A. Morphology

I. Cytology

a) General and Molecular Cytology

By WERNER W. FRANKE, ERNST-DIETER JARASCH, WERNER HERTH, ULRICH SCHEER, and HEIDE ZERBAN

The present review discusses some general aspects of membrane structure and problems of membrane isolation and membrane biochemistry, with particular focus on the endoplasmic reticulum.

1. Structures of Endomembranes and Plasma Membranes, with Special Emphasis on Observations Made with the Freeze-Etching Technique

Little information on the general organization of membranes has come from studies using plant cells, except from contributions concerning the inner mitochondrial and the thylakoidal membranes (for reviews see SITTE, 1, 2). For example very few botanical studies have contributed to the recent cascade of examinations and elaborations of the "fluid mosaic model" concept that emphasizes the lateral mobility of membrane components (SINGER and NICOLSON; EDIDIN and FAMBROUGH; EDIDIN and WEISS; FRYE and EDIDIN; for specific problems of fluidity in surface membranes see also the recent reviews by KOPP; EDIDIN; NICOLSON), again with the exception of some detailed studies in thylakoidal membranes such as those by GOODENOUGH and STAEHELIN; OJAKIAN and SATIR (see also the freeze-etch studies by MÜHLETHALER; ARNTZEN et al.; NEUSHUL; MILLER and STAEHELIN; EYTAN and OHAD; PARK and PFEIFHOFER).

The advent and widespread application of the freeze-cleavage-etching techniques, during whose development plant cells have been used from the early days on (MOOR and MÜHLETHALER; BRANTON and MOOR), has resulted in a series of studies in which freeze-cleavage images of endomembranes and plasma membranes are compared in a variety of plant cell systems.

Although regular, membrane type-specific differences in the location of the cleavage planes as well as in the overall distribution of intramembraneous particles in comparable fracture faces have not been convincingly and consistently demonstrated (BRANTON, 1, 2; BRANTON and DEAMER; SATIR et al., 2; PLATTNER et al., 1; MEYER and WINKELMANN; PINTO DA SILVA and BRANTON; PINTO DA SILVA et al., 2; DEMPSEY et al.; FINEAN et al.; BULLIVANT; VIAN; BENEDETTI et al.; ORCI et al.; TILLACK and MARCHESI; FLOWER, 1-3; for historical review of the interpretation of frozen etched membranes see also STAEHELIN; for bacterial membranes c.f. NANNINGA; for examples in plant cells see MAYER; NORTHCOTE and LEWIS; HEREWARD and NORTHCOTE, 2; KARTENBECK et al.; FINERAN, 1-3; KIERMAYER and STAEHELIN; SCHWELITZ et al.; STAEHELIN and KIERMAYER; WERZ and KELLNER; MATILE, 1; MATILE and MOOR; SOUTHWORTH and BRANTON; BRANTON and SOUTHWORTH; ZERBAN et al.; ZERBAN and WERZ; BRANTON, 2; WILLISON and COCKING; FRANKE et al., 1; for refs. see also ROLAND, 1; for problemes of the influence of specific pretreatment in freeze-etch studies of plant material see FINERAN, 1; PLATTNER et al., 2; SAKAI et al.; RICHTER, 1, 2; for similar problems in animal cells see also McINTYRE et al.; KIRK and TOSTESON; BACHMANN and SCHMITT; REITH and

OFTEBRO; BANK and MAZUR), some minor and gradual differences of particle densities and arrays have been reported, for example as occurring across dictyosomal stacks (STAEHELIN and KIERMAYER; FINERAN, 2; as to animal cells see, however, also CUNNINGHAM et al.). Particle-free membranes such as those of the myelin sheath (BRANTON, 3; however, BISCHOFF and MOOR, 1, 2, have described coarse "humped aspects", probably protrusions and finely granulated faces in fractures through myelin sheath lamellae) and in viral envelopes (c.f. BÄCHI et al., 1, for influenza viruses; SHEFFIELD, 1, 2, for B-type particles; c.f. also the study on the envelope of the Respiratory Syncytial Virus by BÄCHI and HOWE, 2; have not been shown in plant cells.

Somewhat perplexing is the situation in fungal membranes where "freeze-etch particles" have been repeatedly described but may not be demonstrable in all species or developmental stages with the same clarity and consistency (for references on freeze-etching observations in fungal cells see MOOR and MÜHLETHALER; BRANTON and SOUTHWORTH; MATILE and WIEMKEN; MATILE et al.; STREIBLOVA; FUHRMANN et al.; GRIFFITHS; SLEYTR et al.; GIESBRECHT and EMEIS; NEČAS; BRONCHART and DEMOULIN; COLE and ALDRICH; HEREWARD and NORTHCOTE, 1; J.V. ALLEN et al.; HOLT and LEADBETTER; HESS and STOCKS; HESS and WEBER, 1, 2; HESS et al., 1-4; HESS; SASSEN et al.; TAKEO, 1, 2; TAKEO and NISHIURA; TAKEO et al.; KOPP; MALHOTRA and TEWARI.

The particles which have been noted in fractures of the plasma membranes have frequently been interpreted as representing sites containing the enzymes involved in the formation of cell wall components, especially of structural polysaccharides such as cellulose (for detailed discussion see FRANKE et al., 2; WILLISON and COCKING) but it has to be reemphasized that there is not a trace of biochemical evidence to support this identification. In general, all freeze etch-studies suffer from the same problem, namely that of what particles are. The intramembraneous particles have been repeatedly speculated as representing protein complexes of a perplexing variety of properties and functions (particles should be, for example, "sialoglycoproteins", "band III protein","concanavalin A receptors", "sites which bear A and B antigens", receptors for other lectins, proteinaceous sites for glucose and water passage, calcium transport ATPase in sarcoplasmic reticulum, glycoproteinaceous receptors for certain viruses such as the influenza viruses and myxoviruses, proteins involved in cell-to-cell contact, glucan synthetases etc., see, e.g., PINTO DA SILVA and NICOLSON; PINTO DA SILVA et al., 2; TILLACK et al.; MATILE et al.; SCOTT et al.; MARCHESI et al., 1, 2; DEAMER and BASKIN; PINTO DA SILVA, 1; BÄCHI et al., 2; ROBINSON; ROBINSON and PRESTON, 1-4; LOOR; ROLAND, 1; for some bacterial membranes see also TOURTELOTTE and ZUPNIK; a correlation to DNA-attachment sites has been speculated by MEYER et al.). However, it has to be clearly stated that at the moment there is neither a convincing demonstration of the chemical nature of such particles in any cell system, nor have the ideas that the patterns of such particles are correlated with the distribution of distinct membrane components been confirmed in recent reports on the specific distribution of surface receptors of lectins in mammalian lymphocytes (McINTYRE et al.; YAHARA and EDELMAN), in cultured mouse fibroblasts (PINTO DA SILVA and MARTINEZ-PALOMO, 2), and probably also in mammalian erythrocytes (BÄCHI and SCHNEBLI). The present situation is so puzzling that it might best be characterized and summarized by the following quotations: (a) *"Freeze-fracture, freeze-etch and molecular labeling techniques localize concanavalin A receptors to the membrane intercalated particles of human erythrocyte ghost membranes"* (PINTO DA SILVA and NICOLSON; article submitted March 18th, 1974); (b) *"When cells are fixed after con A-peroxidase treatment, ... freeze-fracture examination of these cells does not reveal a corresponding accumulation of membrane particles"* (PINTO DA SILVA and MARTINEZ-PALOMO, 1; article submitted January 16th, 1974; (c) *in studies using normal and transformed mouse fibroblast 3T3 cells "our results probably imply independence of*

membrane particles and concanavalin A receptors" (PINTO DA SILVA and MARTINEZ-PALOMO, 2; article submitted November 29th, 1974); (d) *"Changes in intramembranous particles concomitant with capping of various entities have not been found"* (KARNOVSKY and UNANUE; lecture given April 11-13, 1972); (e) *"The topographic distribution of ferritin-labelled concanavalin A bound to the surface membrane of mouse lymphocytes has been analyzed by examining ultrathin sections and ghost membranes in the electron microscope. .... Analyses using freeze-fracture methods indicated that the distribution of intramembranous particles is not correlated with either the movement of surface receptors or the modulation events"* (YAHARA and EDELMAN; article submitted July 26th, 1974).

The most that can be said at the moment about these structures is that intramembranous particles of unknown identity are consistently observed in specific, defined and characteristic densities and patterns.

Obviously, the distribution of the various size-classes of particles recognized in freeze-etched membranes can be greatly variable within a membrane, as shown in the plasma membrane of pancreatic acinar cells (DE CAMILLI et al.) and as illustrated by the various forms of characteristic clusters such as in the ordered arrays of junctional complexes and desmosomes (KREUTZIGER, 1, 2; CHALCROFT and BULLIVANT; FLOWER, 2; FRIEND and GILULA; GILULA et al.; ORWIN et al., 1-3; NOIROT-TIMOTHÉE and NOIROT; GOODENOUGH and REVEL; REVEL et al.; SPYCHER; CLAUDE and GOODENOUGH; McNUTT and WEINSTEIN, 1, 2; REVEL and KARNOVSKY; STAEHELIN et al., 1; for heterocellular junctions see JOHNSON et al.; for dynamic of gap junction formation during cell-to-cell contact see also SCOTT et al.), at plasma membrane sites that are in contact or association with mucocysts or trichocysts in some ciliates (SATIR; SATIR et al., 1, 2; WUNDERLICH and SPETH; SATIR and SATIR; PLATTNER et al., 1), in the form of the so-called ciliary or flagellar "necklaces" and other ordered arrays (SPETH and WUNDERLICH, 3; WUNDERLICH and SPETH; SATIR et al., 3; BERGSTRÖM and HENLEY; BERGSTRÖM et al.; GILULA and SATIR; FLOWER, 1, 2; for further structural differentiation in cilia see also SATTLER and STAEHELIN), in certain regions of the astrocyte surface in mammalian cerebellar cortex (LANDIS and REESE; see also DERMIETZEL; GEMNE; for neoplastic astrocytes see also TANI et al.), in regions of enhanced pinocytosis in intestinal smooth muscle cells (ORCI and PERRELET), in the "apical endocytic complex" of ileal absorptive cells in some mammals (KNUTTON et al.), and in the surface "plaques" of epithelia of the urinary tract (VERGARA et al.; STAEHELIN et al., 2; PORTER et al.; CHLAPOWSKI et al.; see also the review of HICKS et al.). Paracrystalline clusters of ca. 11 nm particles have also been described in the plasma membrane of special plant cells, the "transfer cells" of the root nodules of the clover, *Trifolium repens* (BRIARTY). LITTLEFIELD and BRACKER have reported that the particles are consistently absent in regions where the plasma membrane of the flax, *Linum usitatissimum*, is invaginated around haustoria" of the penetrating pathogenic rust fungus, *Melampsora lini* (for host-symbiont interrelationships as revealed by freeze-etching see also the study on orchid mycorrhiza by HADLEY et al.). Specific arrays of membrane components are usually revealed in freeze-etched gas vacuoles of some blue-green algae (JONES and JOST; JOST and JONES; JOST; JOST and MATILE; JONES et al.; STOECKENIUS and KUNAU; WAALAND and BRANTON). A variety of decorative particle arrays (e.g. strands) has also been described in plasma membrane and acrosomal membranes of sperm cells, in particular in mammals (FRIEND and FAWCETT; PLATTNER; KOEHLER, 1-3). Conspicuous particle arrays and "segregation clusters" appear to be relatively rare in endoplasmic reticulum membranes (c.f., however, BREATHNACH et al.; REITH and OFTEBRO) but somewhat more frequent in specific regions (MEYER et al.; MOOR et al.; STEIDLE and HUHN; STEARNER and SANDERSON), especially of nuclear envelopes in sperm cells (PLATTNER; LIU; FRIEND and FAWCETT; for reviews see KARTENBECK et al.; FRANKE, 1; FRANKE and SCHEER, 1). Highly ordered particle arrays are also known in various bacterial groups, especially among the Gram-positive species (for reviews see THORNLEY et al.; VAN ITERSON). A large number of reports have described physiological and cell developmental changes in particle distributions as well as alterations induced experimentally or during viral in-

fections in both plasma membranes and various endomembranes including the nuclear envelope (WUNDERLICH et al., 1-3; BÄCHI and HOWE, 1; BÄCHI et al., 2; SPETH and WUNDERLICH, 1; SPETH et al.; BROWN and BURLINGHAM; ALDRICH and GREGG; PINTO DA SILVA, 2; KIRK and TOSTESON; TILLACK and KINSKY; ORCI and PERRELET; ELGSAETER and BRANTON; VERKLEIJ et al., 1, 2; JAMES and BRANTON; SHECHTER et al.; KLEEMAN and McCONNELL; FIIL and BRANTON). During immunolysis of mammalian erythrocyte membranes, when special ca. 10 nm-wide ringlike depressions appear on the outer surface, the internal particle pattern does not significantly change (ILES et al.).

