A. Morphology

I. Cytology

a) General and Molecular Cytology

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The present article had originally been conceived as a review on endomembranes, the plasma membrane, and the major product of membrane-bound activities, the cell wall material. However, limitations of space and the cascading number of pertinent literature articles made it necessary to confine this to one group of membranes and one type of cell wall components. Therefore, we shall begin our survey on the biochemical and cytological aspects of membranes by a review of the class of the pore complex bearing endomembranes, i.e. the nuclear envelope and the annulate lamellae (AL). Next year the membranes of the endoplasmic reticulum and the dictyosomes will be dealt with in conjunction with a discussion of the various intracellular vesicles, the tonoplast and the plasmalemma.

I. Pore Complex Containing Endomembranes

1. The Nuclear Envelope

This structure has only been dealt with briefly in the 1972 issue of this series (SITTE). Therefore, a more comprehensive summary on the progress in research on this structure is presented here. Several reviews have recently appeared covering both structural and biochemical aspects of the nuclear envelope (Table 1). The rapid progress during the past seven years has been promoted primarily by the development of methods for isolating nuclear membrane material from various cell types in sufficient yield and purity. Table 2 lists the hitherto published attempts to prepare nuclear membrane fractions, among them a few studies in which plant material was used (FRANKE, 1; YOO and BAYLEY; STAVY et al.). The particular problem with plant material in general is, apart from the higher resistance to cell breakage due to the presence of the cell wall, the limited quantities of nuclear membrane material obtained and, in recovery and contamination studies, the lack of knowledge as to the composition of the other membrane fractions.

Pore complexes are distinct, highly symmetrical structures which define nuclear envelopes and also annulate lamellae, be they cytoplasmic annulate lamellae (CAL) or intranuclear annulate lamellae (INAL). Although pore-like fenestrations with similar diameters can occur widely in other cisternae as, for example, in ER and dictyosomes, and these pores frequently reveal certain associated substructural details which are similar to those described for true pore complexes (instances for plant cells are given by FRANKE and SCHEER, 1; and COLE and WYNNE; for further references see FRANKE et al., 1; ORCI et al., FRANKE and SCHEER, 2), there are distinct structural features such as the annulus and the precise and sharp Gaussian distribution of pore diameters which make a true pore complex easily distinguishable for the cytologist.

Table 1. Review articles on the nuclear envelope

Reference	Special emphasis on
BERNHARD, 1958	structure
WISCHNITZER, 1960 (1)	structure
BRIEGER, 1963	structure
CLAUDE, 1964	structure
DAVID, 1964	structure and cytopathology
FELDHERR and HARDING, 1964	nucleocytoplasmic exchange
GALL, 1964 (1)	structure
GOLDSTEIN, 1964	nucleocytoplasmic exchange
LOEWENSTEIN, 1964	permeability
GOURANTON, 1969	structure
STEVENS and ANDRÉ, 1969	structure and functions
FRANKE, 1970 (3)	structure
FRANKE and SCHEER, 1970 (3, 4)	structure and functions
BLACKBURN, 1971	structure, functions and cytopathology
KARTENBECK, ZENTGRAF, SCHEER, and FRANKE, 1971	structure
ROBERTS and NORTHCOTE, 1971 (1)	structure and functions
FELDHERR, 1972	structure and functions
ZBARSKY, 1972	preparation and biochemistry
KAY and JOHNSTON, 1973	biochemistry and functions
KESSEL, 1973 (1)	structure, biochemistry and functions
BEREZNEY, 1974	preparation and biochemistry
FRANKE, 1974 (2)	preparation, biochemistry and functions
FRANKE, 1974 (5)	structure, biochemistry and functions
FRANKE, 1974 (6)	structure and biochemistry
FRANKE and SCHEER, 1974 (6)	nucleocytoplasmic exchange
FRANKE and SCHEER, 1974 (2)	structure, biochemistry and functions
KASPER, 1974	biochemistry

a) Membrane Ultrastructure and Biochemistry of the Nuclear Envelope

The nuclear envelope is, in most cell types, in luminal continuity with the endoplasmic reticulum (ER) system, although there are some cell systems known in which this connection is not only lacking, due to the absence of ER elements such as in mature erythrocytes and sperm cells (for literature see reviews listed in Table 1), but is specifically excluded by special membrane arrangements in the perinuclear zone (most prominent examples for plants being perhaps the primary nucleus of *Acetabularia* and related genera and the tetraspore mother cells of some red algae such as *Corallina*; e.g. BOLOUKHÈRE; ZERBAN et al., WOODCOCK and MILLER; FRANKE et al., 2; PEEL et al.). In general, the membrane ultrastructure and the biochemical composition and activity of nuclear membranes is so closely related to those of the endoplasmic reticulum membranes that it seems more reasonable to discuss this in connection with the general review on the endomembrane-plasma membrane system in the forthcoming issue (for reviews which especially focus on the biochemical relationship see, e.g. MORRÉ et al.; KASPER; FRANKE, 2).

b) Ultrastructure of the Pore Complex

There has been almost one decade of controversy about the substructural architecture of the pore complex (reviews in Table 1). However,

