Biosynthesis and Biodegradation of Cellulose

edited by
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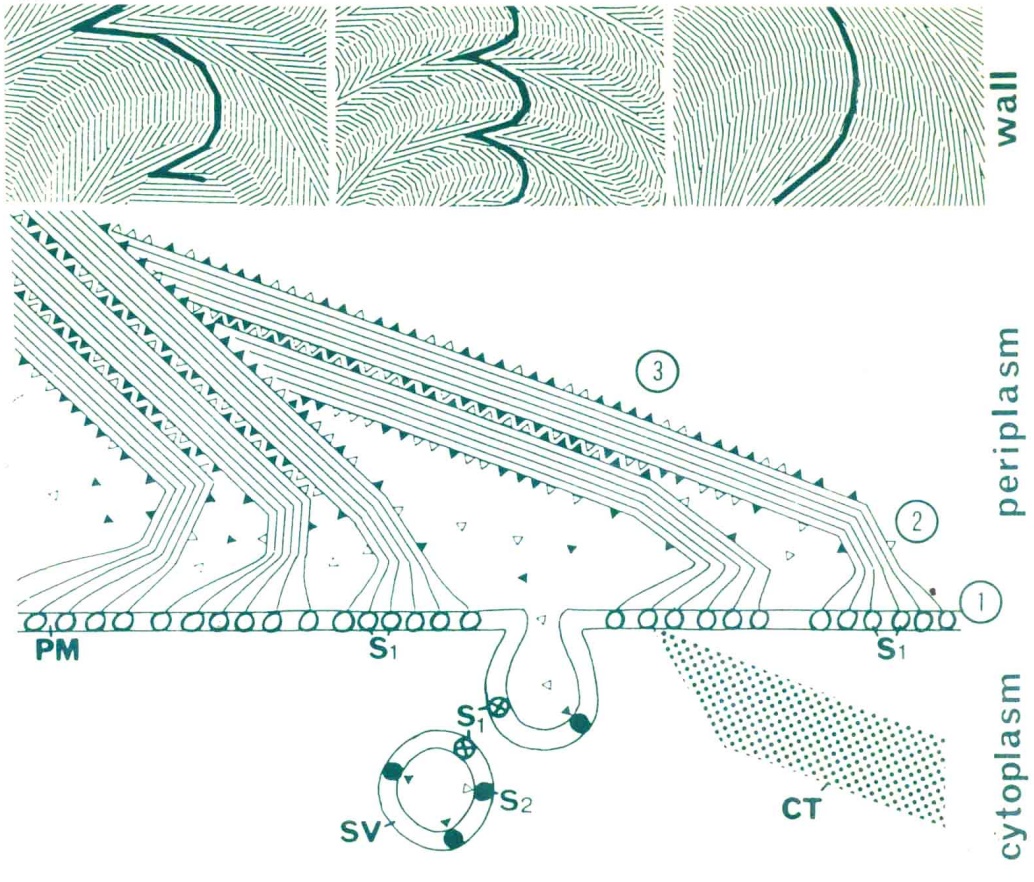

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Preface

This book assembles into a single balanced volume the vast and widely dispersed literature on the biosynthesis and biodegradation of cellulose, an abundant biopolymer of great natural and industrial importance. Included are comprehensive summaries of the biosynthesis of cellulose in both in vitro and in vivo systems, along with reviews of the phenomenon of cellulose degradation as practiced by a variety of microbial groups and their component enzymes. Unlike other books on the biology of cellulose, this volume aims to provide an equal treatment of biosynthesis and biodegradation, which, unfortunately, are usually regarded as separate phenomena with little in common. Thus, a major aim of the book is to broaden the horizons and knowledge of cellulose researchers who have until now lacked a systematic and balanced source of information on aspects of cellulose biology outside of their immediate research area. With respect to biosynthesis, all aspects of the field—including cell biology, biophysics, biochemistry, and molecular biology—are covered to the proportional level of knowledge in each area. Within the biodegradation domain, a broad canvas of microbial degradation has been included, instead of the narrow focus on fungal cellulolytic enzymes that is normally encountered in technology-oriented books on biomass conversion or methods-oriented books on cellulose biochemistry. Because its material is presented in the form of synthetic reviews

and commentaries on the most current research approaches, the book should be appreciated by both established and beginning researchers in the field.

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Biosynthesis and Biodegradation of Cellulose

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Biosynthesis

CANDACE H. HAIGLER

INTRODUCTION

This introduction provides an overview of Part I of the volume, which deals with the biosynthesis of cellulose. Specific chapters are mentioned where appropriate in a brief overview of the field. A similar introduction can be found after Chap. 12 for Part II of the volume on the biodegradation of cellulosic materials.

Because of the abundance of cellulose in the natural world (Chap. 1), understanding the mechanism of its biosynthesis has been a goal for several decades. Recently, interest in this process has been stimulated by increasing evidence that plant cell walls have critical roles in plant development and stress responses, the realization that microbial cellulose is a source of cellulose with special properties for commercial use, and the possibility of improving renewable resources such as cotton and wood through genetic engineering. It is clear to all who work in this field that there is a large deficiency of basic knowledge without which practical improvements are impossible. Of equal importance to applied aims, understanding cellulose synthesis will help illuminate how cells coordinate complex processes. Even now we know or can predict that cellulose synthesis involves differential gene expression, the endoplasmic reticulum, the Golgi apparatus, the cytoskeleton, vesicle transport and fusion with the plasma membrane, endocytosis of excess plasma membrane, specialized fluidity domains in the plasma membrane, geometrical aggregation of membrane proteins, localized ion concentrations, and a precisely

regulated biochemical pathway. After synthesis, the cellulose is integrated into walls by a biophysical process of which we have only rudimentary understanding (Chap. 2).