In summary, the broadening of our knowledge on the structural organization of membranes from data obtained with the freeze-etch technique is rather limited. *First*, we have learned that the majority of structures described with the use of other electron microscopic preparation techniques are not due to artificial changes, especially during the fixation and dehydration procedures. *Secondly*, some structures and structural dimensions described from ultrathin sections and negatively or positively stained preparations of isolated cell components obviously are influenced and altered by these treatments (see the refs. quoted above; as to the particularly illustrative example of nuclear pore distributions see the evaluations contained in: FRANKE, 1, 2; FRANKE and SCHEER, 2; KARTENBECK et al.; SPETH and WUNDERLICH, 2; MAUL et al.; ZERBAN and WERZ, 1). *Thirdly*, we have learned a lot from freeze-etch studies about special problems of the interpretation of freeze-etch images, in particular as to intramembranous fracture planes and particles. In other words, freeze-etch studies have solved some problems which they had created.

2. Isolation and Characterization of Fractions of Isolated Membranes

The current state of research in plant cytology and biochemistry is characterized by the fact that the development of cell fractionation techniques is largely behind that already achieved with animal cells. Two reviews on plant cell fractionation procedures and on the methods that can be recommended for the critical examination and characterization of plant cell fractions have recently been published (JACOBI; PRICE). Among the membranous particles only the procedures for isolating and subfractionating thylakoids and mitochondrial membranes are sufficiently developed and have produced fractions comparable in quality to the best fractions obtained in animal cytology (for reviews see JACOBI; MEHARD; HONDA; LATIES; NOBEL; SCHATZ and KOVÁC). As for the other cellular membranes, the present state of advancement varies greatly.

a) Nuclear Membranes

Procedures for isolating plant cell nuclei and nuclear envelope fragments were mentioned in last year's review. Meanwhile, one further review on the isolation of nuclei has appeared (MASCARENHAS et al.), as well as a detailed study on the isolation and characterization of nuclear membranes from onion root tips and stems (PHILIPP et al.). In addition, SPRING et al. (see also FRANKE et al., 3) have demonstrated that the nuclear envelope from the giant primary nucleus of the vegetative cells of the green alga *Acetabularia* (for review see WERZ) can be manually isolated and freed from most of the adherent nuclear and cytoplasmic material by the use of the same microtechniques that were originally developed for isolating nuclear envelopes from the giant

nuclei of amphibia, echinoderms and insects (for reviews see, e.g. GALL, 1, 2; FRANKE and SCHEER, 1, 2; TRENDELENBURG; FRANKE, 1; FRANKE et al., 2). Although in principle it seems possible to prepare fairly well-purified nuclear membrane fractions from plant tissue (see also FRANKE, 2-4; STAVY et al.; PHILIPP et al.) the yield is relatively low so that it is difficult to obtain enough membrane material for biochemical characterizations. The comparative data obtained by PHILIPP et al. show a marked resemblance of lipid composition and enzyme content to those in rough microsomes prepared in parallel, but emphasize the retention of some nuclear DNA to the membranes, even after treatment with high salt concentrations.

b) Elements from the Endoplasmic Reticulum ("Rough" and "Smooth" Microsomes): Isolation and Composition

For more than two decades the rough microsomal fraction has been one of the best defined and most frequently-used membrane fractions in a variety of animal cells (for recent reviews see KREIBICH and SABATINI; KREIBICH et al.; WIBO et al.; TATA; GRAM; FLEISCHER and KERVINA; EL-AASER et al.; DALLNER; AMAR-COSTESEC et al., 1, 2; BEAUFAY et al., 1, 2; ADELMAN et al.). Therefore, it is surprising to see how little effort has been made to develop methods of preparing a similar highly-purified fraction from plant material, although a variety of plant cells possess a quite well-developed system of endoplasmic reticulum (ER) cisternae with ribosomes attached.

Since the early attempts in the midfifties (MARTIN and MORTON, 1-5; LOENING; AKAZAWA and BEEVERS; HODGE et al.) there has been very little progress and most of the biochemical studies on plant "microsomes" have been done with insufficiently characterized fractions, especially the morphological examinations (of various descriptions of "rough" and "smooth" and total microsomes see, e.g., NAKANO and ASAHI; MOREAU et al.; SHORE and MacLACHLAN; DONALDSON et al.; ABDELKADER and AUDERSET; ABDELKADER, 1, 2; BOWLES and NORTHCOTE, 1, 2; ABDELKADER and MAZLIAK, 1, 2; KANPP et al.; KEMP and MERCER; FERNE et al.; KNAPP et al.; BRANDT and BENVENISTE; DUPÉRON et al.; MEUNIER and MAZLIAK; HIRAI AND ASAHI; the "membrane-like" fraction which BERGQUIST et al. isolated from supernatants obtained after 150,000 g x 30 min in homogenates of *Neurospora crassa* mycelia might well contain ER-derived elements but this remains to be proved.) Only the very recent studies on castor bean endosperm by BEEVERS and co-workers (MOORE et al.; LORD et al., 2) and on *Phaseolus* hypocotyls by DOBBERSTEIN et al. really did document that some of the fractions they used for their investigations on phospholipid metabolism and polyribosome membrane attachment, respectively, were sufficiently purified rough microsomes (see below). PHILIPP et al. have recently compared structural and biochemical data of rough microsomes with and without subsequent treatment with high salt concentrations from two onion tissues. They presented fractions which consisted mostly of ribosome-covered vesicles or elongated sacs, but contained as a minor but inevitable contamination a form of "heavy microsomes" represented by smooth-surfaced vesicles which enclose ribosomes and most probably are derived from the plasma membrane and/or the tonoplast.

Summarizing the data of several authors from various plant materials one can now conclude that the phospholipid composition of rER membranes is (*a*) similar to that of nuclear membranes and (*b*) characterized by the predominance of lecithin and phosphatidylethanolamine (PHILIPP et al. report that these two components constitute more than 80% of onion root tip rER) and the virtual absence of sphingomyelin and cardiolipin (for refs. on phospholipid patterns see PHILIPP et al.; MOREAU et al.; ABDELKADER and MAZLIAK, 1, 2; DONALDSON et al.). Traces of cardiolipin which sometimes may be found in such fractions (see, however, MEUNIER and MAZLIAK; McCARTY et al.) are most probably due to mitochondrial contaminations.

It is very interesting to note the similarity of the typical phospholipid pattern of membranes of the ER-system to that reported by ALLEN et al. in the "lipid vesicles" isolated from pea and bean seeds, i.e. to the phospholipid composition of a structure that represents a non-membranous reserve-lipid aggregate (MOLLENHAUER and TOTTEN, 1-3; cf. GURR et al., 2; fatty acid analyses are also presented in these articles and by GURR et al. (1). This conspicuous similarity in composition is particularly interesting as MOLLENHAUER and TOTTEN (2) have stated (*a*) that such lipid spheres are *"separated from the cytoplasmic ground substance by a thin interfacial structure"* which they think is *"a bounding membrane"* (for detailed discussion of the problems of the interpretation of such boundary structures as membranous structures or "precipitation lines" around lipid globules see also the following articles: GURR et al., 2; YATSU and JACKS; YATSU et al.; MOLLENHAUER and TOTTEN, 3; PHILIPP et al.), and (*b*) that *"lipid vesicles appear to be synthesized in association with, but external to, the endoplasmic reticulum"*. These authors, however, make strong reservations as to the suggestive concepts that such lipid aggregates, which during germination transformed into sac-like structures, represent storages that are used for the rapid production of ER-elements.

A special problem in analyzing plant membrane phospholipids is that of hydrolysis by various lipases, in particular by the phospholipase D which is so common in plants, and results in the artificial accumulation of phosphatidic acid during preparation (KATES; GALLIARD; PHILIPP et al.; DOUCE and LANCE; CLERMONT and DOUCE; NACHBAUR and VIGNAIS). This degradation, however, can be minimized if certain precautions are taken, including, for example, inclusion of phospholipase D inhibitors in the isolation media (see the references given above). Some authors have also reported data on labeling kinetics of phospholipids which suggest an intramembranous exchange of lipids and fatty acids between different membrane types (MAZLIAK and ABDELKADER; ABDELKADER, 2; ABDELKADER and MAZLIAK, 1, 2), especially from ER into mitochondria, similar to what has long been proposed in animal cells (KADENBACH; WIRTZ and ZILVERSMIT, 1, 2; for recent review see WIRTZ).

It has also been shown that many of the enzymes involved in the biosynthesis of phospholipids are located in the endoplasmic reticulum membranes (for refs. see VANDOR and RICHARDSON; CASTELFRANCO et al.; KAGAWA et al.; LORD et al., 1, 2; JOHNSON and KENDE; DEVOR and MUDD; MOORE, T.S., et al.), in some contrast to earlier notions of MORRÉ and co-workers (MORRÉ; MORRÉ et al., 3, 4) who ascribed a major role in the biosynthesis of these lipids to the Golgi apparatus (see below; for data suggesting age-dependent shifts of labeling kinetics of phospholipids see CASTELFRANCO et al.).

Results on the sterol contents of such membrane fractions are much less clear although a large number of sterol analyses in plant membrane material including more or less defined subcellular fractions have been published (KEMP and MERCER; FERNE et al.; BRANDT and BENVENISTE; DUPÉRON et al.; KNAPP et al.; STAVY et al.; PAULET et al.; RUESINK; GRUNWALD; CHENG and SHEEN; KEMP et al.; POINCELOT; MEUNIER and MAZLIAK; HODGES et al.; KEENAN et al.; PHILIPP et al.; for data on fungal plasma membranes see HOLTZ et al.; for general reviews see NES). The amounts of sterols found in rER and nuclear membranes were relatively high, compared with the cholesterol contents in the corresponding fractions from animal cells. Reports of exceptionally high amounts of cholesterol in pea plumule nuclei and nuclear membranes (STAVY et al.) are particularly perplexing since in microsomal, mitochondrial and plastidal membranes of all higher plants so far studied, cholesterol has been found only as a minor component relative to sito-

sterol, campesterol and stigmasterol (see the refs. quoted above; for controversial data as to the sterol contents of plastids compare, e.g. BRANDT and BENVENISTE; PAULET et al.; GRUNWALD; KEMP and MERCER; POINCELOT; NES). In this context it should perhaps be called to mind that KEMP and MERCER have reported an enrichment of cholesterol and cholesterol esters in nuclei isolated from corn shoots, compared to the contents in microsomes.

Data on the occurrence of glycolipids and other nonpolar lipids in microsomal membranes are still too sparse. Some authors have presented data on the pattern and biosynthesis of fatty acids from total microsomal membranes and from the isolated sterols and phospholipids (e.g. ABDELKADER and MAZLIAK; ABDELKADER; THIBAUDIN; MOREAU et al.; KEMP and MERCER; MAZLIAK and ABDELKADER), but the crucial question of whether the patterns found are constitutive or whether they might be greatly influenced by physiological factors (as this is well known in many animal cell types) remains open.

Due to the relatively few comparative studies on membrane lipid moieties among various endomembranes and plasma membranes (for data from fractions enriched in plasma membrane see, e.g., BARTHOLOMEW and MACE; HODGES et al.; KEENAN et al.) isolated from the same cell in parallel it cannot yet be concluded that nuclear and ER membranes were characterized by lower sterol contents than Golgi apparatus and plasma membrane as is the case in various animal cells (MORRÉ et al., 1, and below).