Table 2. Procedures reported for isolating nuclear membranes

Reference	Material	Method of nuclear disruption	Purification by	Type of study
CALLAN and TOMLIN (195o)	Giant nuclei of amphibian and echinoderm oocytes	Manual	-	Structural
GALL (1954, 1956, 1959, 1967; 2-5)	"	"	-	"
MERRIAM (1961, 1962; 1-2)	"	"	-	"
FRANKE and SCHEER (197o, 3)	"	"	-	"
SCHEER (1972; 1)	"	"	-	Structural and Biochemical
FABERGÉ (1973)	"	"	-	Structural
FRANKE et al. (1974; 2)	Primary nucleus of *Acetabularia*	"	-	"
FRANKE (1966; 1)	Onion roots and leaves	Hypotonic shock and/or sonication	Differential and sucrose gradient centrifugation	"
FRANKE (1967; 4)	Mouse liver, macro-nuclei of *Tetrahymena pyriformis*	Sonication	Differential and sucrose gradient centrifugation	"
BORNENS (1968; 1)	Rat liver	Glass-Teflon homogenization and incubation in citric acid medium (pH 3.7)	Differential centrifugation	Structural and Biochemical
FRANKE and KARTENBECK (1969)	Rat brain	Hypotonic shock and sonication	Differential and sucrose gradient centrifugation	Structural
KASHNIG and KASPER (1969)	Rat liver	Sonication plus citrate treatment (1o% w/v)	Sucrose gradient centrifugation	Structural and Biochemical

Table 2 (continued)

Reference	Material	Method of nuclear disruption	Purification by	Type of study
ZBARSKY et al. (1969) ZBARSKY (1972)	Rat liver, Ascites tumors	Hypotonic shock or sonication	Differential and sucrose gradient centrifugation	Biochemical
BEREZNEY et al. (197o;2)	Bovine liver	None	DNase and high-salt ($MgCl_2$) treatment	Structural and Biochemical
COMES and FRANKE (197o)	HeLa cells	Hypotonic shock and sonication	Differential and sucrose gradient	Structural
FRANKE et al. (197o; 3)	Rat liver, pig liver	Sonication	High salt (KCl) treatment, differential and sucrose gradient centrifugation	Structural and Biochemical
HARRIS and AGUTTER (197o)	Rat liver	Resuspension and stirring	None	Structural
KAY et al. (1972)	Rat liver	None	DNase, low-salt treatment in alkaline buffer (pH 8.5), differential and sucrose gradient centrifugation	Structural and Biochemical
KARTENBECK et al. (1971, 1973; 1-2), FRANKE et al. (1973; 5)	Rat liver	Hypotonic shock plus sonication or glass-Teflon homogenization	Differential and/or sucrose gradient centrifugation, without previous high salt (KCl) treatment	Structural and Biochemical
ZENTGRAF et al. (1971)	Hen erythrocytes	Sonication	High-salt treatment (with or without DNase), differential and sucrose gradient centrifugation	Structural and Biochemical

Table 2 (continued)

Reference	Material	Method of nuclear disruption	Purification by	Type of study
AGUTTER (1972)	Rat liver	Sonication	Low-salt treatment, sorbitol gradient centrifugation	Biochemical
BEREZNEY et al. (1972;1) KEENAN et al. (1972)	Beef liver	See BEREZNEY et al. (197o; 2)	DNase and high-salt ($MgCl_2$) treatment, differential and high-salt sucrose gradient centrifugation	Structural and Biochemical
MOORE and WILSON (1972)	Rat ventral prostate	Freeze-thawing plus shearing through canules	High-salt (CsCl) treatment combined with CsCl gradient centrifugation-flotation	Biochemical
JARASCH et al. (1973)	Rat and calf thymus	Sonication	High-salt (NaCl or KCl) treatment, differential and sucrose gradient centrifugation	Structural and Biochemical
HARRIS and BROWN (1971)	Hen and turkey erythrocytes	Sonication	Differential centrifugation	Structural
FAKAN et al. (1972)	Cultured mouse cells	Dounce homogenizer	High-salt (NaCl) treatment, centrifugation in CsCl or in sucrose gradients	Biochemical
MONNERON et al. (1972)	Rat liver	None	High-salt ($MgCl_2$) treatment, flotation in sucrose gradient	Structural and Biochemical
PRICE et al. (1972)	Rat liver and hepatomas	None	Low-salt treatment and sucrose gradient centrifugation	Structural

Table 2 (continued)

Reference	Material	Method of nuclear disruption	Purification by	Type of study
HARLOW et al. (1972)	Hen erythrocytes and late erythroblasts	Saponin-treated nuclei, Dounce homogenizer	Low-salt treatment, sucrose gradient centrifugation	Structural and Biochemical
MATSUURA and UEDA (1972)	Calf thymus	Stirring	DNase and high-salt (NaCl) treatment, sucrose gradient centrifugation	"
STAVY et al. (1973)	Pea plumules	Sonication	Low-salt treatment, sorbitol gradient centrifugation	"
BORNENS (1973; 2)	Rat liver	Swelling in hypotonic solutions, glass-Teflon homogenization	Addition of heparin, sucrose gradient centrifugation	"
NOZAWA et al. (1973)	Macronuclei of *Tetrahymena pyriformis*	None	High-salt (NaCl) treatment, differential centrifugation and flotation steps	"

