David D. Fang *Editor*

Cotton Fiber: Physics, Chemistry and Biology

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Preface

Cotton fiber is the most important natural fiber used in the textile industry. The physical structure and chemical compositions of cotton fibers have been extensively studied. Newer high-speed spinning instruments are being deployed around the world that demand longer, stronger, and finer fibers. Consequently, genetic improvement in fiber quality has been stressed. With improvement in fiber quality has come the realization that further fiber improvement will require a better understanding of fiber development and biology. As a consequence, cotton fiber developmental biology, genetics, and genomics have become focal points in the cotton research community. As the longest single-celled plant hair, cotton fiber has been used as an experiment model to study trichome initiation and elongation in plants. This book provides a comprehensive update on cotton fiber physics, chemistry, and biology that naturally separate the book into three sections. In the physics section, the physical structure of cotton fiber is first illustrated in great detail. Then a suite of fiber properties and their measuring methods are described. The pros and cons of each method are outlined. New methods to measure physical properties of single fiber and young developing fibers are included. In the chemistry section, the chemical compositions of cotton fibers are described in detail. This knowledge is necessary for efficient modification of cotton fibers for better and broader utilization. The advancement in cotton fiber modification using chemical and enzymatic methods opened new ways to utilize cotton fibers. In the biology section, the book first introduces the utilization of naturally occurring color cottons. Color cottons possess unique attributes such as better fire retardant ability. Advancement in understanding fiber color genetics and biochemical pathways and new utilization of color cottons are discussed. Recent technological advancements in molecular biology and genomics have enabled us to study fiber development in great depth. Many genes and quantitative trait loci related to fiber quality attributes have been identified and genetically mapped. Some of these genes and QTLs are being used in breeding. Progresses in cotton fiber improvement using breeding and biotechnology are discussed in the last chapter. This book serves as a reference for researchers, students, processors, and regulators who either conduct research in cotton fiber improvement or utilize cotton fibers.

I greatly appreciate all the authors who contributed excellent work to this book. I also thank my employer, USDA-ARS Southern Regional Research Center (SRRC), where most contributors are employed. Cotton fiber research and utilization has been a major research component at SRRC since its establishment in 1939. Many research results described in this book are from SRRC.

New Orleans, LA, USA David D. Fang

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About the Editor

David D. Fang is a Supervisory Research Geneticist and Research Leader of the Cotton Fiber Bioscience Research Unit, USDA-ARS Southern Regional Research Center in New Orleans, Louisiana, USA. He leads a team to study cotton fiber development using biochemical, genetic, molecular, and genomic methods and technologies. His research interests focus on two aspects: (1) identification of superior fiber alleles and quantitative trait loci based on the analysis of MAGIC populations, and use them in breeding with the assistance of DNA markers; (2) elucidation of the molecular mechanisms of fiber development especially elongation and secondary cell wall thickening through comparative analyses of fiber mutants and their respective wild types. Prior to the current position, he was the Molecular Cotton Breeder, and Director of Molecular Cotton Breeding at Delta and Pine Land Company. He obtained his Ph.D. in 1990 from Huazhong Agricultural University, China.

Chapter 1
General Description of Cotton

David D. Fang

Throughout the world, cotton fiber is the most widely used plant-produced fiber for apparel, home furnishings, and industrial products. In 2016, about 106.5 million bales (218 kg or 480 pounds per bale) of cotton fiber were produced from more than 50 countries around the world. The economic value of the worldwide raw cotton fiber is estimated at $35 billion annually. India, China, the United States, Pakistan, and Brazil account for over 75% of world cotton production (www.cottoninc.com). Of the entire world production, about 36 million bales were destined to the export market with the United States being the largest exporter and Vietnam as the largest importer in 2016.

Naturally, a cotton plant grows as a perennial in tropical and subtropical regions, often reaching the size of a small tree. However, for commercial production of raw fibers, most if not all cotton cultivars are grown as annuals, i.e., the crop is harvested in the same year of planting. An "annual" cotton is not a true annual because the death of a plant is not a natural consequence of seed ripening, rather due to application of chemicals or mechanical destruction. A cotton plant can be maintained indefinitely under a warm environment such as a glasshouse. Indeed, cotton germplasm repositories in several countries use glasshouses to maintain live cotton plants (Percy et al. 2014).