To our knowledge only one study (DOBBERSTEIN et al.) has examined the character of the ribosome-membrane interaction in plant rER. The results obtained with high salt treatments, chelating agents, puromycin, and ribonuclease, following the strategy developed by BLOBEL, SABATINI and co-workers (BLOBEL and POTTER; ADELMAN et al.; BLOBEL and SABATINI), suggest that in bean hypocotyls about 20% of the ribosomes are in membrane structure by both electrostatic interaction and the nascent polypeptides. It remains to be clarified whether in plant cells there is also a selectivity or specificity of the synthesis of export and/or membrane-bound proteins on membrane-attached polyribosomes, as this might be suggested by some preliminary studies (see also the refs. contained in ABDELKADER and AUDERSET).

c) Elements from the Endoplasmic Reticulum: Redox Components and Phytochrome

The endoplasmic reticulum in a wide variety of cells contains two electron transport systems independent of the mitochondrial respiratory chain, one involving NADH, a flavoprotein (NADH-cytochrome b_5 reductase or rotenone- and antimycin-insensitive NADH-cytochrome c reductase) and cytochrome b_5, and the other involving NADPH, a specific flavoprotein (NADPH-cytochrome c reductase) and cytochrome P-450. Possibly, other components such as non-haem iron proteins and other flavoproteins are also integrated into these redox chains (SIEKEVITZ; OMURA et al.; LOVERDE and STRITTMATTER; HILDEBRANDT and ESTABROOK; LU and LEVIN). There is evidence that similar electron transport systems are also present in the endoplasmic reticulum of plant cells, although the constituents have not yet been satisfactorily characterized. Antimycin-insensitive NADH cytochrome c reductase activities have been reported in the microsomal fractions from silver beet petioles (MARTIN and MORTON, 1, 2), wheat roots (MARTIN and MORTON, 3), oat roots (LEONARD et al.), onion stems and root tips (PHILIPP et al.), from roots, cotyledons and stems of pea seedlings (RAGLAND and HACKETT, 1; HIRAI and ASAHI; NAKANO and ASAHI), from cauliflower buds (CRANE),

castor bean endosperm (DONALDSON et al.), and soybean cell cultures (MORRÉ and BEEVERS). ELDAN and MAYER, who measured NADH-cytochrome c reductase activity in germinating lettuce seeds, postulated its presence in the mitochondrial compartment; it is likely, however, that this activity was primarily due to a microsomal enzyme. The specific activities reported for the microsomal NADH-cytochrome c reductase were in the same range or slightly less than those given for the microsomal fractions of animal cells (FRANKE, 1; KASPER). PHILIPP et al. reported an enrichment of rotenone-insensitive NADH-cytochrome c reductase activity in the rough endoplasmic reticulum from onion root tips, compared to total microsomes, and also its localization in nuclear membrane fractions, in agreement with the studies on nuclear membranes from animal cells (ZBARSKY et al.; KASHNIG and KASPER; BEREZNEY et al., 1; FRANKE et al., 4).

A b-type cytochrome with peaks in the "reduced versus oxidized" difference spectrum (room temperature) at approximately 559, 527, and 427 nm has been described from the microsomal fractions of beet petioles and wheat roots (MARTIN and MORTON, 4), mung beans (BONNER; KASINSKY et al.), castor bean endosperm (LORD et al., 1, 2), turnips (RUNGIE and WISKICH), and from the microsomal and nuclear membrane fractions of onion root tips (PHILIPP et al.). The α-band of this pigment splits - in the low-temperature difference spectrum - into two symmetrical peaks at about 552 and 558 nm. This property, together with its antimycin-insensitivity, its reduction by NADH and, to a lesser degree, by ascorbate, and its slow virtually absent autoxidizability show that this b-cytochrome is closely related to cytochrome b_5 described from mammalian and yeast microsomes (STRITTMATTER and VELICK; ESTABROOK; YOSHIDA et al.). The nomenclature of these b-cytochromes in plants is very confusing: MARTIN and MORTON (1, 4); also RUNGIE and WISKICH called it cytochrome b_3, a name already given to a soluble pigment from broad bean leaves (HILL and SCARISBRICK; SHICHI et al., 1) which, according to LUNDEGARDH (1, 2), is also localized in chloroplasts and wheat root mitochondria. The definite localization of these cytochromes remains to be established. KASINSKY et al. designated their particle-bound cytochrome from microsomes and mitochondria of mung bean seedlings cytochrome b-555 (BONNER). These authors, however, also described a cytochrome b-555 with similar or identical properties from the soluble fraction of etiolated mung bean seedlings (SHICHI and HACKETT, 1, 2; SHICHI et al., 2). An ascorbate-reduced cytochrome b-559 has been described from plant chloroplasts by BOARDMAN and ANDERSON (1, 2). This is probably identical with LUNDEGARDH's cytochrome b_3, while the cytochrome b_3 of SHICHI et al. (1) might be identical with the mitochondrial cytochrome b_7 from *Arum maculatum* (BENDALL and HILL, 1; BENDALL; the localization of cytochrome b_7 in the mitochondria, however, has been questioned by BONNER et al.) and the mitochondrial cytochromes b-557 and b-559 from various other plant tissues (LANCE and BONNER; STOREY, 1, 2; LAMBOWITZ and BONNER). The complex situation with the plant cytochromes has been covered in several reviews (HARTREE; SMITH and CHANCE; HACKETT; BONNER; BENDALL and HILL, 2; CHANCE et al.; LEMBERG and BARRETT). However, little information about the authentic intracellular location of these enzymes is available.

Apart from the many uncertainties concerning the identity and compartmentalization of the cytochromes in plants, it seems to be fairly well established that a microsomal redox system exists in many plants, involving a NADH-cytochrome c reductase which is insensitive to rotenone and antimycin and at least one b-cytochrome, similar to the NADH-dependent microsomal electron transport chain from animal cells. While the discussion on the functions of this redox system is still open (for example a second electron donor system in certain mixed-function

oxidations might not even exist in mammalian liver: HILDEBRANDT and ESTABROOK; CORREIA and MANNERING; or it might be responsible for the desaturation of fatty acids: SHIMAKATA et al.), virtually nothing is known about its function in plant cells (see the discussion below).

NADPH-cytochrome c reductase activity has been observed in microsomal fractions from potato tuber (HACKETT et al.), silver beets (MARTIN and MORTON, 2), castor bean endosperm (LORD et al., 1, 2), endosperm of *Echinocystis macrocarpa* (MURPHY and WEST), and in microsomal and nuclear membrane fractions from onion stems and root tips (PHILIPP et al.). This last report, which again stresses the similarity of nuclear membrane and membranes of the rough endoplasmic reticulum is especially interesting in view of the continuing debate on the presence of NADPH-cytochrome c reductase activity in these membranes in a variety of animal cells (BEREZNEY et al., 1, 2; FRANKE, 1; JARASCH and FRANKE; KASPER). The specific activity of this enzyme was variable in the range of a few per cent up to more than three times the level of the NADH-cytochrome c reductase in the same fraction. As PHILIPP et al. pointed out, the microsomal NADH-cytochrome c reductase was more inactivated by preparative stresses than the NADPH-cytochrome c reductase. Therefore, the enormous differences in the specific activities of these two enzymes do not necessarily reflect physiological differences. NADPH diaphorase activity (usually measured with dichlorophenol indophenol or ferricyanide as electron acceptors) has been also found in plant microsomes (MARTIN and MORTON, 2; RAGLAND and HACKETT, 2). This activity, however, was higher in mitochrondrial and soluble fractions than in the microsomes. The dependence on NADPH of certain mixed-function oxidase reactions in plant microsomes has been investigated by RUSSELL for cinnamic acid 4-hydroxylase in pea seedlings, by DENNIS and WEST (see also MURPHY and WEST) for the kaurene hydroxylation in *Echinocystis* endosperm, and by COOLBAUGH and MOORE for the same reaction in pea seedlings. In all these examples, NADH was less or not effective as electron donor. The N-demethylation of certain herbicides in cotton hypocotyl microsomes (FREAR et al., 1, 2; TANAKA et al.) is affected by NADH or NADPH equally well, whereas the oleyl coenzyme A hydroxylase from castor bean seedlings has been reported to be specific NADH (GALLIARD and STUMPF). These mixed-function oxidase reactions could be interpreted as indications of the presence of microsomal electron transport systems similar to those described in mammalian liver (e.g. GILLETTE et al.; BOYD and SMELLIE; KING et al.).

The terminal oxidase of most mixed-function oxidations in animal cells is a carbon monoxide binding pigment, cytochrome P-450 (KLINGENBERG; OMURA and SATO; ESTABROOK et al.). It is also present in some microorganisms (e.g. CARDINI and JURTSHUK; LEBEAULT et al.; GUNSALUS and LIPSCOMB). It has been found in higher plants as well. MARKHAM et al. reported its presence in the microsomal fractions of bean and maize cotyledons and pea seedlings but not in microsomes from mung bean or citrus fruits. FREAR et al. (2) and LORD et al. demonstrated it in the microsomal fractions from cotton hypocotyl and castor bean endosperm respectively, and MURPHY and WEST in *Echinocystis* endosperm. These authors showed the inhibition by carbon monoxide of the kaurene hydroxylation and the reversion of this inhibition by light. The photochemical action spectrum was that of a typical cytochrome P-450. Similar results were obtained by MOORE, T.C., et al., and RUSSELL in pea seedlings. Recently, the dependence on cytochrome P-450 of the cinnamic acid 4-hydroxylation has been reported from microsomes of *Sorghum* seedlings (POTTS et al.). MOORE, who was the first author to demonstrate the presence of cytochrome P-450 in higher plants (CHANCE et al.), noted that plants which contained cytochrome b_5 (cytochrome b-555) also contained cytochrome P-450. This review seems to contradict the

report by PHILIPP et al., who could not find cytochrome P-450 in onion root tip microsomes and nuclear membranes, in spite of the presence of cytochrome b_5 as well as of NADH- and NADPH-dependent cytochrome c reductase activities. In this tissue NADPH is as effective as a reducing agent of cytochrome b_5 as NADH. Therefore it is possible that both redox chains feed into the same pathway. In the light of the insufficient knowledge of plant cytochromes it is premature to draw general conclusions concerning the presence and function of the microsomal electron transport systems. One has to keep in mind various alternatives such as i) that either a cytochrome b_5- or a cytochrome P-450-dependent electron transport chain is present in the cells, ii) that both systems interact and function in concert, and iii) that some components of one system might be integrated into the other system while other components are lacking. Furthermore, the components of these electron transport chains may not necessarily be firmly bound to the membranes (as in mammalian liver) but might be partly (as in the mammalian adrenal) or altogether (as in bacteria) extracted into the "soluble" fraction.