recently a majority of authors have come to farreaching agreement as to the general principles of the pore complex construction, and a variety of contributions have been made by studies of plant cells (e.g.: FRANKE, 1, 3; ROBERTS and NORTHCOTE, 1, 2; LaCOUR and WELLS; HANZELY and OLAH; BAJER and MOLÈ-BAJER; ENGELHARDT and PUSA). According to the models of these authors (e.g. WATSON; MERRIAM, 1; GALL, 1; FRANKE, 2, 3; FRANKE and SCHEER, 2, 3; ROBERTS and NORTHCOTE, 1, 2; LaCOUR and WELLS; WUNDERLICH and SPETH; HANZELY and OLAH), the pores are constituted by fusions of inner and outer nuclear membrane which leave fenestrations of a sharply distributed internal diameter with mean values between 6o and 8o nm, the specific diameter being characteristic of both the cell type studied and the preparation method employed (see e.g. GALL, 1, 2; MERRIAM, 1, 2; FRANKE, 2, 3; FRANKE and SCHEER, 2, 3; SPETH and WUNDERLICH; KARTENBECK et al., 1). There have been discussions as to whether the pore perimeter is circular (e.g. FRANKE, 1-4; FRANKE and SCHEER, 3; ROBERTS and NORTHCOTE, 1, 2) or polygonal (e.g. GALL, 2; KESSEL, 1; ABELSON and SMITH; MAUL, 1, 2). Associated with the membrane surfaces in this pore region are the typical granular and fibrillar stuctures which define the pore complex (WATSON). On either margin of the pore lie eight granular components 1o-2o nm in diameter, which are symmetrically spaced and represent the structured components within the ring of the "annulus" (CALLAN and TOMLIN; AFZELIUS, 1; WATSON; MERRIAM, 1; GALL, 1, 2; BAJER and MOLÈ-BAJER; DANIELS et al.; FRANKE, 1-4; FRANKE and SCHEER, 2, 3; ROBERTS and NORTHCOTE, 1, 2; LaCOUR and WELLS; FABERGÉ). These annular granules appear either compact or as relatively loose fibril coils, the specific aspect possibly depending on both the preparation method and the cell type studied (FRANKE, 3). Eight dense particles lie within the pore and are tightly attached to the pore wall. These particles appear as distinct globules (see ROBERTS and NORTHCOTE, 1) or as centripetally projecting cones (FRANKE, 3; FRANKE and SCHEER, 3). They are sometimes fused and constitute a whole massive pore plug in the equatorial plane. These peripheral granules or projecting tips are also arranged in an eightfold radial symmetry which corresponds to that of the granules in the inner and outer annulus. In a great many preparations these eight centripetally protruding structures appear to taper into fibrils indicating a spokelike pattern (WATSON; MERRIAM, 1; VIVIER; YOO and BAYLEY; DANIELS et al.; FRANKE, 2, 3). In the pore interior some other fibril arrangements such as an inner ring and a variety of pore-traversing filaments have been noted (e.g. YOO and BAYLEY; WUNDERLICH and FRANKE; KESSEL, 1; FRANKE, 2, 3; FRANKE and SCHEER, 2, 3). The very center of the pore is often, though not always, occupied by a specific electron-opaque granule or rod, the "central granule" (POLLISTER et al.; for reviews see GALL, 1; FRANKE, 2, 3; FELDHERR; KESSEL, 1; FRANKE and SCHEER, 2). Fibrillar structures which terminate at the annular or central granules are usually more conspicuous on the nuclear side (see e.g. FRANKE, 2, 3; FRANKE and SCHEER, 2-4; SCHEER, 1). This architecture of the pore complex is universal to all eukaryotes (WATSON; FRANKE, 3). Minor modifications such as a predominance of the fibrillar aspect, a lack or reduction of annular and internal pore complex substructures and intracisternal electron dense appendages at the pore wall have been noted in special cell types (for references see, e.g. FAWCETT; PICHERAL; FRANKE, 3; KARTENBECK et al., 1; WUNDERLICH and SPETH; FRANKE and SCHEER, 2).

All the pore complex components described are rather firmly attached to the membrane proper and remain in their fixed positions even during nuclear envelope isolation procedures (FRANKE, 1-4; MENTRÉ; FRANKE and SCHEER, 1, 2; SCHEER, 1; PRICE et al.; FABERGÉ). Some of them are removed, however, by washing with high or very low salt concentrations,

especially in the absence of divalent cations (MENTRÉ; FRANKE et al., 3; AGUTTER).

Analysis of pore patterns has shown that in most nuclei, even in those which at first view appear to have a non-ordered pore distribution, the pores are arranged in a non-random pattern, probably a consequence of an existing minimal interpore distance (e.g. MAUL et al.; MARKOVICS et al.). Furthermore, highly ordered pore arrays have been observed in a variety of cell types, diverse plant cells included, namely pore rows as well as hexagonal and square packings (DRAWERT and MIX; NORTHCOTE and LEWIS; WUNDERLICH and FRANKE; FLICKINGER; WECKE and GIESBRECHT; FOLLIOT and PICHERAL; KARTENBECK et al., 1; NEUSHUL and WALKER; ROBERTS and NORTHCOTE, 1, 2; THAIR and WARDROP; LOTT et al.; LaFOUNTAIN and LaFOUNTAIN; TEIGLER and BAERWALD; LIU; for specific references see also KESSEL, 1; and FRANKE and SCHEER, 2). An ordered pore distribution has also been noted in the marine dinoflagellate *Noctiluca*, in the nuclei of which pore complexes are confined to special indentations of the nuclear envelope, the "ampullae" (e.g. AFZELIUS, 2; SOYER).