To the reader uninitiated in this field of research, the implication that there is so much left to know about the polymerization of glucose into a homopolymer probably seems strange. The deficiency can in part be explained by the complexity of the process. Not only is a molecule being made, but the molecule is being assembled under cellular control into a structural fibril at the cell surface (Chaps. 6 and 8). A major contributor to the deficiency, however, was the longstanding inability to synthesize cellulose in vitro from cell-free extracts of any organism (Chap. 9). Because of the intractable nature of the problem, many researchers turned to more productive areas of research. The few cellulose biochemists who persevered have at last seen some rewards of their efforts as will be discussed below.

Meanwhile, beginning in 1976 microscopists discovered intriguing particle arrays in freeze-fracture replicas of plasma membranes of nearly all cellulose-synthesizing organisms prepared without the use of artifact-inducing cryoprotectants (Chaps. 3 and 4). Although conclusive assignment of a biochemical function to these particles is a major goal, most everyone now agrees that they are important participants in microfibril formation. Investigation of the relationship between polymerization and crystallization in *Acetobacter xylinum* (Chap. 5) established the theoretical basis for predicting that the different geometrical arrangements of the membrane particles determine the size and crystallinity of microfibrils. Similarly, microscopists observed a coincidence between the alignment of microtubules and cellulose microfibrils in certain cell types (Chap. 7). The extent of this coincidence in different cell types is still being examined, and a mechanism by which cytoplasmic microtubules might affect alignment of extracellular microfibrils is still being sought. The possible participation of other cytoskeletal components in cellulose deposition is largely unexamined. The development of cytochemical techniques based on enzymes (Chap. 2) or antibodies specific for one type of polysaccharide has begun to provide new insights into biophysical and cell biological aspects of wall assembly.

In 1981, the first major breakthrough in the area of biochemistry was realized through the ability to synthesize cellulose in vitro from membrane preparations and solubilized extracts of *A. xylinum* (Chap. 11). The key to this breakthrough was in maintaining a guanyl cyclase that converts GTP to an activator of the enzyme in the in vitro assay system. Unfortunately, application of the same strategies to higher plants has been unsuccessful, causing in vitro synthesis of cellulose from higher plant enzymes to remain as one of the great challenges in the field (Chaps. 8 and 9). The success

with *A. xylinum* and very recent success with in vitro synthesis in *Dictyostelium discoideum* have renewed optimism that an elegant solution can be found to the problem in higher plants. Continuing efforts to find out more about the competing enzyme in higher plants, the callose synthase (Chap. 10), will certainly speed the resolution of the problem. The success with in vitro synthesis in *A. xylinum* has allowed research in cellulose biosynthesis to move into the molecular realm (Chap. 12). Polypeptides that participate in the process have now been tentatively identified in *A. xylinum* and higher plants. Some of the genes in the biosynthetic pathway in *A. xylinum* have been cloned.

This is an opportune time for other scientists to join in the research on cellulose biosynthesis. Part I of this book is designed to review the current status of the field as we move into the 1990s. In Part I, Chaps. 1 and 2 cover the occurrence and natural role of cellulose, Chaps. 3–7 deal with cell biological and biophysical factors in the cellulose synthesis, Chaps. 8–11 cover the biochemistry of cellulose synthesis, and Chap. 12 covers the newly emerging investigation of the molecular biology of cellulose synthesis. Some very recent developments briefly mentioned in this introduction are not covered in detail in the book chapters. This is a sign that a field that once moved very slowly is beginning to move more rapidly. We can expect that immunological and molecular probes will be developed to answer many longstanding questions about cellulose biochemistry and molecular biology. For example, antibodies to polypeptides involved in the process can be used in freeze-fracture labeling to test the identity of the organized membrane particles and as probes to investigate the molecular regulation of cellulose biosynthesis. This work is in its infancy in the public sector, but we can expect rapid progress in the future. In the excitement over biochemistry and molecular biology, we should make sure that the cell biological aspects of this process, which promise to reveal much about coordination of cell functions, are not ignored. As also indicated in the introduction to Part II on the biodegradation of cellulosic materials, research on cellulose biosynthesis will proceed most rapidly if a multidisciplinary approach is taken.

1

Occurrence and Functions of Native Cellulose

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I. INTRODUCTION

Cellulose is synthesized by all higher plants as well as by a wide variety of other organisms. The amount of synthesis is enormous, making cellulose the most abundant biopolymer on earth (1). Most native cellulose is found in cell walls in the form of structural microfibrils. Although considerable effort has gone into investigating the various aspects of cellulose biosynthesis, and not without many exciting successes, much is yet to be understood about cellulose synthesis, microfibril structure, and microfibril deposition within cell walls.

Cellulose is a linear homopolymer composed of β-1,4-linked D-glucopyranosyl units (2). These residues form long chains with variable degrees of polymerization (DP; number of monomeric residues per polymer molecule). Although reliable measurements are difficult to obtain (3–5), DP values in higher plants appear to generally range between 7000 to 14,000 or more for secondary walls (3,6), whereas they are as low as 500 for primary walls (3,7). These extended glucan chains strongly associate by hydrogen bonding and van der Waals forces (8), laterally aggregating into crystalline cellulose microfibrils (4,9). Degrees of polymerization have not been correlated with native microfibril lengths. Length determination is difficult since microfibril ends are rarely seen in electron