As to other pigments that might be present in endomembranes, it has been recently reported by COLEMAN and PRATT that an antibody against phytochrome binds to various membranous structures including profiles of the ER-systems, a finding which seems to correlate with the demonstrations of the binding of phytochrome *in vitro* to membrane surface (MARMÉ et al.).

d) Elements from the Endoplasmic Reticulum: Phosphohydrolases

Apart from such ubiquitous and poorly-characterized activities as the "acid phosphatase" which seems to occur in the nuclear envelope and the endoplasmic reticulum as well as in vacuoles, vesicles, dictyosomes, and along the plasma membrane in various cell types (for biochemical data see, e.g., HIRAI and ASAHI; MATILE, 2; LAU and LYGRE; PHILIPP et al.; RAY et al. LEONARD et al.; see various chapters in the book by JACOBI; for *in situ* localization see, e.g., ROLAND, 2; LIVINGSTON et al.; BRÅTEN; POUX, 1-3; MICALEF; BERJAK; for reviews see DAUWALDER et al., 1, 2) a variety of more specific phosphohydrolase activities have been reported in the ER. The most intensely studied enzyme activity is the ATPase. While basal Mg^{2+}-stimulated ATP-ase activity is clearly localized in both the nuclear envelope and the ER (e.g. PHILIPP et al.; LEONARD et al.; STAVY et al.; WILKINS and THOMPSON; MASLOWSKI and KOMOSZYNSKI) it is by no means a specific enzyme for these structures. It occurs not only in various other plant cell membranes (LEONARD et al.; HANSSON and KYLIN; RAY et al.; LEONARD and HANSSON; HODGES et al.; GILDER and CRONSHAW; FUHRMANN et al.; SCHIBECI et al.; HOLTZ et al.; for fungal plasma membranes cf. also the reviews in the textbook by JACOBI) but also in a variety of nonmembranous structures. In view of the morphological relationship and even - in some situations - direct continuity between the endoplasmic reticulum and the outer plastidal membrane (CROTTY and LEDBETTER) the localization of divalent cation stimulated ATPase on the outer membrane of chloroplasts (SABNIS et al.) should be called to mind. As to the occurrence of Mg^{2+}-ATPase activity that can be stimulated by monovalent cations, there are several reports indicating that this activity is present in fractions enriched in plasma membrane or other smooth-surfaced membrane components such as tonoplast fragments (HODGES et al.; WILKINS and THOMPSON; LAI and THOMPSON, 1, 2; LEONARD et al.; KUIPER; FISCHER and HODGES; HANSSON and KYLIN; for fungal plasma membranes, however, see HOLTZ et al.) but is absent from nuclear membranes and from rER (PHILIPP et al.; see, however, MASLOWSKI and KOMOSZYNSKI). It is still

subject to debate whether such activity is sensitive to ouabain (for controversial findings see HODGES et al.; MASLOWSKI and KOMOSZYNKSI; FISCHER and HODGES; FUHRMANN et al.; SPANSWICK and WILLIAMS; JANÀCEK and RYBOVA; MacROBBIE). A detailed study of the electron microscopic localization of nucleoside triphosphatase activities in phloem parts of tobacco has been presented by GILDER and CRONSHAW.

Among the other nucleotidases several have been localized with great significance in ER-elements and the nuclear envelope using both assays in isolated membrane fractions and localization *in situ*. This holds in particular for inosinediphosphatase (IDPase) (PHILIPP et al.; MARUYAMA; POWELL and BREW; ZAAR and SCHNEPF; ZERBAN and WERZ; GOFF and KLOHS; KLOHS and GOFF; RAY et al.; MOORE and BEEVERS; for review see MORRÉ et al., 1, 2; DAUWALDER et al., 1, 2; for the localization of this enzyme in the ER-system in animal cells see e.g. GOLDFISCHER et al.). This enzyme activity was considered for some time to be a potential marker enzyme for the Golgi apparatus, but recent studies have shown that it is spread over the endomembrane system and that the various reported cases of specific localizations in dictyosomes most probably reflect just the most stable form of activity, i.e. most resistance to fixation or isolation procedures (for examples in plant cells see e.g. RAY et al.; DAUWALDER et al., 1, 2; ZERBAN and WERZ; GOFF and KLOHS). In addition, most studies show that the capability of these endomembranes to hydrolyze IDP is not specific at all, because other nucleoside diphosphates are split in ER-membranes as well, although frequently at somewhat lower rates (for refs. see e.g. DAUWALDER et al., 1, 2; PHILIPP et al.; GOFF and KLOHS; RAY et al.; ZAAR and SCHNEPF) so that some authors prefer to speak of a non-specific nucleoside diphosphatase (NDPase) activity. Such NDPase activity may be related to the glycosyltransferase activities in these membranes (e.g. RAY et al.; POWELL and BREW; DAUWALDER et al., 1, 2).

It is an open question whether true specific nucleoside monophosphatase activities do exist in plant membranes at all. Although hydrolysis of 5'-nucleotides such as 5'-AMP has been occasionally reported in various plant materials, including determinations in ER type membranes (GILDER and CRONSHAW; LAI et al.; STAVY et al.; see however, ZAAR and SCHNEPF; HOLTZ et al.; PHILIPP et al.), the enzymic specificity of the activities measured still remains to be clarified.

A particularly well studied enzyme activity is the thiamine pyrophosphatase (TPPase) which has frequently been localized with great preference and apparent specificity in dictyosomes, sometimes even with a polar distribution (for animal cells see GOLDFISCHER et al.; and, in *Amoeba*, WISE and FLICKINGER; for plant cells see DAUWALDER et al., 1, 2; ZERBAN and WERZ; POUX, 3; MARUYAMA; MORRÉ et al., 1, 3) but again there are some indications that this enzyme might be present, though with lower activity, in other endomembranes as well (ZERBAN and WERZ, 2).

A special controversy has arisen about the question of whether plant cells contain true glucose-6-phosphatase (G-6-Pase) and whether this is a membrane-associated activity. Some authors have reported the localization of G-6-Pase in the endoplasmic reticulum and the nuclear envelope by biochemical (STAVY et al.; LAI et al.) or cytochemical (ROLAND, 2) techniques, respectively. Others have reported G-6-Pase activity in plant cells which, however, was "soluble" and definitely not membrane-associated (cf. e.g. MORRÉ et al., 3). On the contrary, some authors (LAU and LYGRE; PHILIPP et al.) conclude from their experiments that the apparent hydrolysis of G-6-P is due to a non-specific acid phosphatase activity.

e) Elements from the Endoplasmic Reticulum: Glycosyltransferases

The enzyme activities that transfer carbohydrate residues from nucleotides to endogenous or artificial acceptors and contribute *in vivo* to the formation of cell wall and storage polysaccharides as well as to the synthesis of membrane-bound glycolipids and glycoproteins are of special interest to botanists. While several reports have suggested the localization of such activities in the Golgi apparatus (for earlier references see BROWN et al.; NORTHCOTE; RAY et al.; see also last year's review in FRANKE et al., 2; POWELL and BREWER; BRETT and NORTHCOTE) and in the plasma membrane (e.g., BRETT and NORTHCOTE; SHORE and MacLACHLAN; MORRÉ et al., 3; HODGES et al.) only relatively few studies mention the occurrence of glycosyltransferases in elements isolated from the ER, including smooth microsomal fractions that could well contain either dictyosomal or plasmalemma fragments (BOWLES and NORTHCOTE, 1, 2; SHORE and MacLACHLAN; for discussion see also DAUWALDER et al., 2). Certainly, the localization of the various transferase activities and the identification of their products *in situ* will be major problems in plant biochemistry and cytology in the future, especially the elucidation of the "classic" question as to where in the secretory pathway the structural and crystalline cell wall polysaccharides are formed (see last year's review). At the moment, the possibility cannot be excluded that such processes are also carried in the ER-system or are at least initiated there.

References

ABDELKADER, A.B.: (1) C.R. Acad. Sci. (Paris) 275, 51-54 (1972); - (2) C.R. Acad. Sci. (Paris) 277, 1455-1458 (1973). - ABDELKADER, A.B., AUDERSET, G.: C.R. Acad. Sci. (Paris) 274, 1311-1314 (1972). - ABDELKADER, A.B., MAZLIAK, P.: (1) Europ. J. Biochem. 15, 250-262 (1970); - (2) C.R. Acad. Sci. (Paris) 269, 697-700 (1969). - ADELMAN, M.R., BLOBEL, G., SABATINI, D.D.: Nondestructive separation of rat liver rough microsomes into ribosomal and membraneous components, 201-215. In: Methods in Enzymology, eds. S. FLEISCHER, L. PACKER, Vol. 31. New York-San Francisco-London: Academic Press 1974. - AKAZAWA, T., BEEVERS, H.: Biochem. J. 67, 110-114 (1957). - ALDRICH, H.C., GREGG, J.H.: Exp. Cell Res. 81, 407-412 (1973). - ALLEN, C.F., GOOD, P., MOLLENHAUER, H.H., TOTTEN, C.: J. Cell Biol. 48, 542-546 (1971). - ALLEN, J.V., HESS, W.M., WEBER, D.J.: Mycologia 63, 144-156 (1971). - AMAR-COSTESEC, A., BEAUFAY, H., WIBO, M., THINÈS-SEMPOUX, D., FEYTMANS, E., ROBBI, M., BERTHET, J.: (1) J. Cell Biol. 61, 201-212 (1974). - AMAR-COSTESEC, A., WIBO, M., THINÈS-SEMPOUX, D., BEAUFAY, H., BERTHET, J.: (2) J. Cell Biol. 62, 717-745 (1974). - ARNTZEN, C.J., DILLEY, R.A., CRANE, F.L.: J. Cell Biol. 43, 16-31 (1969).

BACHMAN, L., SCHMITT, W.W.: Proc. Nat. Acad. Sci. US 68, 2149-2152 (1971). - BÄCHI, Th., HOWE, C.: (1) Proc. Soc. Exp. Biol. Med. 141, 141-149 (1972); - (2) J. Virol. 12, 1173-1180 (1973). - BÄCHI, Th., SCHNEBLI, H.P.: Exp. Cell Res. 91, 285-293 (1975). - BÄCHI, Th., GERHARD, W., LINDENMANN, J., MÜHLETHALER, K.: (1) J. Virol. 4, 769-776 (1969). - BÄCHI, Th., AGUET, M., HOWE, C.: (2) J. Virol. 11, 1004-1012 (1973). - BANK, H., MAZUR, P.: J. Cell Biol. 57, 729-742 (1973). - BARTHOLOMEW, L., MACE, K.D.: Cytobios 5, 241-247 (1972). - BEAUFAY, H., AMAR-COSTESEC, A., FEYTMANS, E., THINÈS-SEMPOUX, D., WIBO, M., ROBBI, M., BERTHET, J.: (1) J. Cell Biol. 61, 188-200 (1974). - BEAUFAY, H., AMAR-COSTESEC, A., THINÈS-SEMPOUX, D., WIBO, M., ROBBI, M., BERTHET, J.: (2) J. Cell Biol. 61, 213-231 (1974). - BENDALL, D.S.: Biochem. J. 109, 46P-47P (1968). - BENDALL, D.S.,