Pore complexes of the same fine structural architecture as in the nuclear envelopes of plant and animal nuclei (FRANKE, 3) occur in CAL and INAL (for details see the following section on AL and KESSEL, 2; WISCHNITZER, 2; SCHEER and FRANKE, 1; MAUL, 2).

c) Quantitative Aspects

The frequency of pores on the nuclear surface can, in some nuclei, vary in different regions and the average nuclear pore frequency, i.e. the number of pores per unit surface area, can differ among different nuclear types. In some nuclei, pore complexes have been said to be totally lacking (for special discussion and difficulties in making such a negative statement see FRANKE and SCHEER, 2). Nuclei with a rather low RNA synthetic activity usually contain fewer pores per unit surface, as well as in absolute figures, compared to very active nuclei where pore frequencies of up to 60-75 pores per square micron have been observed, for example in the giant nuclei of amphibian oocytes and of *Acetabularia* (e.g. FRANKE and SCHEER, 3, 4; SCHEER, 1, 2; ZERBAN et al.; FRANKE et al., 2; for methodological problems in pore frequency determinations see, e.g. FRANKE, 3; SPETH and WUNDERLICH; KARTENBECK et al., 1; SCHEER, 2). However, despite clear demonstrations of dramatically decreased pore numbers in some cell differentiation processes characterized by nuclear inactivation such as erythropoiesis and spermiogenesis and the pore number increase in some nuclear activation processes a general, simple and strict correlation of the relative frequency of both pore complexes and central granules with nuclear activity does not seem to exist (for discussions of the controversial data with both plant and animal cell systems see, e.g. MERRIAM, 2; FRANKE and SCHEER, 3, 4; LaFOUNTAIN and LaFOUNTAIN; MAUL et al., 2; SCHEER, 1, 2; ECKERT et al.; WUNDERLICH; WUNDERLICH and SPETH; SPREY and HASCHE, 1-3; JORDAN and CHAPMAN; LOTT et al.).

Rates and mechanisms of pore complex formations are unknown. At least in some cells pore formation can be rather rapid; for example a net nuclear pore formation rate of about 500 pores per min has been calculated in *Xenopus laevis* oocytes nuclei during midlampbrush phase (SCHEER, 2). Increases in pore numbers are apparently lower in most other cell systems, the giant nuclear growth of *Acetabularia* included (e.g. MAUL et al., 1, 2; SCOTT et al.; FRANKE et al., 2). It has been proposed that pore complex formation results from localized membrane disinte-

gration and refusion processes, during which a part of the former membrane material is excluded and might reconstitute in the form of the fibrillo-granular structures associated with the pores (e.g. FRANKE et al., 1; FRANKE, 2).

d) Interaction of Nuclear Membranes with DNA and RNA Containing Structures

The concept of a membrane attachment of chromosomal DNA and of a functional role of this relationship in replication (and also transcription processes) in prokaryotic cells is now about one decade old (JACOB et al.). Similar concepts have, during the past five years, also been pursued and experimentally probed for eukaryotic cells. In addition, various studies have correlated analysis of, for instance, nuclear membrane-bound RNA with possible functions in nucleocytoplasmic exchange. However, the reported results and interpretations were very controversial and contradictory. Therefore a critical evaluation requires a special and detailed chapter on nucleic acid-membrane interactions and nucleocytoplasmic translocations which will be given in next year's review.

II. Annulate Lamellae (AL)

Annulate lamellae are known as characteristic structures of various animal cells, especially of germ line cells including oocytes and spermatocytes, and of embryonic cells and other rapidly growing systems such as cancer cells (for references see KESSEL, 2; WISCHNITZER, 2; FRANKE and SCHEER, 5). The first documentation of the existence of AL in a plant cell was published by GIANORDOLI. He described single cisternae of typical AL in the cytoplasm of the central cell of the developing archegonium of *Sciadopitys verticillata* (Taxodiaceae), a cell system which is chracterized by an extensive volume increase (by ca. 3ooo x) before the egg cell is produced by the final mitotic division. (This is an interesting similarity to the development of animal oocytes which are AL-containing cells par excellence.) KESSEL (2) mentioned, in an addendum to his review on AL, unpublished observations of SKVARLA of "extensive profiles of annulate lamellae in pollen of *Canna* during pollen wall formation". SCHEER and FRANKE (2) confirmed this and further demonstrated the occurrence of extensive stacks of AL in *Canna* pollen mother cells with a high package density of pore complexes. The question as to whether AL in plant cells were possibly confined to male and female gametogenesis has also been answered through studies of plant cell suspension cultures: In *Haplopappus gracilis* cells, AL-cisternae were found not only in the cytoplasm but also in the nucleoplasm (intranuclear AL; FRANKE et al., 4). The cytoplasmic AL in *Haplopappus* consisted of single cisternae of rough ER with few pore complexes. Recently, FOWKE et al. (1) described cytoplasmic AL with similar ultrastructural features in multinucleate protoplasts of *Ammi visnaga* which had formed by spontaneous fusion during protoplast culture. Intranuclear AL have also been reported in postmeiotic tetraspore mother cells of the red alga, *Corallina officinalis* (PEEL et al.).

