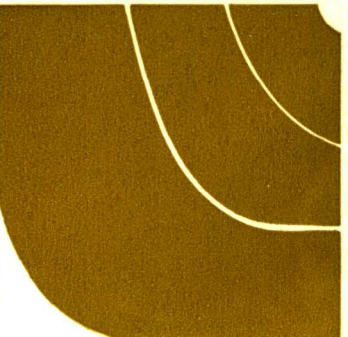
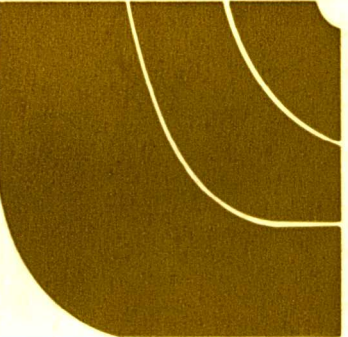
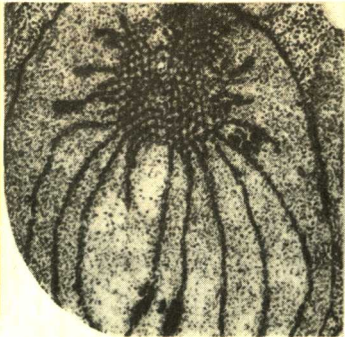
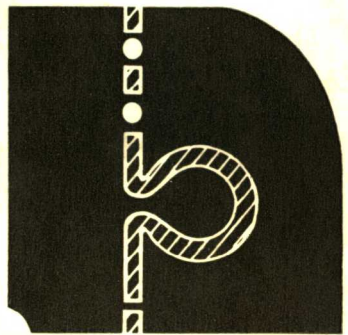
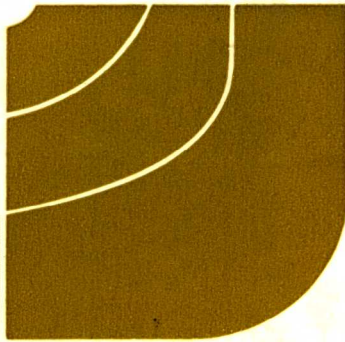
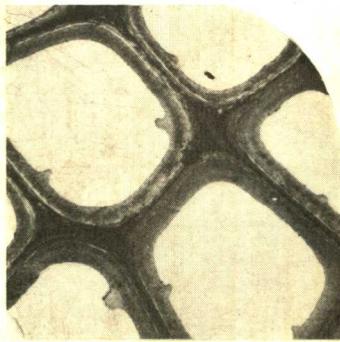


THE MOLECULAR BIOLOGY OF PLANT CELLS

Botanical
Monographs
Volume 14

Edited by
H. Smith

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THE MOLECULAR BIOLOGY
OF PLANT CELLS

EDITED BY

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PREFACE

The discoveries and concepts of molecular biology have, during the past 25 years, provided a radical new basis for our understanding of biological processes. The application of this theoretical framework, formulated essentially from studies on prokaryotes, to multicellular eukaryotic organisms is perhaps the major challenge in biology, offering the exciting possibility of achieving an understanding of growth, development and behaviour in mechanistic terms. Progress in the molecular biology of animals has been rapid and spectacular, with discoveries being made which lie outside the predictions derived from analogies with prokaryote studies. Up till quite recently, this has not been so far plants, largely due to the inherent difficulties of working with higher plants, but also to the "mammalian chauvinism" practised by so many biochemists. Attitudes change with fashions, however, and the generally increased awareness of the vitally important role of plants in the survival of the human race has led, in the last few years, to an enhanced availability of funds, and a heightened enthusiasm, for basic research on plant biochemistry, cell physiology and genetics. Following upon the so-called "Green Revolution", the much-publicized potential for the genetic manipulation of plant cells towards greater agricultural productivity has caught the imagination of scientists and laymen throughout the world. Unfortunately, much of the speculation has been ill-informed and sensationalist; nevertheless, the theoretical possibilities are limitless and much research investment is being injected into this area. A need exists, therefore, for the training of competent research workers with specialist knowledge of plant cells, their structure, function, biochemistry and genetics. This book aims to provide a sound factual basis for a training of this nature.