Cotton belongs to the family Malvaceae, the tribe Gossypieae, and the genus *Gossypium* (Wendel and Grover 2015). The cotton genus (*Gossypium* L.) consists of about 45 diploid species (2n = 2x = 26) classified as 8 genome groups (A–G and K) and 6 allotetraploid (AD) species (2n = 4x = 52) (Fryxell 1992; Wendel and Grover 2015). The haploid genome size of diploid species ranges from 885 Mbp of a D-genome species to 2570 Mbp of a K-genome species. A tetraploid species has

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a genome size of about 2400 Mbp (Hendrix and Stewart 2005; Zhang et al. 2015). A preponderance of evidence has demonstrated that the six tetraploid cotton species (*G. barbadense*, *G. darwinii*, *G. ekmanianum*, *G. hirsutum*, *G. mustelinum*, and *G. tomentosum*), which are entirely New World distribution, originated from a single hybridization event between an A-genome species (either *G. herbaceum* or *G. arboreum*) and a D-genome species (possibly *G. raimondii*) 1–2 million years ago (Endrizzi et al. 1985; Paterson et al. 2012; Wendel and Cronn 2003; Wendel and Grover 2015; Zhang et al. 2015). Four species, i.e., *G. arboreum*, *G. barbadense*, *G. herbaceum*, and *G. hirsutum*, are cultivated for their ability to produce high fiber yield.

G. hirsutum (Fig. 1.1), native to Mexico and Central America, was introduced into the United States as early as the sixteenth century shortly after Columbus' discovery of the Americas (Beckert 2014). Tremendous efforts in introduction, selection, and breeding significantly improved *G. hirsutum* plants to better adapt to commercial production under new environments in subsequent centuries (Fig. 1.2). Eli Whitney's invention of a saw gin to mechanically separate cotton fiber from seeds in 1793 greatly helped the expansion of cotton production in the United States and the spread of American cotton varieties to other countries (Lee and Fang 2015). Of the four commercially cultivated species, *G. hirsutum* commonly known as upland cotton or American upland cotton is grown on the most acres and accounts for over 90% of the world's raw cotton fiber production. In general, upland cottons have fiber length ranging from 20 to 32 mm, micronaire value falling between 3.5 and 5.5, and bundle fiber strength between 27 and 32 g/tex.

G. barbadense (Fig. 1.3) originated in South America and has a wide range of distribution across the continent. This species includes commercial varieties commonly known as Egyptian, Sea Island, Pima (also called American Pima), American

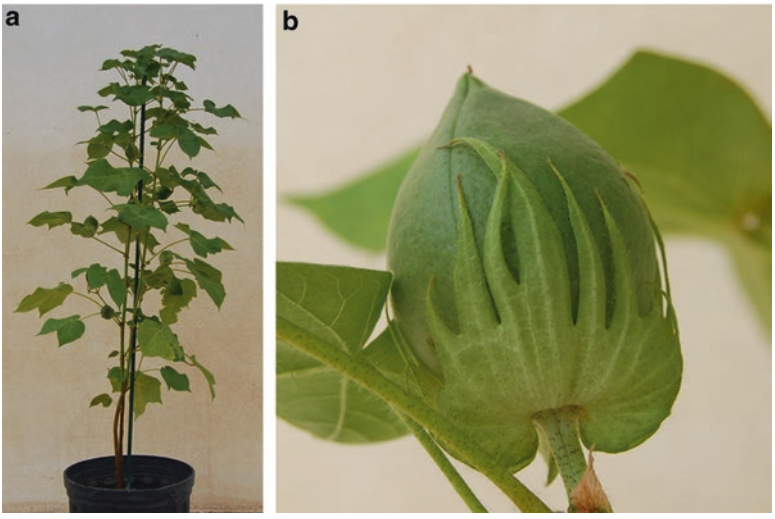

Fig. 1.1 *G. hirsutum* plant (**a**) and boll (**b**) (courtesy of Doug Hinchliffe)