The plant AL have properties identical of animal AL:
1. They can occur in the cytoplasm as well as in the nucleoplasm.
2. They occur either in single cisternae or in form of stacks of regularly spaced cisternae (stacking periodicity ca. 1oo nm; SCHEER and FRANKE, 2).

3. Dimensions as well as substructural details of the AL pore complexes are identical to those of the nuclear envelope (see previous paragraph).
4. The pore frequency in AL ist mostly higher than in the nuclear envelope of the same cell; sometimes it is very high, resulting in closely packed hexagonal pore arrangements (GIANORDOLI; for animal cells see KESSEL, 2, and SCHEER and FRANKE, 1).
5. The pore complexes of adjacent cisternae in stacked AL are often arranged in register across the stacks; granulo-fibrillar strands appear to connect adjacent annuli.
6. Frequently, AL-cisternae show luminal continuity with the ER-system.
7. Often, ribosomes are seen attached to surfaces of AL-cisternae and of the ER with which it is continuous.
8. In many cell types one notes a conspicuous relationship of AL to specific electron dense aggregates (c.f. for plants: GIANORDOLI; SCHEER and FRANKE, 2), which resembles the classic AL-"heavy body" relationship described in sea urchin oocytes (e.g. AFZELIUS, 3; HARRIS).
9. Intranuclear AL are frequently attached to blocks of condensed chromatin, especially the perinucleolar heterochromatin (FRANKE et al., 4).

The genesis of AL is still an unsolved question. At least in the plant cells studied there is no indication of a formation from outfoldings of the nuclear envelope. On the contrary, it is assumed that the pore complexes are formed in pre-existing ER cisternae, possibly by interaction of the associated electron-dense (granulofibrillar) material (ribonucleoprotein?) with these membranes (for details see SCHEER and FRANKE, 2) in local membrane breakage and fusion. Such an AL-origin could well explain the occurrence in some cells of long cisternae of rough ER containing a few isolated pore complexes (GIANORDOLI; FRANKE et al., 4; FOWKE et al., 1).

III. Structure and Biosynthesis of the Plant Cell Wall

The plant cell wall was last generally treated in this review series in 1972 (SITTE, see there for earlier literature). Since then several special aspects of polysaccharide synthesis and of plant cell wall structure and formation have been reviewed in detail (NIKAIDO and HASSID; SHAFIZADEH and McGINNIS; O'BRIEN; NORTHCOTE, 1, 2). In addition, a comprehensive review of studies on biosynthesis, assembly and structural organization of plant cell wall polysaccharides, based on a symposium held at the 164th National Meeting of the American Chemical Society in New York in August 1972, has appeared (LOEWUS).

The present article places emphasis on structural polysaccharides and the question of their structure and biosynthesis and the importance of wall proteins.

a) Morphology of Structural Polysaccharides

A variety of electron microscopic investigations has confirmed the earlier concept of two distinct polysaccharide wall components, an amorphous matrix material and fibrillar components (for review see e.g. MÜHLETHALER; SHAFIZADEH and McGINNIS). In most higher plants the predominating resistant structural polysaccharide is cellulose. In fungi it may be replaced by chitin or β-1,3-glucan (e.g. BARTNICKI-

GARCIA; KREGER and KOPECKA). In some algae β-1,3-xylan and β-1,4-mannan have been shown to be the exclusive structural polysaccharide (review: PRESTON, 1, 2). The β-1,3-xylan from the green seaweed *Penicillus dumetosus* and the β-1,4-mannan from the related green alga *Codium fragile* have degrees of polymerization of up to more than ten-thousand, values much higher than earlier reported and comparable to those known for cellulose (MACKIE and SELLEN, 1-2). The β-1,4-mannan has been shown to occur as fibrils in the cell walls of *Codium* and *Acetabularia*, provided that mild treatments are used for isolation (MACKIE and PRESTON). Xylan microfibrils have been earlier described from metal shadowed preparations (FREI and PRESTON). By negative staining, PARKER and LEEPER found microcrystal widths of 2o-3o Å for cellulose, 1o-25 Å for β-1,4 mannan and 22 or 34 Å for β-1,3-xylan. These values are similar to those reported for elementary cellulose fibrils (review: FREY-WYSSLING). Ultrastructural reinvestigations of the cellulose fibrils from *Valonia* and other sources (GARDNER and BLACKWELL; HANNA) have confirmed earlier findings of a particularly high frequency of 3o-4o Å wide microfibrils, but also demonstrated the occurrence of thinner fibrils ("subelementary fibrils") down to the limits of detection with the present electron microscopic technique (e.g. FRANKE and FALK; FRANKE and ERMEN; KRAMER). Similar small diameters have also been reported for the cellulose-containing fibrils of the *Pleurochrysis scherffelii* scales (BROWN et al., 1-3) and for the probably cellulosic fibrils of the lorica of *Dinobryon* (FRANKE and HERTH). The existence of such "subelementary fibrils" makes folded arrangements of the cellulosic glucan chains (e.g. BITTIGER and HUSEMANN) still less probable (see also MUGGLI et al.).