The Molecular Biology of Plant Cells is a text-book written by a number of authors, not merely a collection of unconnected articles. No one author could hope to cover the very wide subject area treated here in the necessary depth and thus each topic is dealt with by a specialist. In inviting the many distinguished scientists to contribute, however, I provided a relatively well defined "common pattern" for each chapter such that, as far as is possible in a multi-author book, uniformity of treatment and continuity of style would be achieved. I am most grateful to the authors, all of whom agreed to restrict themselves to my overall guidelines, and most of whom managed to complete their chapters within a year of my original deadline!

The book is intended as a text-book for senior undergraduate and post-graduate students in biology, biochemistry, botany, molecular biology and agricultural science. It covers the basic cellular physiology, biochemistry and

genetics of plant cells, but does not deal with metabolic pathways, or with the physiology and biochemistry of the intact plant. Plant development has only been lightly touched upon in this book, since a companion volume, to be called "The Molecular Biology of Plant Development" is currently being prepared. Together, the two volumes should provide a sound textual basis for teaching and research in the molecular biology of higher plants.

In the preparation and editing of this volume I have received much support, and I would like to express my thanks for secretarial help to Miss Marjorie Bentley, Mrs. Laurel Dee, Mrs. Jane Squirrell and Mrs. Elizabeth Horwood; to many scientific colleagues, particularly Professor M. B. Wilkins, Dr. D. Grierson, Dr. D. T. Clarkson and Professor D. H. Northcote for advice on content and authors; and to Mr. Robert Campbell of Blackwells for his patience and fortitude in dealing with an erratic editor.