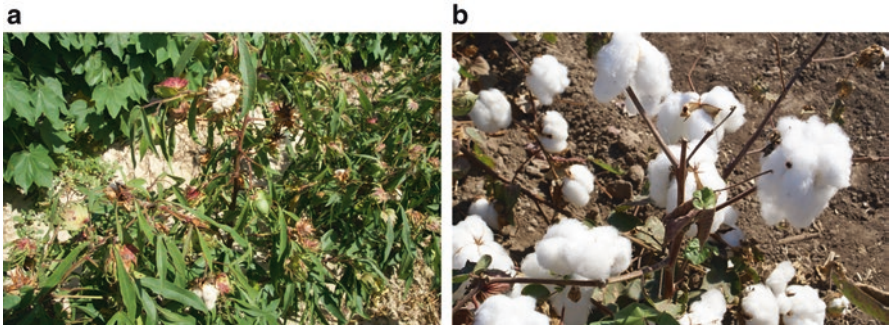

Fig. 1.2 *Gossypium hirsutum*. (**a**) Landrace, (**b**) cultivar (courtesy of James Frelichowski)

Fig. 1.3 *G. barbadense* (courtesy of James Frelichowski)

Egyptian, and extra-long staple. This species provides about 3–5% of the world's cotton production. *G. barbadense* varieties are mainly grown in Egypt, Sudan, and the western United States. The fiber of *G. barbadense* is longer (>33 mm), stronger (>32 g/tex), and finer (micronaire <4.0) than that of *G. hirsutum*. *G. barbadense* fibers are mainly used to produce high-quality apparel that can command a premium price. Although *G. barbadense* has better fiber, its low yield and poor adaptability to variable environments limit its cultivation. Since the beginning of the twentieth century, a lot of breeding efforts in the United States have been dedicated to introgression of *G. barbadense* fiber traits into *G. hirsutum* varieties. Many germplasm resources including commercially successful Acala-type varieties with variable levels of *G. barbadense* introgression have been developed (Smith et al. 1999).

G. arboreum and *G. herbaceum* are known as Asiatic or old world cottons (Figs. 1.4 and 1.5). They are also called "desi" cottons. These two diploid species have been cultivated by mankind for thousands of years (Lee and Fang 2015).

Fig. 1.4 *G. arboreum* plant (**a**) and boll (**b**) (courtesy of Doug Hinchliffe)

Fig. 1.5 *G. herbaceum* plant (**a**) and boll (**b**) (courtesy of Doug Hinchliffe)

Their fibers are short (<20 mm), coarse (micronaire >5.0), and weak (<22 g/tex) (Reddy and Reddy 2011), and yield is low. However, desi cottons have favorable traits such as resistance or immunity to leaf curl virus (a destructive disease affecting cottons in India and Pakistan) (Nazeer et al. 2014), blue disease (a virus disease prevalent in South American countries) (Fang et al. 2010), and bunchy top disease (another virus disease in Australia) (Ellis et al. 2016). In addition, desi cottons are drought tolerant which makes these two diploid species suitable to the arid

sub-Indian continent. Desi cottons account for less than 2% of the world's cotton production and are mainly cultivated in India and Pakistan.

Cotton originated in the tropics, and the plant becomes inactive at temperatures below 16 °C. Cotton plants need about 160 days above 16 °C to produce a crop (Snider and Oosterhuis 2015; Waddle 1984). Planting time for cotton varies by locality. Planting recommendations are generally based on soil temperature greater than 16 °C at a certain planting depth and favorable air temperature forecasts for the next 3–7 days after planting. In the United States, the planting season can start as early as February in the lower Rio Grande valley in Texas and continue as late as May in the southeast regions. Soil temperature greatly affects seed germination. Seedlings emerge from the soil within a week after planting. Flower buds or squares are visible near the top part of the plant about 5–6 weeks after seedling emergence. Blossoms appear in another 3–4 weeks. The time interval from the day of anthesis (flowering) to open boll ready for harvesting is about 50–80 days depending on genotypes and environments.

Cotton fibers are unicellular trichomes or plant hairs that differentiate from epidermal cells of developing cotton seeds. Cotton fiber development occurs in a temporally ordered series of developmental stages and divides into four distinctive yet overlapping stages: initiation, elongation, secondary cell wall (SCW) biosynthesis, and maturation (Haigler et al. 2012; Lee et al. 2007). Visible signs of fiber initiation are first evident 1 or 2 days before anthesis; therefore, developmental events are staged by the number of days post-anthesis (DPA). Over a 2–3-week period, fiber cells elongate up to 25–40 mm, making them among the longest cells in the plant kingdom (Kim and Triplett 2001). During the elongation stage, only a thin (0.1–0.2 μm) primary cell wall with a waxy cuticle surrounds each fiber cell. Depending on genotypes, there are 10,000 to 20,000 fibers per seed (Zhang et al. 2011). In each boll (ovary), there can be over half a million synchronously elongating fiber cells that are in a sole cell type (Bowman et al. 2001).