Various other high molecular weight polysaccharides have been found to appear fibrillar in the electron microscope (e.g., acidic algal polysaccharides: RAMUS, 1, 2; polygalacturonic acid: LEPPARD and COLVIN; COLVIN and LEPPARD). Apparently any linear polysaccharide of high degree of polymerization may form paracrystalline fibrils. Therefore it is no longer justifiable to morphologically distinguish a fibrillar component as cellulosic, not even if it is ribbon-shaped and reveals "kinking sites", unless confirmed by chemical and X-ray analysis.

b) Molecular Components of the Cell Wall

In addition to the well known components of higher plant cell walls (lignin, cellulose, callose, the hemicelluloses xylans and glucomannans, the pectic substances galacturonans, arabinans, galactans and arabinogalactans and fuco-or galacto-xyloglucans, for review see SHAFIZADEH and McGINNIS; ASPINALL), there has recently been an increasing number of reports suggesting a rather widespread occurrence of noncellulosic glucans (e.g. FRASER and WILKIE; LOESCHER and NEVINS; SMITH and STONE; BUCHALA and MEIER, 1, 2). These, however, are rather alkali-soluble and have β-1,3- and β-1,4 linkages, with a predominance of β-1,4, as earlier described for lichenan and barley glucan (e.g. FLEMING and MANNEPS).

A series of publications by ALBERSHEIM and coworkers (TALMAGDE et al.; BAUER et al.; KEEGSTRA et al.; WILDER and ALBERSHEIM) based on detailed methylation analyses of components of primary cell walls from exponentially growing sycamore *(Acer pseudoplatanus)* cells cultured in suspension have been summarized in a tentative model of molecular arrangements and cross-linkages in primary cell wall (KEEGSTRA et al.). These authors conclude that the sycamore primary wall is composed of 1o% arabinan, 2% 3,6-linked arabinogalactan, 23% cellulose, 9% oligo-ara-

binosides (bound to hydroxyproline), 8% 4-linked galactan, 1o% protein rich in hydroxyproline, 16% rhamnogalacturonan and 21% xyloglucan, that covalent linkages exist between the xyloglucan and the pectic components and that the wall protein is bound via hydroxyproline to the oligo-arabinosides which are covalently bound to the pectic components. The sycamore primary cell wall is therefore regarded as one giant complex macromolecule ("protein-glycan-network" in the terminology of LAMPORT, 1-3). In this concept only the cellulosic fibrils are envisaged to be not covalently bound, but attached to the xyloglucan by numerous hydrogen-bonds. The authors proposed sliding of the xyloglucan past the cellulosic fibrils after lowering of the pH as a possible non-enzymatic way of extension growth, and they suggested that auxin might act in such manner (see SCHRAUDOLF for review of auxin effects).

Neither the alkali-resistant structural polysaccharide moiety of this wall nor of the secondary wall of sycamore have been fully characterized with similar adequate methods, nor has any other plant cell wall been similarly studied. Therefore, many questions as, for example, that of the species specificity of wall composition, and that of the specific arrangement of the cellulosic moiety still remain to be solved.

The question of wall proteins associated with the so-called α-cellulose fraction has been further studied in the last years. The extraction procedures used by various authors are so different that this divergence of preparations could explain the differences in results reported. The claim of ISRAEL et al. that negligible amounts of hydroxyproline-rich proteins were contained in the cell wall was refuted by ROBERTS and NORTHCOTE (3) who showed with both autoradiography and chemical analysis that most of the hydroxyproline-rich protein of suspension cultured sycamore cells is located within the cell wall. This seems to confirm the earlier ultrastructural findings of SAVADA and CHRISPEELS (1-3). There is still some controversy as to the question of covalent linkages of the hydroxyproline-rich protein to the major wall polysaccharides: HEATH and NORTHCOTE (1, 2) investigated the glycopeptides which were released from the α-cellulose associated hydroxyproline-rich glycoprotein, and claimed that this glycoprotein cannot act as covalent cross-link between the major polysaccharides of the wall as postulated repeatedly by LAMPORT and coworkers (LAMPORT, 1-3; LAMPORT and MILLER; LAMPORT et al.) since these glycopeptides were released without cleavage of glycosidic bonds and only short oligosaccharides were attached to the hydroxyproline residues. On the other hand, not only hydroxyproline-arabinose linkages but also serine-galactose linkages have been clearly demonstrated (LAMPORT, 3; LAMPORT et al.), and other covalent sugar-amino acid linkages might also exist in the wall (MONRO et al.). Analyses of various types of alkali-resistant structural polysaccharides (a β-1,4-glucan-containing one from the scales of *Pleurochrysis scherffelii*: HERTH et al.; BROWN et al., 1-3; β-1,3-xylan from *Caulerpa prolifera*, β-1,4-mannan from *Acetabularia mediterranea*, a mixed crystal β-1,3- and β-1,4-glucan polysaccharide from lily pollen tube walls, and cotton cellulose: BROWN et al., 3; HERTH) demonstrated a consistently present nitrogen-containing moiety, even in the most resistant fractions, which upon acidic hydrolysis was identified as being amino acids the pattern of which shows a predominance of hydroxyamino acids, especially of serine, and of glu-X and asp-X. At present one can at least not exclude that the types of covalent linkages found in glycoproteins (for review see, e.g., MARSHALL and NEUBERGER; SPIRO) might also occur in the amino acid linkages to sugars in the plant cell wall polysaccharides. Investigations of the biosynthesis of the hydroxyproline-rich wall protein (e.g., CHRISPEELS; SAVADA and CHRISPEELS, 1-3) and its secretion