July 1977

Harry Smith

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SECTION ONE
PLANT CELL STRUCTURE AND
FUNCTION

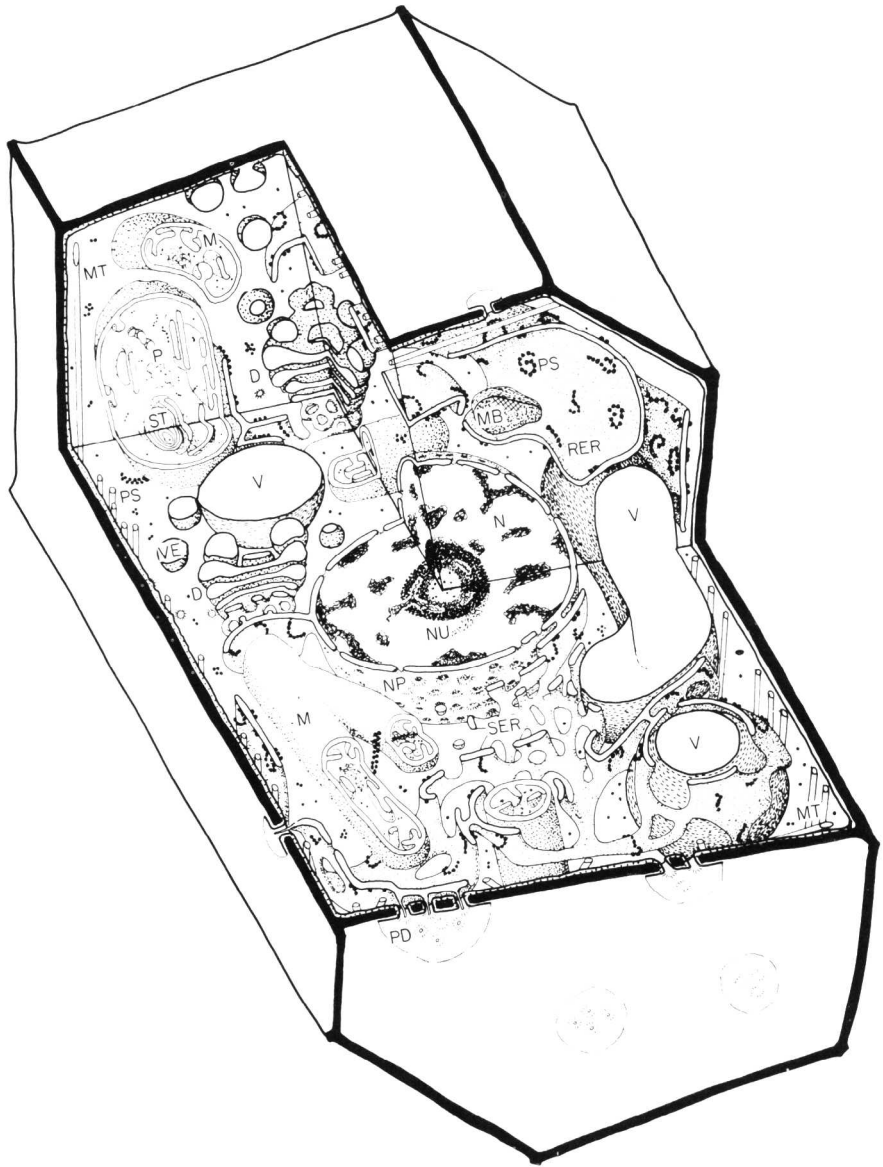


Fig. 1.1. A diagram of an undifferentiated cell showing the principal components. Some of the constituents are illustrated by only a few examples (e.g. ribosomes). The components may be identified by letters which refer to those given in the text. Figure taken from *Ultrastructure and the Biology of Plant Cells* by B.E.S. Gunning and M.W. Steer, published by Edward Arnold, London. The drawing was generously provided by Dr. Steer and is reproduced with the kind permission of the authors and copyright holders.

INTRODUCTION

The term *cell*, as first used by Robert Hooke in 1665 signified an apparently empty space or lumen, surrounded by walls. We now know, of course, that the space is far from empty, and that rigid cell walls as seen by Hooke in thin slices of cork, are not ubiquitous in multicellular organisms. Indeed, the wall became to be regarded as the definitive structure of the cell, and when in the 1830s, the zoologist Schwann was able to recognise structures in cartilage resembling plant parenchymatous cell walls, the concept of the cell as the basic biological unit common to all organisms was born. Definitions have changed considerably in the subsequent century and a half, and, in particular, the cell wall is now seen in its proper perspective as being a structure, albeit of great importance, but restricted to plants and existing only outside the true cell. Nevertheless, the general concept of the cell as the basic minimum unit of life remains.

Since all organisms need to perform a number of essential functions merely in order to survive, both as individuals and as species, it should not be surprising to find a basic unity between the cells of all organisms. Each cell, at least in the early stages of its development, possesses the capacity to synthesize complex substances from simple ones, to liberate and transform the potential chemical energy of highly reduced compounds, to react to internal and external stimuli, to control the influx and efflux of materials across the limiting cell membranes and to regulate its activities in relation to the information contained in its individual store, or stores, of hereditary genetic material. Evolution has solved the problems posed by these requirements in more or less identical ways in all organisms, and thus the basic processes, activities, and structures of each individual plant cell are similar, not only to other plant cells, but also to all other eucaryotic cells. This book concentrates on the unifying features of plant cells and relates them to present knowledge and general theories of molecular biology. It should not be forgotten, however, that cells are characterised as much by their diversity as their unity. A wide range of different cell types with varying specialized functions are necessary for the life of the higher green plant; however, the origin of cell heterogeneity is a topic outside the scope of this present book.