In upland cotton, fiber cell starts to elongate as early as on the first day of anthesis and continues up to 20 DPA. Fiber length is largely determined at the elongation stage especially the length of elongation period as demonstrated by Avci et al. (2013) based on comparison of *G. hirsutum* and *G. barbadense* fiber length development. SCW biosynthesis begins approximately 12 to 16 DPA and continues until approximately 35 DPA or later. This stage is critical for fiber strength and maturity. The fiber SCW is deposited between the plasmalemma and the primary cell wall and is 1.5–3.0 μm thick at maturity. At or around 45 DPA, fiber development enters into maturation stage. Fiber development ceases when the fruit wall dehisces and the fibers dry upon exposure to the environment. The cytoplasmic contents of the living cell adhere to the drying cell wall.

Fiber primary cell walls (PCW) are a composite of carbohydrate polymers (cellulose, hemicellulose, and pectin) and structural wall proteins. The cellulose content of the expanding PCW is less than 15% by weight, whereas mature fibers have thickened secondary cell wall composed of nearly pure cellulose (>95%). Cellulose is a linear β-1,4-D-glucopyranose polymer that aggregates into higher-order structures called microfibrils (5–15 nm diameter and 10 μm long) (Fig. 1.6).

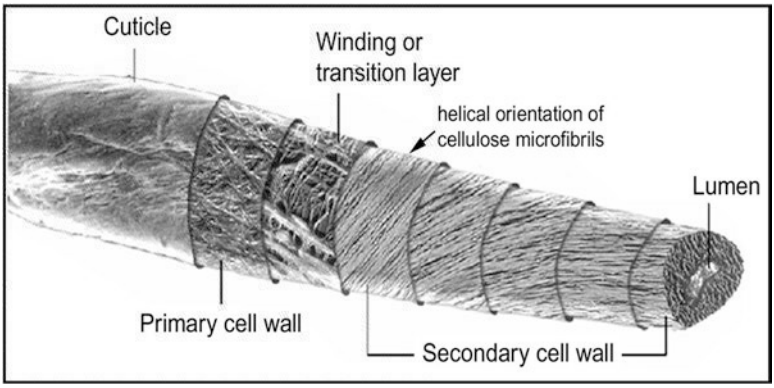

Fig. 1.6 Cotton fiber structure (courtesy of Cotton Structure and Quality Research Unit, USDA-ARS, New Orleans, LA)

Cellulose microbrils (CMF) are helically arranged around the longitudinal axis of the fiber in layers. Periodically the gyre of the helix reverses direction, and a reversal is formed. There is a strong association between the orientation of CMF in the fiber secondary cell wall and fiber strength (Moharir 1998; Moharir et al. 1999; Warwicker et al. 1966). Fibers with CMF oriented with a shallow angle relative to the long axis of the fiber are stronger than fibers with larger orientation angles. The orientation of CMF in the fiber cell wall, as in other plant cells, appears to be influenced by the cytoskeleton (Seagull 1991); however, how the cytoskeleton exerts an influence on cell wall structure remains an unanswered question in plant cell biology. The degree of polymerization (number of glucose molecules per polymer) of cellulose is much larger in the secondary cell wall than in the primary cell wall (Timpa and Triplett 1993). As the cellulose degree of polymerization increases in the secondary cell wall, fiber strength increases (Timpa and Ramey 1994).

Cotton harvesting methods vary from different regions in the world. Almost the entire cotton crop in the United States is mechanically harvested; however, manual harvesting is still prevalent in many cotton-growing countries where labor cost is relatively low. There are two major types of mechanical cotton harvesters: picker and stripper. A picker harvester selectively collects seed cotton from open bolls, leaving much of the bur and other plant materials in fields. A stripper harvester collects seed cotton along with significant amount of other plant materials. Cottons harvested by a stripper tend to be dirtier and require more cleaning in the subsequent ginning process (Wanjura et al. 2015). After harvesting, seed cotton will be transported to a ginning facility. During the peak of harvesting, ginning facilities cannot immediately process all the seed cottons that were harvested. Thus, the harvested seed cottons are temporarily stored in fields in compacted modules wrapped in plastic films or covered with tarps.

Although the main function of the ginning operation is to separate fibers from seeds, the ginning process also includes conditioning (to adjust moisture), cleaning (to remove non-fiber trash), and packing into bales for transportation and marketing.