(same authors; and DASHEK; DOERSCHUG and CHRISPEELS; BRYSK and CHRISPEELS) show that the hydroxyproline rich peptide moiety is synthesized in three steps: hydroxylation of peptidyl proline, glycosylation of peptidyl hydroxyproline and secretion of the completed glycoprotein into the wall. Glycosylation and secretion occurs within membraneous compartments (vesicles), the identification of which as being derived from Golgi apparatus or smooth endoplasmic reticulum is still controversial (see, e.g., RAY et al.; SAVADA and CHRISPEELS, 1; RAY, 1, 2; DASHEK). The stepwise synthesis is very similar to animal glycoprotein synthesis (compare, e.g., MARSHALL and NEUBERGER; SPIRO; BENNET et al.) and would allow for genetic control of the kind of wall polysaccharide synthesized (for discussion of structural polysaccharides see also HERTH et al.) via certain "recognition sequences" for the sugar transferases (e.g. SPIRO). From the studies of hydroxyproline content during and after cell wall extension (CLELAND and KARLSNESS; RIDGE and OSBORNE; WINTER et al.; SAVADA and CHRISPEELS, 2; SAVADA et al.) several authors have concluded that the hydroxyproline-rich cell wall protein is involved in the cessation of elongation. Externally supplied hydroxyproline or hydroxyproline analogues should interfere with cell wall protein synthesis and enhance wall growth (VAUGHAN and CUSENS; VAUGHAN) by inhibition of the synthesis of wall proteins which would crosslink the polysaccharides and thus counteract wall extension (see discussion by WINTER et al.). The nature of the labile bonds which have to be cleaved during cell extension according to LAMPORT's "extensin"-hypothesis is still not clear. Structural functions of the wall protein have also been suggested for the proteins or peptides associated with algal walls (THOMPSON and PRESTON) and for the peptides maintaining the structural integrity of the cellulosic scales of *Pleurochrysis scherffelii* (HERTH et al.; BROWN et al., 2). Some of the wall proteins described in the literature could also include artificially entrapped or (fragments of) wall associated enzymes (for ideas of enzyme-substrate linkage compare BELL and KOSHLAND) which were resistant to the extraction procedures used in the specific case. There is accumulating evidence for the widespread occurrence of wall associated enzymes (for green plants see, e.g., KNOX and HESLOP-HARRISON; KNOX; BARNETT; UEDA et al.).

c) Self Assembly

The refined investigations of the cell wall of the green alga *Chlamydomonas reinhardi* using wild type as well as mutants defective in various stages of wall formation (ROBERTS et al.; HILLS et al.; HILLS) have demonstrated that there is no cellulose in the wall, in contrast to earlier reports by other groups, but that it is composed of distinct hydroxyproline-rich glycoproteins. There exist seven wall layers, two rather variable fibrillar layers on the plasma membrane and, at the exterior side sandwiched between these two layers, an elaborate three dimensionally structured lattice. The most exciting finding was the reassembly of the same defined lattice, which was achieved from solutions of these glycoproteins in 8 M lithium chloride by dialysis against water when nucleation centers from the non-dissolved wall material were provided. This is the first report on *in vitro* assembly of the major constituents of a plant cell wall. *In vivo*, the wall seems to be assembled extracellularly from glycoprotein subunits synthesized within the cell. Similar self assembly processes are known for virus particles, bacterial cell walls, flagella and microtubules (e.g. BANCROFT et al.; BUCKMIRE and MURRAY; ADA et al.; BORISY and OLMSTED; KUSHNER; SHELANSKI; ERICKSON). Whether certain components of higher plant cell walls are also self assembly structures is not yet known. In most cases additional secondary modifications, for example

cross-linking (SAVADA and CHRISPEELS) or degradation of certain wall components (e.g. KIVALAAN et al.; NEVINS et al.; BARTNICKI-GARCIA and LIPPMAN), seem to be involved and make wall assembly a more complex phenomenon.