The basic structures of an undifferentiated plant cell can be seen in Fig. 1.1. The cell proper is delimited by the *plasma membrane* (or *plasmalemma*) which is of unit membrane construction (chapters 2 and 8). Outside the plasma membrane, and thus actually *extra-cellular*, is the *cell wall* (chapter 1). The cell wall is normally closely appressed to the plasma membrane and in meristematic cells is thin and relatively weak. During differentiation various specialized

wall structures develop; depending on the function of the mature cell, the walls may become relatively massive and extremely strong through the deposition of rigid, highly cross-linked polymeric substances. Adjacent protoplasts (i.e. the cells proper) are connected across the cell walls by narrow cytoplasmic channels, bounded by the plasma membrane, known as *plasmodesmata* (PD).

Within the cell a number of separate compartments, and interconnecting compartments, delimited by membranes, may be recognised (chapter 8). *Vacuoles* (V) are prominent, apparently empty spaces, spherical and numerous in the meristematic cell but irregular, very large, and coalescent in the mature expanded cell. Vacuoles serve as intracellular dust-bins—repositories for unwanted and often toxic byproducts of metabolism—and may also have functions similar to the *lysosomes* of animal cells. They are bounded by a single membrane known as the *tonoplast* (chapters 2 and 8).

The nucleus (N) (chapter 9), a major compartment in most cells, comprises a *nuclear envelope* possessing many large *nuclear pores* (NP) and *nucleoplasm*, the ground substance in which the hereditary material, *chromatin*, and the *nucleolus* (NU) lie. The nucleus is the principal site of the hereditary material of the cell, although both plastids and mitochondria also contain DNA. The material outside the nuclear envelope is commonly known as *cytoplasm*.

Ramifying throughout the cytoplasm, and occasionally connected to the outer membrane of the nuclear envelope, the cisternae of the *endoplasmic reticulum* act to integrate the biosynthetic functions of the cell (chapter 8). The endoplasmic reticulum is generally classified into two types: *rough endoplasmic reticulum* (RER), which has *ribosomes* attached to its outer face (chapter 10); and *smooth endoplasmic reticulum* (SER) which is not involved in protein synthesis. The endoplasmic reticulum may also, on occasion, be seen to be associated with stacks of vesicles (VE) known collectively as *dictyosomes* (D) or *Golgi bodies*. The endoplasmic reticulum and the dictyosomes are responsible for the formation and secretion of cellular membranes.

Three other membrane-bound compartments remain, each concerned with an aspect of energy or intermediary metabolism. *Plastids* (P), undifferentiated in meristematic cells and present only as *proplastids*, represent a general class of organelle in which the *chloroplast* is the characteristic member (chapters 3 and 4). *Mitochondria* (M) are smaller, but also bounded by a double membrane, and similarly involved in energy metabolism (chapter 5). As mentioned above, both mitochondria and plastids contain their own stores of hereditary material (chapter 11). The final compartments, in contrast, are bound by only a single membrane and do not contain hereditary material; these are known as *microbodies* (MB) and often contain dense, granular, or even crystalline contents (chapter 6). Within the cytoplasm just inside the plasma membrane lie long narrow cylinders known as microtubules (MT); microtubules function in a number of processes in which orientation of cellular components is important (chapter 7). Finally, plant cells contain many fine fibrils, known as

microfilaments, which appear to be contractile in function and to be composed of a material similar to actin, one of the contractile components of muscle.

The 'typical' plant cell does not exist, of course, and the meristematic cell shown in Fig. 1.1 has only been chosen since it possesses all the essential characteristics of plant cells. Many of the cellular components are only present in very simple forms in meristematic cells, however, and the subsequent chapters in Section I necessarily involve a consideration of a variety of more specialized cell types.

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CHAPTER 1

PLANT CELL WALLS

1.1 INTRODUCTION

Plant cell walls establish a home, and indeed a city, for plant protoplasts. They serve many specialized functions in plant tissues, and form the skin, the skeleton, and the circulatory system of plants.

There are many variations in the form and substance of plant cell walls. The walls may be plastic or they may be rigid, permeable or impermeable, impregnated with plastics or coated with slime, cemented in layers to form fibres or dissolved in spots to form pores. These variations are of vital importance to the proper biological functioning of plant cells and organs, and thus the structure of the cell wall is often our best indication of the nature of the protoplast which dwells inside.