Inappropriate ginning can break fibers and greatly affects fiber quality. Upland cottons are usually ginned on saw gins, while Pima cottons are often ginned using roller gins. A cotton bale varies in dimension, volume, and weight. A typical US cotton bale weighs 218 kg (480 pounds) with dimension of 1400 mm (length) × 533 mm (width) × 736 mm (height). A cotton bale is wrapped with plastic films or cotton fabric (Wanjura et al. 2015).

Quality of upland cotton raw fibers is becoming a critical factor in cotton production in the United States. Nearly every bale of cotton produced in the United States is classed by the USDA Agricultural Marketing Service using high volume instruments (HVI) that rapidly measure fiber physical properties including length, length uniformity, strength, micronaire (MIC), and trash content. Fiber length is largely influenced by the genetic background of each cultivar, but adverse environmental conditions will reduce fiber length below a genotype's potential (Bradow and Davidonis 2000; Kelly et al. 2015; Meredith et al. 2012). Fiber length and length uniformity are important determinants for yarn strength, evenness, fineness, and spinning efficiency (Kelly et al. 2015; Thibodeaux et al. 2008). Short fibers may be generated during ginning process if the fibers are weak. Fiber strength is highly influenced by cotton genotype and may also be negatively affected by poor growing conditions (Hinchliffe et al. 2011; Zhang et al. 2017). High-speed textile processing machinery, especially rotor-spinning equipment that spins cotton fiber into yarn found in most US textile plants, puts an increased demand on higher cotton fiber strength. MIC is a measurement of the air permeability through a mass of fiber compressed to a fixed volume and is influenced by both fiber fineness and maturity. Modern high-yield cotton varieties produce high-MIC cotton (>5.0) because the yield is positively correlated to MIC value (Nichols et al. 2012). The high-MIC cotton is composed of coarse and thick fibers, and it is unfavorable to both textile manufacturers and consumers. Cotton fibers with intermediate MIC values ranging from 3.7 to 4.2 are classified as premium cotton. Buyers discount the value of high- (>5.0) and low-MIC (<3.4) cotton. In summary, the value of cotton fiber in the market, regardless of its end use, is directly related to the combination of its physical properties. These combined physical attributes of cotton fiber have a direct and significant impact on the economical return to cotton farmers and other related downstream entities. The highly mechanized production and processing of cotton products at increasingly higher speed demand that the raw fiber be as uniform, long, and strong as possible.

There are three primary products derived from cotton production: cotton lint, linters, and cottonseed. Cotton lint is long (>25 mm) fiber that can be spun into yarn. This product is used in clothing, denim, towels, and dollar bills. The lint fibers can be easily separated from seeds through the ginning process. Linters are short fibers (usually <15 mm) that are still attached to the seeds after ginning. The linter fibers are removed during the delintering process. Linters are used in plastics, paper products, films, and cosmetics. Besides length, there are many notable differences between lint and linters in physical and chemical properties (Wakelyn et al. 1998). Linters are coarser and thicker and often show pigmentation. Lint fiber cells usually initiate before or on the day of anthesis and elongate as late as 20 DPA (Avci et al.

2013). In contrast, linter fiber cells initiate at 3–4 DPA and stop elongation as early as 12 DPA. There are cotton varieties or mutants that are linter-free (e.g., *G. barbadense* varieties, N_1 and n_2 mutants) and fiberless (e.g., XZ142 *fl*) (Zhang and Pan 1991). These mutants are widely used to study the biology of fiber development (Naoumkina et al. 2016). Cottonseed is crushed into three separate products—oil, meal, and hulls (the outer covering of a seed). The oil is the cottonseed's most valuable by-product and is purified and used in cooking. The hulls are used in livestock feed, fertilizer, fuel, and packing materials. The meal is made by grinding the cottonseed and is used in livestock and poultry feed, as well as in natural fertilizers for lawns, gardens, and flower beds.