HILL, R.: (1) New Phytologist 55, 206-212 (1956); - (2) Ann. Rev. Plant Physiol. 19, 167-186 (1968). - BENEDETTI, E.L., DUNIA, I., DIAWARA, A.: Europ. J. Cancer 9, 263-272 (1973). - BEREZNEY, R., FUNK, L.K., CRANE, F.L.: (1) Biochim. Biophys. Acta 223, 61-70 (1970). - BEREZNEY, R., MACAULAY, L.K., CRANE, F.L.: (2) J. Biol. Chem. 247, 5549-5561 (1972). - BERGQUIST, A., EAKIN, E.A., VAN WINKLE, B., WAGNER, R.P.: Proc. Nat. Acad. Sci. US 59, 1136-1143 (1968). - BERGSTRÖM, B.H., HENLEY, C.: J. Ultrastruct. Res. 42, 551-553 (1973). - BERGSTRÖM, B.H., HENLEY, C., COSTELLO, D.P.: Cytobios 7, 51-60 (1973). - BERJAK, P.: Ann. Bot. 36, 73-81 (1972). - BISCHOFF, A., MOOR, H.: (1) Z. Zellforsch. 81, 303-310 (1967); - (2) Z. Zellforsch. 81, 571-580 (1967). - BLOBEL, G., POTTER, V.R.: J. Mol. Biol. 26, 279 (1968); - BLOBEL, G., SABATINI, D.D.: J. Cell Biol. 45, 146 (1970). - BOARDMAN, N.K., ANDERSON, J.M.: (1) Nature 203, 166-167 (1964); - (2) Biochim. Biophys. Acta 143, 187-203 (1967). - BONNER, W.D., Jr.: The Cytochromes of Plant Tissues, 479-500. In: Haematin Enzymes, eds. J.E. FALK, R. LEMBERG, R.K.MORTON, Vol. 2. Oxford-New York: Pergamon Press 1961. - BONNER, W.D., CHRISTENSEN, E.L., BAHR, J.T.: Cyanide and Antimycin-Insensitive Respiration, 113-119. In: Biochemistry and Biophysics of Mitochondrial Membranes, eds. G.F. AZZONE, E. CARAFOLI, A.L. LEHNINGER, E. QUAGLIARIELLO, N., SILIPRANDI. New York-London: Academic Press 1972. - BOWLES, D.J., NORTHCOTE, D.H.: (1) Biochem. J. 130, 1133-1145 (1972); - (2) Biochem. J. 142, 139-144 (1974). - BOYD, G.S., SMELLIE, R.M.S. (eds.): Biological Hydroxylation Mechanisms, pp. 1-250. London-New York: Academic Press 1972. - BRANDT, R.D., BENVENISTE, P.: Biochim. Biophys. Acta 282, 85-92 (1972). - BRANTON, D.: (1) Proc. Nat. Acad. Sci. US 55, 1048-1056 (1966); - (2) Exp. Cell Res. 45, 703-707 (1967); - (3) Ann. Rev. Plant Physiol. 20, 209-238 (1969). - BRANTON, D., DEAMER, D.W.: In: Protoplasmatologia, eds. M. ALFERT, H. BAUER, W. SANDRITTER, P. SITTE, 1-70. Wien-New York: Academic Press 1972. - BRANTON, D., MOOR, H.: J. Ultrastr. Res. 11, 401-411 (1964). - BRANTON, D., SOUTHWORTH, D.: Exp. Cell Res. 47, 618-653 (1967). - BRÅTEN, T.: J. Cell Sci. 17, 647-653 (1975). - BREATHNACH, A.S., STOLINSKI, C., GROSS, M.: J. Cell Sci. 11, 477-489 (1972). - BRETT, C.T., NORTHCOTE, D.H.: Biochem. J. 148, 107-117 (1975). - BRIARTY, L.G.: Planta 113, 373-377 (1973). - BRONCHART, R., DEMOULIN, V.: Planta 94, 229-232 (1970). - BROWN, D.T., BURLINGHAM, B.T.: J. Virol. 12, 386-396 (1973). - BROWN, R.M., HERTH, W., FRANKE, W.W., ROMANOVICZ, D.: The Role of the Golgi Apparatus in the Biosynthesis and Secretion of a Cellulosic Glycoprotein in Pleurochrysis: A Model System for the Synthesis of Structural Polysaccharides, 207-257. In: Biogenesis of Plant Cell Wall Polysaccharides, ed. F. LOEWUS. New York: Academic Press 1973. - BULLIVANT, S.: Phil. Trans. R. Soc. Lond. B 268, 5-14 (1974). - BUTTROSE, M.S.: Planta 96, 13 (1971).

CARDINI, G., JURTSHUK, P.: J. Biol. Chem. 243, 6070-6072 (1968). - CASTELFRANCO, P.A., TANG, W.J., BOLAR, M.L.: Plant Physiol. 48, 795-800 (1971). - CHALCROFT, J.P.: Bullivant, S.: J. Cell Biol. 47, 49-60 (1970). - CHANCE, B., BONNER, W.D., Jr., STOREY, B.T.: Ann. Rev. Plant Physiol. 19, 295-320 (1968). - CHENG, A.L.S., SHEEN, S.J.: Theor. App. Genetics 42, 181-186 (1972). - CHLAPOWSKI, F.J., BONNEVILLE, M.A., STAEHELIN, L.A.: J. Cell Biol. 53, 92-104 (1972). - CLAUDE, P., GOODENOUGH, D.A.: J. Cell Biol. 58, 390-400 (1973). - CLERMONT, H., DOUCE, R.: FEBS Letters 9, 284-286 (1970). - COLE, G.T., ALDRICH, H.C.: J. Cell Biol. 51, 873-874 (1971). - COLEMAN, R.A., PRATT, L.H.: J. Histochem. Cytochem. 22, 1039-1047 (1974). - COOLBAUGH, R.C., MOORE, T.C.: Phytochemistry 10, 2401-2412 (1971). - CORREIA, M.A., MANNERING, G.J.: Mol. Pharmacol. 9, 470-485 (1973). - CRANE, F.L.: Plant Physiol. 32, 619-625 (1957). - CROTTY, W.J., LEDBETTER, M.C.: Science 182, 839-841 (1973). - CUNNINGHAM, W.P., STAEHELIN, L.A., RUBIN, R.W., WILKIN, R., BONNEVILLE, M.: J. Cell Biol. 62, 491-504 (1974).

DALLNER, G.: Isolation of Rough and Smooth Microsomal - General, 191-201. In: Methods in Enzymology, eds. S. FLEISCHER, L.PACKER, Vol. 31. New York - San Francisco-London: Academic Press 1974. - DAUWALDER, M., WHALEY, W.G., KEPHART, J.E.: (1) J. Cell. Sci. 4, 455-497 (1969); - (2) Sub-Cell. Biochem. 1, 225-275 (1972). - DEAMER, D.W.: J. Biol. Chem. 248, 5477-5485 (1973). - DEAMER, D.W., BASKIN, R.J.: J. Cell. Biol. 42, 296-307 (1969). - DE CAMILLI, P., PELUCHETTI, D., MELDOLESI, J.: Nature 248, 245-246 (1974). - DEMPSEY, G.P., BULLIVANT, S., WATKINS, W.B.: Sciences 179, 190-192 (1973). - DENNIS, D.T., WEST, C.A.: J. Biol. Chem. 242, 3293-3300 (1967). - DERMIETZEL, R.: Naturwissenschaften 60, 208 (1973). - DEVOR, K.A., MUDD, I.B.: J. Lipid Res. 12, 403-411 (1971). - DOBBERSTEIN, B., VOLKMANN, D., KLÄMBT, D.: Biochim. Biophys. Acta 374, 187-196 (1974). - DONALDSON, R.P., TOLBERT, N.E., SCHNARRENBERGER, C.: Arch. Biochem. Biophys. 152, 199-215 (1972). - DOUCE, R., LANCE, C.: Physiol. Veg. 10, 181-198 (1972). - DUPÉRON, R., BRILLARD, M., DUPÉRON, P.: C.R. Acad. Sci. (Paris) 274, 2321-2324 (1972).

EDIDIN, M.: Transport at the Cellular Level. In: Symposia of the Society for Experimental Botany, Vol. 28, 1-14. Cambridge: University Press 1974. - EDIDIN, M., FAMBROUGH, D.: J. Cell Biol. 57, 27-53 (1973). - EDIDIN, M., WEISS, A.: Proc. Nat. Acad. Sci. US 69, 2456-2459 (1972). - EL-AASER, A.A., FITZ-SIMONS, J.T.R., HINTON, R.H., NORIS, D.A., REIO, E.: Histochem. J. 5, 199-223 (1973). - ELDAN, M., MAYER, A.M.: Physiol. Plant. 26, 67-72 (1972). - ELGSAETER, A., BRANTON, D.: J. Cell Biol. 63, 1013-1030 (1974). - ESTABROOK, R.W.: Spectrophotometric Studies of Cytochromes Cooled in Liquid Nitrogen, 436-460. In: Haematin Enzymes, eds. J.E. FALK, R. LEMBERG, R.K. MORTON, Vol. 2. Oxford-New York: Pergamon Press 1961. - ESTABROOK, R.W., BARON, J., FRANKLIN, M., MASON, I., WATERMAN, M., PETERSON, J.: Cytochrome P-450-Panacea or Plaque, 197-230. In: The Molecular Basis of Electron Transport, eds. J. SCHULTZ, B.F. CAMERON. New York-London: Academic Press 1972. - EYTAN, G., OHAD, J.: J. Biol. Chem. 247, 112-121 (1972).

FERNE, M., BENVENISTE, P., STOECKEL, M.E.: C.R. Acad. Sci. (Paris) 272, 2385-2388 (1971). - FIIL, A., BRANTON, D.: J. Bacteriol. 98, 1320-1327 (1969). - FINEAN, J.B., FREEMAN, R., LIMBRICK, A.R.: Phil. Trans. R. Soc. Lond. B. 268, 15-21 (1974). - FINERAN, B.A.: (1) J. Microscopy 92, 85-97 (1970); - (2) Cytobiologie 8, 175-193 (1973); - (3) J. Ultrastr. Res. 43, 75-87 (1973). - FISCHER, J.D., HODGES, T.K.: Plant Physiol. 44, 385-395 (1969). - FLEISCHER, S., KERVINA, M.: Subcellular Fractionation of Rat Liver, 6-41. In: Methods in Enzymology, eds. S. FLEISCHER, L., PACKER, Vol. 31. New York-San Francisco-London: Academic Press 1974. - FLOWER, N.E.: (1) J. Cell Sci. 9, 435-442 (1971); - (2) J. Ultrastruct. Res. 37, 259-268 (1971); - (3) J. Cell Sci. 12, 445-452 (1973). - FRANKE, W.W.: (1) Structure, Biochemistry, and Function of the Nucelar Envelope, 71-236, In: Int. Rev. of Cytology, eds. G.H. BOURNE, J.F. DANIELLI, Suppl. 4. New York-San Francisco-London: Academic Press 1974; - (2) Z. Zellforsch. 105, 405-429 (1970); - (3) J. Cell Biol. 31, 619-623 (1966); - (4) Zellkerne und Kernbestandteile, 15-40. In: Biochemische Cytologie der Pflanzenzelle, ed. G. JACOBI. Stuttgart: Thieme 1974. - FRANKE, R.W., SCHEER, U.: (1) J. Ultrastruct. Res. 30, 288-316 (1970); - (2) Structures and Functions Nuclear Envelope, 219-347. In: The Cell Nucleus, ed. H. BUSCH, Vol. 1. New York-London: Academic Press 1974. - FRANKE, W.W., SPRING, H., SCHEER, U., ZERBAN, H.: (1) J. Cell Biol. 66, in press 1975; - FRANKE, W.W., SCHEER, U., HERTH, W.: (2) General and Molecular Cytology, 1-20. In: Progress in Botany, eds. H. ELLENBERG, K. ESSER, H. MERXMÜLLER, E. SCHNEPF, E. ZIEGLER, Vol. 36. Berlin-Heidelberg-New York: Springer 1974. - FRANKE, W.W., BERGER, S., FALK, H., SPRING, H., SCHEER, U., HERTH, W., TRENDELENBURG, M.F., SCHWEIGER, H.-G.: (3)

Protoplasma 82, 249-282 (1974); - FRANKE, W.W., DEUMLING, B., ERMEN, B., JARASCH, E.-D., KLEINIG, H.: (4) J. Cell Biol. 46, 379-395 (1970). - FREAR, D.S., SWANSON, H.R., TANAKA, F.S.: (1) Phytochemistry 8, 2157-2169 (1969); - (2) Herbicide Metabolism in Plants, 225-246. In: Recent Advances in Phytochemistry, eds. V.C. RUNECKLES, T.C. TSO, Vol. 5. New York: Academic Press 1972. - FRIEND, D.S., GILULA, N.B.: J. Cell Biol. 53, 758-776 (1972). - FRIEND, D.S., FAWCETT, D.W.: J. Cell Biol. 63, 641-664 (1974). - FRYE, L.D., EDIDIN, M.: J. Cell Sci. 7, 319-335 (1970). - FUHRMANN, G.F., WEHRLI, E., BOEHM, C.: Biochim. Biophys. Acta 363, 295-310 (1974).