1.2 THE MOLECULAR STRUCTURE OF PLANT CELL WALLS

Polysaccharides are the principal components of all plant cell walls. The polysaccharides of the cell wall are made up of sugars which are linked to each other by glycosidic bonds to form the polymer chains. Each polysaccharide contains particular kinds of sugars which are joined to each other in characteristic patterns of linkage position and sequence. It is now known that the secondary, tertiary and quaternary structures of cell wall polysaccharides are determined by the structures of the component sugars and the linkages between them, just as the three dimensional structure of a protein is determined by the sequence and structures of its component amino acids (Rees, 1972).

The various polysaccharide chains of the plant cell wall are connected to each other in specific ways, and they form an integrated network. The properties of this network depend not only on the amounts, characteristic properties and orientations of the individual polysaccharides, but also on the nature and frequency of the interconnecting linkages between them.

The conformational structures of the nine sugars commonly found in plant cell walls are shown in Fig. 1.1. The three types of polysaccharide normally found in plant cell walls (cellulose, hemicelluloses, and pectic polysaccharides), and the structural protein of primary walls, are described briefly below.

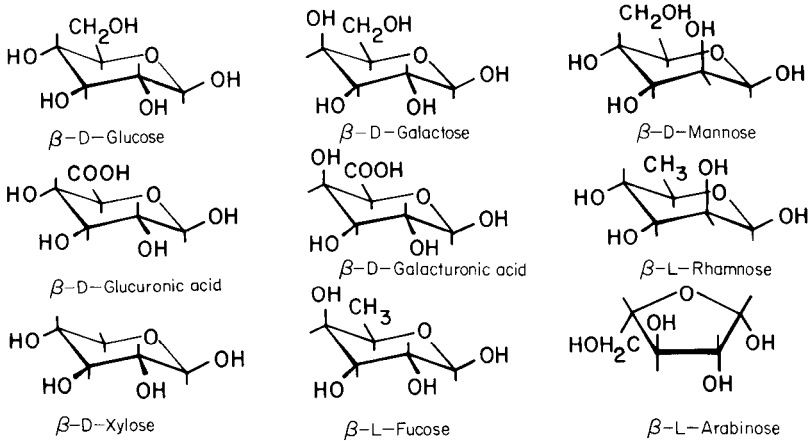


Fig. 1.1. Sugars of plant cell walls.

Conformational line drawings indicate approximate bond angles. β -L-arabinose is shown in its preferred planar furanose ring form. The other sugars are shown in their most stable pyranose chair form. Carbon atoms are numbered as indicated for β -D-glucose. Ring hydrogens are indicated by bonds only. Note that groups attached to a ring may be either axial (projecting above or below the ring) or equatorial (projecting to the side of the ring). Substituents at C_1 project equatorially in the β configuration, but are axial in the α configuration. All 'bulky' groups ($-\text{OH}$, $-\text{CH}_2\text{OH}$, & $-\text{COOH}$) are in equatorial positions in β -D-glucose, β -D-glucuronic acid, and β -D-xylose. Note that these sugars differ only in the group attached to C_5 . Galactose, galacturonic acid and fucose are similarly related (axial $-\text{OH}$ group at C_4), as are mannose and rhamnose (axial $-\text{OH}$ group at C_2).

1.2.1 CELLULOSE

Cellulose occurs as a crystalline, fibrillar aggregate of β -1,4-linked glucan chains (Frey-Wyssling, 1969). Cellulose fibrils give plant cell walls most of their enormous strength, much as glass fibres embedded in an epoxy resin give strength to a fibreglass composite (Northcote, 1972).

