takes the form of large, storage protein-filled multivesicular bodies. Unlike the protein storage vacuole, these structures possess BP-80.

Nadine Paris (Neuchatel, Switzerland) presented results about the sorting of BP-80 itself. The protein is a type I membrane spanning protein which has a cytosolic domain. This contains a tyrosine motif of a type known to interact with the adaptors of Golgi-derived CCV in mammalian cells (Sandoval and Bakke, 1994). Furthermore, when the transmembrane and cytosolic domains of BP-80 were fused to a secretory green fluorescent protein (GFP) construct, the resulting fusion protein also accumulated in the Golgi. Since plant adaptors have not yet been isolated, Leonard Bevers, Nadine Paris and co-workers have performed heterologous binding experiments with pea clathrin, BP-80 and brain adaptors. The latter were shown to bind to both the clathrin and BP-80.

In conclusion, a pattern of similarity for sorting and packaging events at the trans Golgi of animal and plant cells is emerging, and there is good progress towards understanding the sorting machinery of vesicle transport.

**Novel tools**

Immunofluorescence, as visualized by laser scanning confocal microscopy, has often superceded immunogold electron microscopy in animal cell biology, especially in routine situations where antigen distributions are normally quite characteristic, e.g. a punctuate perinuclear staining for the Golgi apparatus or a diffuse perinuclear staining for the ER. However, as vividly demonstrated by Chris Hawes (Oxford, UK), when combined with green fluorescent protein (GFP) as a reporter fluorochrome, and using living cells, this powerful new technology can provide startling new insights into understanding membrane dynamics. The audience was literally stunned by this real-time display of ER morphology and the movement of ER tubules within the living cell, visualized via GFP-constructs containing a signal peptide with and without an ER retention signal. However, it has to be appreciated that 50 nm transport vesicles would not show up individually under the light microscope, and that further progress in this area can only be assured in...
combination with electron microscopy, protein and lipid biochemistry or molecular biology.

Ulrike Homann (Cambridge, UK) has used the patch-clamp technique to study the regulation of exocytosis, by monitoring changes in membrane area under different conditions. Exocytosis and a consequent increase in the membrane area of a clamp is monitored via the increased polypeptide and the C-terminus of the x-type, and vice versa. This suggests that it requires GTP-binding proteins. This might be a ‘regulated’ pathway. The other one is calcium independent, and may be the ‘constitutive’ pathway.

The selection of secretion-defective mutants in yeast has allowed for the identification of a number of proteins involved in vesiculation and fusion events. Prominent examples of these are the Rab/Ypt family of GTPases, and several groups are now addressing the role(s) of corresponding plant homologues. Thus, Ian Moore (Oxford, UK) has obtained a Rab2 Arabidopsis mutant which displays retarded root growth; Gerhard Saalbach (Gatersleben, Germany) has isolated several ypt homologous cDNA clones from Vicia faba and is busy generating double transformants expressing brazil nut 2S albumin; and Takahashi Ueda’s group (Tokyo, Japan) is currently investigating the interactions of proteins with the Golgi-localized Ara4 GTPase.

In order to really understand the secretory pathway in plants it is important to recognize plant-specific gene products. Paul Dupree (Cambridge, UK) and his colleagues have embarked on an ambitious project to identify novel plant proteins by subjecting endomembrane proteins to 2D-PAGE. Enrichment in specific compartments such as the Golgi results in the definition of subsets of spots, recognized by computer-aided analysis.

Industrial and pharmaceutical products of the endomembrane system

To draw attention to a variety of proteins which are manufactured by the plant endomembrane system, a session was devoted to interesting molecules of commercial importance. Peter Shewry (Long Ashton, UK) discussed the structure of high molecular weight (HMW) glutenin subunits in wheat. They seem to be mainly helical, with a globular head. Transgenic wheat has been produced with up to a 5-fold increase in the amount of individual subunits. Increasing the number of subunits results in increased elasticity of the dough, which could be an important step forward in downstream processing and European wheat production. Gad Galili (Rehovot, Israel) studied the role of the Cys residues in HMW glutenin polymerization by exchanging the N-terminus of an x-type polypeptide for that of a y-type. In transgenic wheat, 50% of the recombinant protein formed new heteropolymers with the endogenous subunits, whereas the remainder was monomeric, contained an unusual intramolecular disulphide bond and was soluble rather than insoluble in aqueous buffers. This supports the idea that the large natural glutenin polymers arise mainly through interactions between the N-terminus of a y-type polypeptide and the C-terminus of the x-type, and vice versa. From both presentations it seems possible to alter the food-technological characteristics of wheat by expressing recombinant glutenins (Shimoni et al., 1997).

Julian Ma (London, UK) illustrated that it is very easy to produce antibodies of any kind in transgenic plants. The model antibody used is a mouse IgG1, Guy’s 13 (see above) and it is useful for topical immunotherapy against streptococcal infections in the mouth, eye, etc. Endogenous antibodies which are present in saliva and tears have a ‘secretory component’ which is believed to give resistance to proteases. In mammals, the secretory component is acquired as the antibodies secreted by plasma cells cross epithelial cells by transcytosis. Plants are the first expression system in which complete, functional secretory antibodies have been expressed in a single cell (Ma et al., 1995).

The last speaker of the conference, Johnathan Napier (Long Ashton, UK), discussed a portion of the plant endomembrane system with a very peculiar structure, the oil bodies or oleosomes. They have a diameter of 0.2–2 μm and are composed of a phospholipid monolayer and a protein shell made up of a single protein species, the oleosins. Oleosins are storage-tissue specific and can represent up to 10% of total seed protein. Structurally, oleosin has an amphipathic helix and a hydrophobic tail. Oleosin is targeted to the ER membrane via the signal recognition particle. The model currently favoured is that oil bodies bud from the ER membrane because of the insertion of oleosins. Because they are easily purified, oil bodies could be explored for the production of proteins in plants.

Concluding remarks

In many ways, the secretory pathway can be regarded as a biochemical pathway for the synthesis of proteins, lipids and oligo(poly)saccharides. Whereas most biochemical pathways are composed of a cascade of enzymes, the secretory pathway is more complex as it entails a number of distinct compartments in which specific biosynthetic steps and quality controls occur. Perhaps the most complex steps are those involving the formation, directed transport and fusion of transport vesicles to deliver specific cargoes to their final compartments.

In order to study this pathway, a whole arsenal of techniques, expertise and tools are needed. Perhaps one of the most impressive conclusions of this conference is that a critical mass of researchers to tackle the mysteries...
of the secretory pathway has now been reached. Protein-protein interactions can now be studied with a range of biochemical assays, and the combination of gene-cloning, immunocytochemistry and genetic manipulation via directed modification of proteins and generation of transgenic plants is crucial for further progress. Often, only some of these techniques are available in individual laboratories, but the formation of scientific networks within the EU will help to overcome this problem.

Commercial interest is still modest and there was poor industrial representation at this conference in spite of a separate session on industrial products. Given the high potential of the plant endomembrane system for manufacturing proteins of pharmaceutical or nutritional value this may be surprising. However, it is during conferences such as this that an awareness of such possibilities can be generated. Whereas applied aspects are becoming increasingly important these days, we should not forget the intriguing questions of a more fundamental nature, for example, how is a functionally intact Golgi apparatus maintained in spite of all the vesicles that fuse with and bud off from this compartment? How many targeting mechanisms to the vacuoles exist? Or how does the ER machinery distinguish immature proteins, which must be retained until they have reached the proper conformation, from the permanently defective ones that need to be degraded? It is questions as these which will inspire the field in the future.

References