GALL, J.G.: (1) Protoplasmatologia 5, 4-25 (1964); - (2) J. Cell Biol. 32, 391-399 (1967). - GALLIARD, T.: Techniques for Overcoming Problems of the Lipolytic Enzymes and Lipoxygenases in the Preparation of Plant Organelles, 520-529. In: Methods in Enzymology, eds. L. FLEISCHER, L. PACKER, Vol. 31. New York-San Francisco-London: Academic Press 1974. GALLIARD, T., STUMPF, P.K.: J. Biol. Chem. 241, 5806-5812 (1966). - GEMNE, G.: Life Science 7, 1239-1241 (1968). - GIESBRECHT, P., EMEIS, C.C.: Monatsschr. f. Brauerei 20, 181-183 (1967). - GILDER, J., CRONSHAW, J.: J. Cell Biol. 60, 221-235 (1974). - GILLETTE, J.R., CONNEY, A.H., COSMIDES, G.J., ESTABROOK, R.W., FOUTS, J.R., MANNERING, G.J. (eds.): Microsomes and Drug Oxidations, pp. 1-547. New York-London: Academic Press 1969. - GILULA, N.B., SATIR, P.: J. Cell Biol. 53, 494-509 (1972). - GILULA, N.B., BRANTON, D., SATIR, P.: Proc. Nat. Acad. Sci. US 67, 213-220 (1970). - GOFF, C.W., KLOHS, W.D.: J. Histochem. Cytochem. 22, 945-951 (1973). - GOLDFISCHER, S., ESSNER, E., SCHILLER, B.: J. Histochem. Cytochem. 19, 349-360 (1971). - GOODENOUGH, D.A., REVEL, J.P.: J. Cell Biol. 45, 272-290 (1970). - GOODENOUGH, U.W., STAEHELIN, L.A.: J. Cell Biol. 48, 594-619 (1971). - GRAM, T.: Separation of Hepatic Smooth and Rough Microsomes Associated with Drug-Metabolizing Enzymes, 225-237. In: Methods in Enzymology, eds. S. FLEISCHER, L. PACKER, Vol. 31. New York-San Francisco-London: Academic Press 1974. - GRIFFITHS, D.A.: Arch. Mikrobiol. 76, 74-82 (1971). - GRUNWALD, C.: Plant Physiol. 45, 663-666 (1970). - GUNSALUS, I.C., LIPSCOMB, J.D.: Component Dynamics in Oxygen Reduction by Cytochrome P-450-cam, 179-196. In: The Molecular Basis of Electron Transport, eds. J. SCHULTZ, B.F. CAMERON. New York-London: Academic Press 1972. - GURR, M.J., BLADES, J. APPLEBY, R.S.: (1) Eur. J. Biochem. 29, 362-368 (1972). - GURR, M.J., BLADES, J., APPLEBY, R.S., SMITH, C.G., ROBINSON, M.P., NICHOLS, B.W.: (2) Eur. J. Biochem. 43, 281-290 (1974).

HACKETT, D.P.: Ann. Rev. Plant Physiol. 10, 113-146 (1959). - HACKETT, D.P., HAAS, D.W., GRIFFITHS, S.K., NIEDERPRUEM, D.J.: Plant Physiol. 35, 8-19 (1960). - HADLEY, G., JOHNSON, R.P.C., JOHN, D.A.: Planta 100, 191-199 (1971). - HANSSON, G., KYLIN, A.: Z. Pflanzenphysiol. 60, 270-275 (1969). - HARTREE, E.F.: Advances in Enzymology, Vol. 18, 1-64. New York-London: Interscience 1957. - HEREWARD, F.V., NORTHCOTE, D.H.: (1) J. Cell Sci. 10, 555-561 (1972); - (2) J. Cell Sci. 13, 621-635 (1973). - HESS, W.M.: Canad. J. Microbiol. 14, 205-210 (1968). - HESS, W.M., STOCKS, D.L.: Mycologia 61, 560-571 (1969). - HESS, W.M., WEBER, D.J.: (1) Mycologia 64, 1164-1166 (1972); - (2) Protoplasma 77, 15-33 (1973). - HESS, W.M., SASSEN, M.M.A., REMSEN, C.C.: (1) Naturwissenschaften 53, 708-709 (1966). - HESS, M.M., MÜLLER, E., AUE, R.: Naturwissenschaften 54, 521-522 (1967); - HESS, W.M., SASSEN, M.M.A., REMSEN, C.C.: Mycologia 60, 290-303 (1968); - HESS, W.M., BAIR, R.L., NEUSHUL, M.: (4) Stain Techn. 47, 249-255 (1972). - HICKS, R.M., KETTERER, B., WARREN, R.C.: Phil. Trans. R. Soc. Lond. B. 268, 23-38 (1974). - HILDEBRANDT, A., ESTABROOK, R.W.: Arch. Biochem. Biophys. 143, 66-79 (1971). - HILL, R., SCARISBRICK, R.: New Phytologist 50, 98-111 (1951). - HIRAI, M., ASAHI, T.: Plant Cell Physiol.

14, 1019-1029 (1973). - HODGE, A.J., MARTIN, E.M., MORTON, R.K.: J. Biophys. Biochem. Cytol. 3, 61-69 (1957). - HODGES, T.K., LEONARD, R.T., BRACKER, C.E., KEENAN, T.W.: Proc. Nat. Acad. Sci. US 69, 3307-3311 (1972). - HOLT, S.C., LEADBETTER, E.R.: Bact. Rev. 33, 346-378 (1969). - HOLTZ, R.B., STEWART, P.S., PATTON, S., SCHISLER, L.C.: Plant Physiol. 50, 541-546 (1972). - HONDA, S.J.: Fractionation of Green Tissue, 544-552. In: Methods in Enzymology, Vol. 31. Part A: Biomembranes, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press 1974.

ILES, G.H., SEEMAN, P., NAYLOR, D., CINADER, B.: J. Cell Biol. 56, 528-539 (1973).

JACOBI, G.: Biochemische Cytologie der Pflanzenzelle, 1-97. Stuttgart: Thieme 1974. - JAMES, R., BRANTON, D.: Biochim. Biophys. Acta 323, 378-390 (1973). - JANÀCEK, R., RYBOVA, R.: Cytologia 31, 199-202 (1966). - JARASCH, E.-D., FRANKE, W.W.: J. Biol. Chem. 249, 7245-7254 (1974). -JOHNSON, K.D., KENDE H.: Proc. Nat. Acad. Sci. US 68, 2674-2677 (1971). - JOHNSON, R.G., HERMAN, W.S., PREUS, D.M.: J. Ultrastr. Res. 43, 298-312 (1973). - JONES, D.D., JOST, M.: Arch. Mikrobiol. 70, 43-64 (1970). - JONES, D.D., HAUG, A., JOST, M., GRABER, D.R.: Arch. Biochem. Biophys. 135, 296-303 (1969). - JOST, M.: Arch. Mikrobiol. 50, 211-245 (1965). - JOST, M., JONES, D.D.: Can. J. Microbiol. 16, 159-164 (1970). - JOST, M., MATILE, Ph.: Arch. Mikrobiol. 53, 50-58 (1966).

KADENBACH, B.: Biosynthesis of Mitochondrial Cytochromes, 360-361. In: Biochemical Aspects of the Biogenesis of Mitochondria, eds. E.C. SLATER, J.M. TAGER, S. PAPA, E. QUAGLIARIELLO. Bari: Adriatica editrice 1968. - KAGAWA, T., LORD, J.M., BEEVERS, H.: Plant Physiol. 51, 61-65 (1972). - KARNOVSKY, M.J., UNANUE, E.R.: Fed. Proc. 32, 55-59 (1973). - KARTENBECK, J., ZENTGRAF, H., SCHEER, U., FRANKE, W.W.: The Nuclear Envelope in Freeze-Etching. In: Adv. in Anatomy, Embryology and Cell Biology Vol. 45, 1-55. Berlin-Heidelberg-New York: Springer 1971. - KASHNIG, D.M., KASPER, C.B.: J. Biol. Chem. 244, 3786-3792 (1969). - KASINSKY, H.E., SHICHI, H., HACKETT, D.P.: Plant Physiol. 41, 739-748 (1966). - KASPER, C.B.: Chemical and Biochemical Properties of the Nuclear Envelope, 349-384. In: The Cell Nucleus, ed. H. BUSCH, Vol. 1. New York-San Francisco-London: Academic Press 1974. - KATES, M.: Plant Phospholipds and Glycolipids, 225-265. In: Adv. in Lipid Research, eds. R. PAOLETTI, D. KRITCHEVSKY, Vol. 8. New York-San Francisco-London: Academic Press 1970. - KEENAN, T.W., LEONARD, R.T., HODGES, T.K.: Cytobios 7, 103-112 (1973). - KEMP, R.J., MERCER, E.I.: Biochem. J. 110, 119-125 (1968); - KEMP, R.J., GOAD, L.J., MERCER, E.I.: Phytochemistry 6, 1609-1615 (1967). - KIERMAYER, O., STAEHELIN, L.A.: Protoplasma 74, 227-237 (1972). - KING, T.E., MASON, H.S., MORRISON, M. (eds.): Oxidases and Related Redox Systems. Vol. 2, 431-625. Baltimore-Tokyo-London: University Park Press 1973. - KIRK, R.G., TOSTESON, D.C.: J. Membrane Biol. 12, 273-285 (1973). - KLEEMANN, W., McCONNELL, H.M.: Biochim. Biophys. Acta 345, 220-230 (1974). - KLINGENBERG, M.: Arch. Biochem. Biophys. 75, 376-386 (1958). - KLOHS, W.D., GOFF, C.W.: J. Histochem. Cytochem. 21, 417-422 (1973). - KNAPP, F.F., AEXEL, R.T., NICHOLAS, H.J.: Plant Physiol. 44, 442-446 (1969). - KNUTTON, S., LIMBRICK, A.R., ROBERTSON, J.D.: J. Cell Biol. 62, 679-694 (1974). - KOEHLER, J.K.: (1) J. Ultrastruct. Res. 33, 598-614 (1970); - (2) J. Ultrastruct. Res. 39, 520-539 (1971); - (3) J. Ultrastruct. Res. 44, 355-368 (1973). - KOPP, F.: Cytobiologie 6, 287-317 (1972). - KREIBICH, G., SABATINI, D.D.: Procedure for the Selective Release of Content from Microsomal Vesicles without Membrane Disassembly, 215-224. In: Methods in Enzymology, Vol. 31, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press

1974. - KREIBICH, G., DEBEY, P., SABATINI, D.D.: J. Cell Biol. 58, 436-462 (1973). - KREUTZIGER, G.O.: (1) Proc. 26th Electron Microsc. Soc. Amer. 234 (1968); - (2) J. Ultrastruct. Res. 30, 250 (1970). - KUIPER, P.J.C.: Physiol. Plant 26, 200-205 (1972).

LAI, Y.F., THOMPSON, J.E.: (1) Biochim. Biophys. Acta 233, 84-90 (1971); - (2) Plant Physiol. 50, 452-457 (1972). - LAMBOWITZ, A.M., BONNER, W.D., Jr.: J. Biol. Chem. 249, 2428-2440 (1974). - LANCE, C., BONNER, W.D., Jr.: Plant Physiol. 43, 756-766 (1968). - LANDIS, D.M.D., REESE, T.S.: J. Cell Biol. 60, 316-320 (1974). - LATIES, G.G.: Isolation of Mitochondria from Plant Material, 589-599. In: Methods in Enzymology, Vol. 31, Part A, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press 1974. - LAU, W.P., LYGRE, D.G.: Biochim. Biophys. Acta 309, 318-327 (1973). - LEBEAULT, J.-M., LODE, E.T., COON, M.J.: Biochem. Biophys. Res. Comm. 42, 413-419 (1971). - LEMBERG, R., BARRETT, J.: Cytochromes, pp. 100-111. London-New York: Academic Press 1973. - LEONARD, R.T., HANSSON, G.B.: Plant Physiol. 49, 436-440 (1972). - LEONARD, R.T., HANSON, D., HODGES, T.K.: Plant Physiol. 51, 749-754 (1973). - LITTLEFIELD, L., BRACKER, C.E.: Protoplasma 74, 271 (1972). - LIU, T.P.: Tissue Cell 5, 323-331 (1973). - LIVINGSTON, D.C., COMBS, M.M., FRANKS, L.M., MAGGI, V., GAHAN, P.B.: Histochemie 18, 48-60 (1969). - LOENING, U.E.: Biochem. J. 81, 254-260 (1961). - LOOR, F.: Europ. J. Immunol. 3, 112-116 (1973). - LORD, J.M., KAGAWA, T., BEEVERS, H.: (1) Proc. Nat. Acad. Sci. US 69, 2429-2432 (1972); - LORD, J.M., KAGAWA, T., MOORE, T.S., BEEVERS, H.: (2) J. Cell Biol. 57, 659-667 (1973). - LOVERDE, A., STRITTMATTER, P.: J. Biol. Chem. 243, 5777-5787 (1968). - LU, A.Y.H., LEVIN, W.: Biochim. Biophys. Acta 344, 205-240 (1974). - LUNDEGARDH, H.: (1) Biochim. Biophys Acta 57, 352-358 (1962); - (2) Physiol. Plant. 15, 390-398 (1962).