d) Biosynthesis of Cell Wall Components

The role of the Golgi apparatus in the synthesis of complex molecules, e.g. glycoproteins and matrix polysaccharides, is well documented (reviews: WHALEY et al.; NORTHCOTE; MORRÉ and VANDERWOUDE). A stepwise assembly of a complex cell wall subunit within the Golgi apparatus followed by secretion of this subunit has been shown for the scales of *Pleurochrysis scherffelii* with the aid of the periodic acid-silver methenamine technique (BROWN et al., 1-3). As further investigations have presented clear evidence for the cellulosic nature of the alkali resistant fibrillar component of these scales (HERTH et al.; BROWN et al, 1-3), this is the first evidence for cellulose synthesis within Golgi cisternae. For higher plants the views on the cytological site of cellulose synthesis are still controversial, as there are no spectacular morphological markers for cellulose formation (review: O'BRIEN). There is no information on the primary product of cellulose synthesis, and there is also no specific stain for cytochemical localization of cellulose in electron micrographs. There exist two working hypotheses. One is based on the example of scale formation, and favors the idea of an intracellular synthesis and secretion of the individual glucan chains followed by aggregation and crystallization extracellularly in the wall (BROWN et al., 3), whereas the second concept, which seems to be supported by the majority of authors, favors the assumption that synthesis and crystallization of cellulose occurs nearly simultaneously on the plasma membrane (compare PRESTON, 1, 2). There exists no proof for either of these two hypotheses, which are not mutually exclusive. At least one of the arguments in favour of a membrane-controlled formation of cellulose crystal fibrils, the highly ordered crosswise alternating deposition of layers of cellulose microfibrils, can now be regarded as not pertinent in view of the demonstrations of similarly ordered depositions of collagen fibrils in the extracellular matrix during wound healing processes in, for example, amphibia. The particles on the plasma membrane visible in freeze-etched preparations (BARNETT and PRESTON; ROBINSON and PRESTON) and, according to these authors, supposedly identical with "multienzyme-complexes" synthesizing cellulose fibrils are typical for freeze-etched membranes in general, and therefore are also visible in other intracellular membranes which are certainly not involved in such glucan syntheses (see, e.g. KARTENBECK et al., 1). Moreover, such ordered granules may be absent in some plasma membranes during the phase of highest cellulose (e.g. WILLISON and COCKING) or glucan synthesis (NEČAS). Simple pictures showing some fibrillar material attached to the cell surface membrane (WILLISON and COCKING; WILLISON; PRAT and ROLAND; PRAT) cannot be regarded as proof that (i) the fibrils are synthesized there and (ii) that they contain cellulosic material (compare the above paragraph on polysaccharide fibrils). Unfortunately, very much circumstantial and indirect evidences (wall synthesis without obvious Golgi activity (O'BRIEN; FOWKE et al., 2) or the predominance of radioactive glucose incorporation into the pectic component of vesicular fractions from lily pollen tubes (VANDERWOUDE et al., 1) and maize roots (BOWLES and NORTHCOTE)) have been regarded as demonstrations of cellulose synthesis at the plasma membrane, but this is logically inconclusive. From *in vitro* assays there is no doubt that particulate fractions from various plants contain β-glucan synthetases (NIKAIDO and HASSID), but the origin of such vesicles from the Golgi or from plasma membrane or

from both is subject to controversy, the more since staining procedures used to identify plasma membrane (ROLAND et al.) are apparently also positive for mature Golgi-derived vesicles. Furthermore, it is not clear whether the enzymes present in Golgi apparatus fractions are already active there, or become active on their way to the plasma membrane or after secretion and/or incorporation into the plasma membrane (MORRÉ and VANDERWOUDE; compare also the "template transfer" postulated for *Micrasterias* by KIERMAYER and DOBBERSTEIN). ROBINSON and RAY claim to have achieved a separation of cellulose- and hemicellulose biosynthesis by selective inhibition of hemicellulose synthesis with KCN. They showed different kinetics for the two processes and, therefore, postulated different cytological sites for the two processes.

The matter is further complicated in that the primary wall cellulose might be synthesized by another enzyme system or by a different mechanism than the secondary wall cellulose (e.g. ELBEIN and FORSEE; DELMER et al.) as already proposed by MARX-FIGINI. Other questions of cellulose synthesis are also still unsolved: A high molecular weight fibrillar cellulose I has not been synthesized *in vitro*. The endogeneous acceptors and the eventual primers are unknown (e.g. SPENCER et al.). Perhaps further inhibition experiments or studies of stimulatory effects as those reported for auxin (VANDERWOUDE et al., 2; RAY, 1, 2) will lead to a more detailed picture.

The existence of a lipid intermediate in cellulose biosynthesis, which was postulated by COLVIN in 1961, has become more probable by the demonstration of chloroform-methanol soluble glycolipids consisting of a polyprenol-like compound with covalently linked sugars, and the transfer of the sugars into alkali insoluble polysaccharides in vesicular fractions obtained from cotton hairs (ELBEIN and FORSEE). KJOSBAKKEN and COLVIN proposed that cellulose biosynthesis proceeds by a way involving a transient lipid-pyrophosphate-cellobiose compound. The cellobiose residue would then be transferred to the end of a preexisting polyglucosan chain at the tip of a microfibril. Further experiments must elucidate whether these lipid intermediates are not a byproduct, but really necessary intermediates in cellulose biosynthesis.

e) "Naked Protoplasts" and Wall Regeneration

A new field of investigations has been opened with the use of "naked protoplasts" obtained after enzymatic removal of the cell walls of either mechanically or enzymatically isolated cells from various tissues and subsequent culture of the protoplasts in defined media. Until now mostly cytological events have been studied with electron-microscopy, and the effects of various influences on wall regeneration have been examined (e.g. NEČAS; NAGATA and TAKEBE; PRAT and ROLAND; COCKING; HORINE and RUESINK; WILLISON and COCKING; BURGESS et al.; FOWKE et al., 1, 2; PRAT; WILLISON). The results, however, were meager. "No evidence was obtained for direct participation of any organelle in cell wall formation" (FOWKE et al., 2).

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