The basic structure of the β -1,4-linked glucan chains of cellulose is illustrated in Fig. 1.2 by conformational line drawings and in Fig. 1.3 by molecular models. Residues of β -D-glucose (Fig. 1.1) are glycosidically linked to each other, *from* carbon 1 of one residue *to* carbon 4 of the adjacent residue. The upside-down inversion of every second residue in the chain minimizes contact between atoms of adjacent residues. Close inspection of the models in Fig. 1.3 shows that the $-\text{OH}$ groups at carbon 3 are in very close proximity to the ring oxygens (O_5) of adjacent residues. Hydrogen bonds between O_3 and O'_5 help to stabilize the flat, straight, ribbon-like structure of β -1,4-linked glucan chains.

The flat, ribbon-like structure allows the chains to fit closely together, one on top of the other, over their entire lengths. These *interchain* associations are stabilized by hydrogen bonds between O_6 of a glucose residue in one chain and

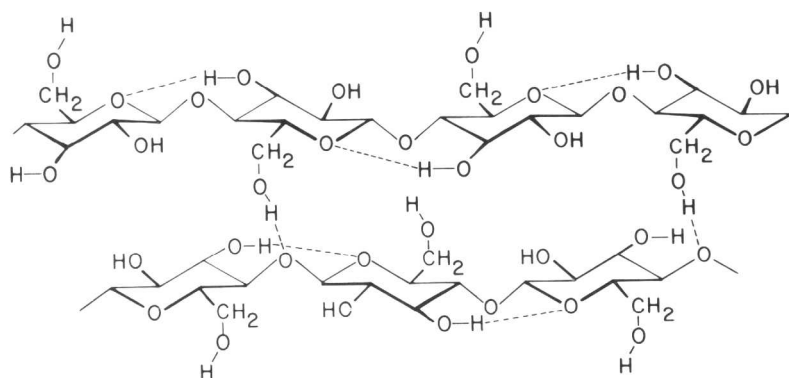


Fig. 1.2. β -1,4-linked glucan chains of cellulose.

Portions of two associated chains are illustrated by conformational line drawings. Distances between atoms are *not* accurately indicated in this illustration, but see Fig. 1.3.

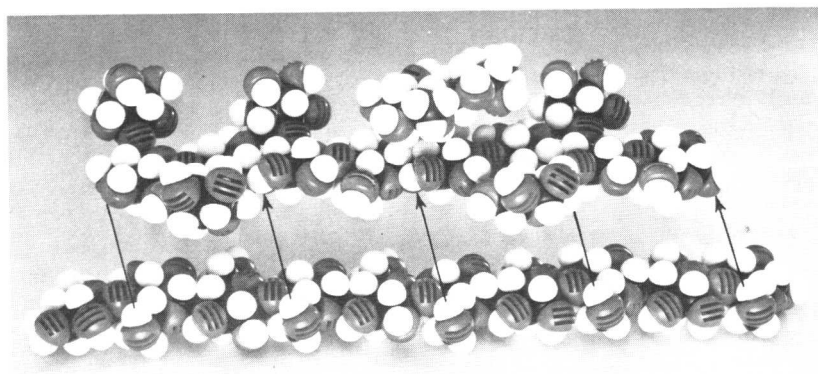


Fig. 1.3. Hemicellulosic xyloglucan associated with cellulose.

The repeating subunit of a hemicellulosic xyloglucan is shown in association with a portion of a β -1,4-linked glucan chain of cellulose (Bauer *et al.*, 1973). Molecular models have been used to accurately indicate interatomic distances and bond angles. Hydrogen bonds from the cellulosic glucan chain to the glucan backbone of the hemicellulose are indicated by arrows.

the oxygen of the glycosidic bond (O_1) between glucose residues in an adjacent chain. Since the glucan chains of cellulose are very long (8,000 to 15,000 residues), the number of hydrogen bonds between adjacent chains is very large. The resultant crystal is extremely stable and so tightly packed that there is no room for water molecules in the crystal structure.

Although there is some controversy as to whether native cellulose fibrils are 3.5 nm or 10 nm in diameter, it is clear that the glucan chains of cellulose