MacROBBI, E.A.C.: J. Gen. Physiol. 45, 861 (1962). - MALHOTRA, S.K., TEWARI, J.P.: Proc. Roy. Soc. 184, 207-216 (1973). - MARCHESI, V.T., TILLACK, T.W., JACKSON, R.L., SEGREST, J.P., SCOTT, R.E.: (1) Proc. Nat. Acad. Sci. US 69, 1445-1449 (1972); - MARCHESI, V.T., JACKSON, R.L., SEGREST, J.P., KAHANE, J.: Fed. Proc. 32, 1833-1837 (1973). - MARKHAM, A., HARTMAN, G.C., PARKE, D.C.: Biochem. J. 130, 90 P (1972). - MARMÉ, D., BOISARD, J., BRIGGS, W.R.: Proc. Nat. Acad. Sci. US 70, 3861 (1973). - MARTIN, E.M., MORTON, R.K.: (1) Nature 176, 113-114 (1955); - (2) Biochem. J. 62, 696-704 (1956); - (3) Biochem. J. 64, 687-693 (1956); - (4) Biochem. J. 65, 404-413 (1957); - (5) Biochem. J. 64, 221-235 (1956). - MARUYAMA, K.: Cytologia 39, 767-776 (1974). - MASCARENHAS, J.P., BERMAN-KURTZ, M., KULIKOWSKI, R.R.: Isolation of Plant Nuclei, 558-564. In: Methods in Enzymology, Vol. 31, Part A, Biomembranes, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press 1974. - MASLOWSKI, P., KOMOSZYNSKI, M.: Phytochemistry 13, 89-92 (1974). - MATILE, Ph.: (1) Planta 79, 181-196 (1968); - (2) Ber. Deut. Botan. Ges. 82, 397-405 (1969). - MATILE, Ph., WIEMKEN, A.: Arch. Mikrobiol. 56, 148-155 (1967). - MATILE, Ph., MOOR, H.: Planta 80, 159-175 (1968). - MATILE, Ph., MOOR, H., MÜHLETHALER, K.: Arch. Mikrobiol. 58, 201-211 (1967). - MAUL, G.G., MAUL, H.M., SCOGNA, J.E., LIEBERMANN, M.W., STEIN, G.S., YEE-LI HSU, B., BORUN, T.W.: J. Cell Biol. 55, 433-447 (1972). - MAYER, F.: J. Ultrastr. Res. 28, 102-111 (1969). - MAZLIAK, P., ABDELKADER, A.B.: Phytochemistry 10, 2879-2890 (1971). - McCARTY, R.E., DOUCE, R., BENSON, A.A.: Biochim. Biophys. Acta 316, 266-270 (1973). - McINTYRE, J.A., GILULA, N.B., KARNOVSKY, M.J.: J. Cell Biol. 60, 192-203 (1974). - McNUTT, N.S., WEINSTEIN, R.S.: (1) J. Cell Biol. 47, 666-668 (1970); - (2) Progr. Biophys. Mol. Biol. 26, 45-102 (1973). - McNUTT, N.S., HERSHBERG, R.A., WEINSTEIN, R.S.: J. Cell Biol. 51, 805-825 (1971). - MEHARD, C.W.: Mitochondrien, 109-126.

In: Biochemische Cytologie der Pflanzenzelle, ed. G. JACOBI. Stuttgart: Thieme 1974. - MEUNIER, D., MAZLIAK, P.: C.R. Acad. Sci. (Paris) 275, 213-216 (1972). - MEYER, H.W., WINKELMANN, H.: Protoplasma 70, 233-246 (1970). - MEYER, H.W., ROTH, J., BOLCK, F.: Protoplasma 313-321 (1972). - MICALEF, H.: C.R. Acad. Sci. (Paris) 275, 2481-2484 (1972). - MILLER, K.R., STAEHELIN, L.A.: Protoplasma 77, 55-78 (1973). - MOLLENHAUER, H.H., TOTTEN, C.: (1) J. Cell Biol. 48, 387-394 (1971); - (2) J. Cell Biol. 48, 395-405 (1971); - (3) J. Cell Biol. 48, 533-541 (1971). - MOOR, H.: Ber. Deut. Bot. Ges. 82, 385-396 (1969). - MOOR, H., MÜHLETHALER, K.: J. Cell Biol. 17, 609-628 (1963). - MOOR, H., RUSKA, C., RUSKA, H.: Z. Zellforsch. 62, 581-601 (1964). - MOORE, C.W.D.: Ph.D. Dissertation University of Cambridge. Cambridge, England 1967. - MOORE, T.C., BARLOW, S.A., COOLBAUGH, R.C.: Phytochemistry 11, 3225-3233 (1972). - MOORE, T.S., BEEVERS, H.: Plant Physiol. 53, 261-265 (1974). - MOORE, T.S., LORD, J.M., KAGAWA, T., BEEVERS, H.: Plant Physiol. 52, 50-53 (1973). - MOREAU, F., DUPONT, J., LANCE, C.: Biochim. Biophys. Acta 345, 294-304 (1974). - MORRÉ, D.J.: Plant Physiol. 45, 791-799 (1970). - MORRÉ, D.J., MOLLENHAUER, H.H., BRACKER, C.E.: (1) Origin and Continuity of Golgi-Apparatus, 82-126. In: Results and Problems in Cell Differentiation, Vol. 2, eds. W. BEERMAN, J. REINERT, H. URSPRUNG. Berlin-Heidelberg-New York: Springer 1971; - MORRÉ, D.J., MERRITT, W.D., LEMBI, C.A.: (2) Protoplasma 73, 43-49 (1971); - MORRÉ, D.J., LEMBI, C.A., VAN DER WOUDE, W.J.: Golgi-Apparat und verwandte Zellbestandteile, 147-172. In: Biochemische Cytologie der Pflanzenzelle, ed. G. JACOBI. Stuttgart: Thieme 1974; - MORRÉ, D.J., NYQUIST, S., RIVERA, E.: (4) Plant Physiol. 45, 800-804 (1970). - MÜHLETHALER, K.: Int. Rev. Cytol. 31, 1-19 (1971). - MURPHY, P.J., WEST, C.A.: Arch. Biochem. Biophys. 133, 395-407 (1969).

NACHBAUR, J., VIGNAIS, P.M.: Biochem. Biophys. Res. Commun. 33, 315-320 (1968). - NAKANO, M., ASAHI, T.: Plant Cell Physiol. (Tokyo) 15, 331-340 (1974). - NANNINGA, N.: J. Cell Biol. 49, 564-570 (1971). - NEČAS, O.: Bact. Rev. 35, 149-170 (1971). - NES, W.R.: Lipid 9, 596-612 (1973). - NEUSHUL, M.: J. Ultrastr. Res. 37, 532-543 (1971). - NICOLSON, G.L.: Int. Rev. Cytol. 39, 89-189 (1974). - NOBEL, P.S.: Rapid Isolation Techniques for Chloroplasts, 600-608. In: Methods in Enzymology, Vol. 31, Part A, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press 1974. - NOIROT-TIMOTHÉE, C., NOIROT, C.: J. Microscopie 17, 169-184 (1973). - NORTHCOTE, D.H., LEWIS, D.R.: J. Cell Sci. 3, 199-209 (1968). - NORTHCOTE, D.H.: Phil. Trans. R. Soc. Lond. B. 268, 119-128 (1974).

OJAKIAN, G.K., SATIR, P.: Proc. Nat. Acad. Sci. US 71, 2052-2056 (1974). - OMURA, T., SATO, R.: J. Biol. Chem. 239, 2370-2378 (1964). - OMURA, T., SATO, R., COOPER, D.J., ROSENTHAL, O., ESTABROOK, R.W.: Fed. Proc. 24, 1181-1189 (1965). - ORCI, L., PERRELET, A.: Science 181, 868-869 (1973). - ORCI, L., PERRELET, A., LIKE, A.A.: J. Cell Biol. 55, 245-249 (1972). - ORWIN, D.F.G., THOMSON, R.W., FLOWER, N.E.: (1) J. Ultrastr. Res. 45, 1-14 (1973); - (2) J. Ultrastr. Res. 45, 15-29 (1973); - (3) J. Ultrastr. Res. 45, 30-40 (1973).

PARK, R.B., PFEIFHOFER, A.O.: J. Cell Sci. 5, 299-311 (1969). - PAULET, A., LECHEVALEUR, D., BAZIER, R., COSTES, C., MONÉGER, R.: C.R. Acad. Sci. (Paris) 277, 565-568 (1973). - PHILIPP, E.-J., FRANKE, W.W., KEENAN, T.W., JARASCH, E.-D.: J. Cell Biol. (1975) in press. - PINTO DA SILVA, P.: (1) Proc. Nat. Acad. Sci. US 70, 1339-1343 (1973); - (2) J. Cell Biol. 53, 777-787 (1972). - PINTO DA SILVA, P., BRANTON, D.: J. Cell Biol. 45, 598-605 (1970). - PINTO DA SILVA, P., DOUGLAS, S.T., BRANTON, D.: (1) Nature 232, 194-196 (1971); - PINTO DA SILVA, P., MOSS, P.S., FUDENBERG, H.H.: (2) Exp. Cell Res. 81, 127-138 (1973); - PINTO DA SILVA, P., MARTINEZ-PALOMO, A.: (1) Nature 249,

170-171 (1974); - (2) Proc. Nat. Acad. Sci. US 72, 572-576 (1975). - PINTO DA SILVA, P., NICOLSON, G.L.: Biochim. Biophys. Acta 363, 311-319 (1974). - PLATTNER, H.: J. Submicrosc. Cytol. 3, 19-32 (1971). - PLATTNER, H., MILLER, F., BACHMANN, L.: (1) J. Cell Sci. 13, 687-719 (1973); - PLATTNER, H., FISCHER, W.M., SCHMITT, W.W., BACHMANN, L.: (2) J. Cell Biol. 53, 116-126 (1972). - POINCELOT, R.P.: Arch. Biochem. Biophys. 159, 134-142 (1973). - PORTER, K.R., KENYON, K., BADENHAUSEN, S.: Protoplasma 63, 262-274 (1967). - POTTS, J.R.M., WEKLYCH, R., CONN, E.E.: J. Biol. Chem. 249, 5019-5026 (1974). - POUX, N.: (1) J. Microsc. 9, 407-434 (1970); - (2) J. Microsc. 14, 183-218 (1972); - (3) Annales Université et A.R.E.S. (Paris) 11, 81-94 (1973). - POWELL, J.T., BREW, K.: Biochem. J. 142, 203-209 (1974). - PRICE, C.A.: Plant Cell Fractionation, 501-519. In: Methods in Enzymology, Vol. 31, eds. S. FLEISCHER, L. PACKER. New York: Academic Press 1974.

RAGLAND, T.E., HACKETT, D.P.: (1) Biochim. Biophys. Acta 54, 577-580 (1961); - (2) Arch. Biochem. Biophys. 108, 479-489 (1964). - RAY, P.M., SHININGER, T.L., RAY, M.M.: Proc. Nat. Acad. Sci. US 64, 605-612 (1969). - REITH, A., OFTEBRO, R.: Exp. Cell Res. 66, 385-395 (1971). - REVEL, J.P., KARNOVSKY, M.J.: J. Cell Biol. 33, C7 (1967). - REVEL, J.P., YEE, A.G., HUDSPETH, A.J.: Proc. Nat. Acad. Sci. US 68, 2924-2927 (1971). - RICHTER, H.: (1) Protoplasma 65, 155-166 (1968); - (2) Protoplasma 66, 63-78 (1968). - ROBINSON, D.G.: J. Cell Sci. 10, 307-314 (1972). - ROBINSON, D.G., PRESTON, R.D.: (1) J. Cell Sci. 9, 581-601 (1971); - (2) J. Exp. Botany 22, 635-643 (1971); - (3) Brit. Phycol. J. 6, 113-128 (1971); - (4) Planta 104, 234-246 (1972). - ROLAND, J.C.: (1) Int. Rev. Cytol. 36, 45-92 (1973); - (2) C.R. Acad. Sci. (Paris) 268, 2052-2055 (1969). - RUESINK, A.W.: Plant Physiol. 47, 192-195 (1971). - RUNGIE, J.M., WISKICH, J.T.: Aust. J. Biol. Sci. 25, 89-102 (1972). - RUSSELL, D.W.: J. Biol. Chem. 246, 3870-3878 (1971).

SABNIS, D.D., GORDON, M., GALSTON, A.W.: Plant Physiol. 45, 25-32 (1970). - SAKAI, A., OETSUKA, K., YOSHIDA, S.: Cryobiol. 4, 165-173 (1968). - SASSEN, M.M.A., REMSEN, C.C., HESS, W.M.: Protoplasma 64, 75-88 (1967). - SATIR, B.: Transport at the Cellular Level, 399-418. Symp. of the Soc. for Exp. Biol., No. 28, eds. M.A. SLEIGH, D.H. JENNINGS. Cambridge: University Press 1974. - SATIR, P., SATIR, B.: Exp. Cell Res. 89, 404-407 (1974). - SATIR, B., SCHOOLEY, C., SATIR, P.: (1) Nature 235, 53-56 (1972); - (2) J. Cell Biol. 56, 153-176 (1973); - (3) Acta Protozool. 11, 291 (1972). - SATTLER, C.A., STAEHELIN, L.A.: J. Cell Biol. 62, 473-490 (1974). - SCHATZ, G., KOVÁČ, L.: Isolation of Promitochondria from Anaerobically Grown Saccharomyces Cerevisiae, 627-632. In: Methods in Enzymology, Vol. 31, eds. S. FLEISCHER, L. PACKER. New York-San Francisco-London: Academic Press 1974. - SCHIBECI, A., RATTRAY, J.B.M., KIDBY, D.K.: Biochim. Biophys. Acta 311, 15-25 (1973). - SCHWELITZ, F.O., EVANS, W.R., MOLLENHAUER, H.H., DILLEY, R.A.: Protoplasma 69, 341-349 (1969). - SCOTT, R.E., FURCHT, L.T., KERSEY, J.H.: Proc. Nat. Acad. Sci. US 73, 3631-3635 (1973). - SHECHTER, E., LETELLIER, L., GULIK-KRZYWICKI, T.: Eur. J. Biochem. 49, 61-76 (1974). - SHEFFIELD, J.B.: (1) J. Virol. 12, 616-624 (1973); - (2) Virology 57, 287-290 (1974). - SHICHI, H., HACKETT, D.P.: (1) J. Biol. Chem. 237, 2955-2958 (1962); - (2) J. Biol. Chem. 237, 2959-2964 (1962); - (3) Nature 193, 776-777 (1962). - SHICHI, H., KASINSKY, H.E., HACKETT, D.P.: (1) J. Biol. Chem. 238, 1162-1166 (1963); - SHICHI, H., HACKETT, D.P., FUNATSU, G.: (2) J. Biol. Chem. 238, 1156-1161 (1963). - SHIMAKATA, T., MIHARA, K., SATO, R.: J. Biochem. 72, 1163-1174 (1972). - SHORE, G., MacLACHLAN, G.A.: J. Cell Biol. 64, 557-571 (1975). - SIEKEVITZ, P.: Ann. Rev. Physiol. 25, 15-40 (1963). - SINGER, S.J., NICOLSON, G.L.: Science 175, 720-731

(1972). - SITTE, P.: (1) Ber. Deut. Botan. Ges. 82, 329-383 (1969); - (2) Fortschr. Botan. 34, 2-6 (1972). - SLEYTER, U., ADAM, H., KLAUSHOFER, H.: Mikroskopie 25, 320-331 (1969). - SMITH, L., CHANCE, B.: Ann. Rev. Plant Physiol. 9, 449-482 (1958). - SOUTHWORTH, D., BRANTON, D.: J. Cell Sci. 9, 193 (1971). - SPANSWICK, R.M., WILLIAMS, E.J.: J. Exp. Bot. 15, 193 (1964). - SPETH, V., WUNDERLICH, F.: (1) Biochim. Biophys. Acta 291, 621-628 (1973); - (2) J. Cell Biol. 47, 772-777 (1970); - (3) Protoplasma 75, 341-344 (1972). - SPETH, V., WALLACH, D.F.H., WEIDEKAMM, E., KNÜFERMANN, H.: Biochim. Biophys. Acta 255, 386-394 (1972). - SPRING, H., TRENDELENBURG, M.F., SCHEER, U., FRANKE, W.W., HERTH, W.: Cytobiologie 10, 1-65 (1974). - SPYCHER, M.A.: Z. Zellforsch. 111, 64-74 (1970). - STAEHELIN, L.A.: J. Ultrastruct. Res. 22, 326-347 (1968). - STAEHELIN, L.A., KIERMAYER, O.: J. Cell Sci. 7, 787-792 (1970). - STAEHELIN, L.A., MUKHERJEE, T.M., WILLIAMS, A.W.: (1) Protoplasma 67, 165-184 (1969); - STAEHELIN, L.A., CHLAPOWSKI, F.J., BONNEVILLE, M.A.: (2) J. Cell Biol. 53, 73-91 (1972). - STAVY, R., BEN-SHAUL, Y., GALUN, E.: Biochim. Biophys. Acta 323, 167-177 (1973). - STEARNER, S.P., SANDERSON, M.H.: Z. Zellforsch. 114, 301-308 (1971). - STECK, T.L.: J. Cell Biol. 62, 1-19 (1974). - STEIDLE, C., HUHN, D.: Blut 20, 90-104 (1970). - STOECKENIUS, W., KUNAU, W.H.: J. Cell Biol. 38, 337-357 (1968). - STOREY, B.T.: (1) Plant Physiol. 44, 413-421 (1969); - (2) Plant Physiol. 45, 447-454 (1970). - STREIBLOVÁ, E.: J. Bacteriol. 95, 700-707 (1968). - STRITTMATTER, P., VELICK, S.F.: J. Biol. Chem. 221, 277-286 (1956).

TANAKA, F.S., SWANSON, H.R., FREAR, D.S.: Phytochemistry 11, 2701-2708 (1972). - TANI, E., NISHIURA, M., HIGASHI, N.: Acta Neuropath. 26, 127-138 (1973). - TAKEO, K.: (1) Arch. Microbiol. 99, 91-98 (1974); - (2) Arch. Microbiol. 99, 99-107 (1974). - TAKEO, K., NISHIURA, M.: Arch. Microbiol. 98, 175-185 (1974). - TAKEO, K., UEHIRA, K., NISHIURA, M.: J. Elect. Micros. 21, 230 (1972). - TATA, J.R.: Preparation and Properties of Microsomal Fractions from Animal Cells, 83-107. In: Subcellular Components, eds. G.D. BIRNIE, S.M. FOX. London: Butterworth 1969. - THIBAUDIN, A.: C.R. Acad. Sci. (Paris) 268, 1399-1402 (1969). - THRONLEY, M.J., GLAUERT, A.M., SLEYTR, U.B.: Phil. Trans. R. Soc. Lond. B. 248, 147-153 (1974). - TILLACK, T.W.: J. Biol. Chem. 249, 624 (1974). - TILLACK, T.W., KINSKY, S.C.: Biochim. Biophys. Acta 323, 43-54 (1973). - TILLACK, T.W., MARCHESI, V.T.: J. Cell Biol. 45, 649-653 (1970). - TILLACK, T.W., SCOTT, R.E., MARCHESI, V.T.: Exp. Med. 135, 1209-1227 (1972). - TOURTELOTTE, M.E., BRANTON, D., KEITH, A.: Proc. Nat. Acad. Sci. US 66, 909-916 (1970). - TOURTELOTTE, M.E., ZUPNIK, J.S.: Science 179, 84-87 (1973). - TRENDELENBURG, M.F.: Chromosoma 48, 119-135 (1974).

VANDOR, S.L., RICHARDSON, K.E.: Can. J. Biochem. 46, 1309-1315 (1968). - VAN ITERSON, W.: The Bacterial Surface, 149-173. In: Handbook of Molecular Cytology, ed. A. LIMA-DE-FARIA. Amsterdam-London: North-Holland Publ. Co. 1969. - VERGARA, J., LONGLEY, W., ROBERTSON, J.D.: J. Mol. Biol. 46, 593-596 (1969). - VERKLEIJ, A.J., VERVERGAERT, P.H.J., VAN DEENEN, L.L.M., ELBERS, E.: (1) Biochim. Biophys. Acta 288, 326-332 (1972); - VERKLEIJ, A.J., DE KRUIFF, B., GERRITSEN, W.F., DEMEL, R.A., VAN DEENEN, L.L., VERVERGAERT, P.H.: (2) Biochim. Biophys. Acta 291, 577-581 (1973). - VIAN, B.: C.R. Acad. Sci. (Paris) 275, 2471-2474 (1972).

WAALAND, J.R., BRANTON, D.: Science 163, 1339-1341 (1969). - WERZ, G.: Int. Rev. Cytol. 38, 319-368 (1974). - WERZ, G., KELLNER, G.: Protoplasma 69, 351-364 (1970). - WIBO, M., AMAR-COSTESEC, A., BERTHET, J., BEAUFAY, H.: J. Cell Biol. 51, 52-71 (1971). - WILKINS, J.A., THOMPSON, J.E.: Physiol. Plant. 29, 181-185 (1973). - WILLISON, J.H.M., COCKING, E.C.: Protoplasma 84, 147-159 (1975). - WIRTZ, K.W.A.: Biochim. Bio-

phys. Acta 344, 95-117 (1974). - WIRTZ, K.W.A., ZILVERSMIT, D.B.: (1) Biochim. Biophys. Acta 193, 105 (1969); - (2) J. Biol. Chem. 243, 3596 (1968). - WISE, G.E., FLICKINGER, C.J.: J. Cell Biol. 46, 620-626 (1970). - WUNDERLICH, F., SPETH, V.: J. Ultrastruct. Res. 41, 258-269 (1972). - WUNDERLICH, F., SPETH, V., BATZ, W., KLEINIG, H.: (1) Biochim. Biophys. Acta 298, 39-49 (1973); - WUNDERLICH, F., HOELZL-WALLACH, D.F., SPETH, V., FISCHER, M.: (2) Biochim. Biophys. Acta 373, 34-43 (1974); - WUNDERLICH, F., MÜLLER, R., SPETH, (3) Science 182, 1136-1138 (1973).

YAHARA, I., EDELMAN, G.M.: Exp. Cell Res. 91, 125-142 (1975). - YATSU, L.Y., JACKS, T.J., HENSARLING, T.P.: Plant Physiol. 48, 675 (1971). - YATSU, L.Y., JACKS, T.J.: Plant Physiol. 49, 937 (1972). - YOSHIDA, Y., KUMAOKA, H., SATO, R.: J. Biochem. 75, 1211-1219 (1974).

ZAAR, K., SCHNEPF, E.: Planta 88, 224-232 (1969). - ZBARSKY, I.B., PEREVOSHCHIKOVA, K.A., DELEKTORSKAYA, L.N., DELEKTORSKY, V.V.: Nature 221, 257-259 (1969). - ZERBAN, H., WERZ, G. (1) Exp. Cell Res. 1975, in press; - (2) Cytobiol. 1975, in press. - ZERBAN, H., WEHNER, M., WERZ, G.: Planta 114, 239-250 (1973).

Dr. WERNER W. FRANKE
Dr. ERNST-DIETER JARASCH
Dr. ULRICH SCHEER
Dr. HEIDE ZERBAN
Division of Membrane Biology and Biochemistry
Institute of Experimental Pathology
German Cancer Research Center
D-6900 Heidelberg
Im Neuenheimer Feld 280

Dr. WERNER HERTH
Lehrstuhl für Zellenlehre
Universität Heidelberg
D-6900 Heidelberg
Im Neuenheimer Feld 230