Although almost all cottons of commerce are white, naturally colored cotton fibers exist in various hues including light to dark brown, red, rust, and green, and they are found in both diploid and tetraploid species (Hinchliffe et al. 2016). Naturally colored cottons have been grown for several thousand years but almost completely disappeared in the mid-twentieth century because of the availability of inexpensive dyes, higher production of white cotton, and cotton ginners' concern of contamination to white cottons. In recent years, there is a renewed interest in growing colored cotton for better stewardship of the environment by reducing the amount of dying chemicals used to artificially color cotton fabrics. Currently, colored cottons are typically grown as a source of fiber for niche textile markets that promote the use of natural colors in textiles as an alternative to dying scoured and bleached cotton fibers. Colored cotton fibers are usually weaker, shorter, and finer and often yield lower. However, these shortcomings associated with colored cotton can be overcome through breeding if demand for naturally colored cotton fibers is increased. A recent finding that naturally colored cotton fibers confer higher flame retardancy may spark new demand for colored cotton (Hinchliffe et al. 2016).

Cotton is one of the first crops that were genetically modified using transgenic technologies. In 1996, the first transgenic cotton variety containing a Bt gene from the bacterium *Bacillus thuringiensis* was introduced to the US market. Since then, transgenic cotton has been grown in more than 15 countries. As of today, transgenic cotton accounts for more than 85% and about 60% of cotton acreages in the United States and the world, respectively (Zhang 2015). There are two major transgenic traits: Bt toxin (a protein from *Bacillus thuringiensis*) expressed in cotton varieties to protect fruit from lepidopteran insects such as boll worms and herbicide tolerance that enables easy management of weeds using herbicides such as glyphosate. Bt cotton includes a variety of genes producing different toxins developed by several companies (Luttrell et al. 2015). The first herbicide-tolerant gene to be commercialized in cotton conferred tolerance to the herbicide bromoxynil (BXN by Stoneville Pedigree Company). The BXN varieties were soon replaced by those containing genes that confer tolerance to glyphosate under the name of RoundUp Ready® (Monsanto Company). Later, RoundUp Ready Flex (Monsanto Company) and LibertyLink (Bayer CropScience) cottons were introduced. Many cotton varieties contain both Bt- and herbicide-tolerant genes. So far, no other genes controlling agronomic traits and fiber properties have been introduced into cotton via transformation with commercial success. Manipulation of fiber properties especially length

and strength via biotechnology is recognized as a potential means to improve quality and develop new products.

Cotton is also on the cutting edge of genomic methods and technologies. Genome sequences of *G. arboreum*, *G. raimondii*, *G. hirsutum*, and *G. barbadense* have been published (Li et al. 2014; Paterson et al. 2012; Yuan et al. 2015; Zhang et al. 2015). Many fiber quality quantitative trait loci have been identified, and some of them are being used in breeding practices (Fang 2015; Said et al. 2013). Genes relating to fiber cell initiation (Wan et al. 2016; Wu et al. 2018; Zhu et al. 2018), elongation (Thyssen et al. 2017), and maturity (Thyssen et al. 2016) have been identified. Many more fiber genes will be identified, and the network of fiber genes regulating fiber development may be elucidated in the foreseeable future. In the subsequent chapters, the physical and chemical characteristics of cotton fiber will be described in detail. How fiber cells initiate and elongate into a 35–40-mm-long hollow tube will be illustrated. Improvement of fiber quality through conventional breeding and marker-assisted selection will be discussed.

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Chapter 2
Cotton Fiber Structure

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2.1 Introduction

Cotton is the most important natural fiber used in textiles, and it also has other uses such as being a component of high-quality paper. Because of its importance, cotton has received a great deal of study. Still, at the time of this writing, much remains to be learned about many of the details of the cotton fiber structure. These structural details must become known to understand the relations between the structure and performance properties of the fiber. That is a prerequisite for knowledge-based improvements.

A reason for failure to learn the entire story about cotton fibers is that they are very small yet have a variable and complex organization. As seed hairs, they are complete cells that undergo a multistage biosynthesis (Chap. 7). Unlike the trunk of a tree, the cotton fiber develops its outer perimeter first and then grows inward toward the lumen at the center of the fiber. The biosynthetic tissues are themselves synthesized within the fibers, but at the end of the fiber development, they have diminished to insignificance as a fraction by weight. During the 45-day or somewhat longer development of the *Gossypium hirsutum* fiber, numerous types of structures must be constructed. Various aspects of the fiber will reflect the influence of the environment during this development. Temperature, sunlight, nutrients in the soil, and especially water are keys to the characteristics of the final product. A primary variant is the amount of secondary wall cellulose within a given fiber. That degree of

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thickening, or maturity, of the fiber is a primary quality parameter (measured as maturity ratio, or by inference, micronaire; see Chap. 3) as it is a major factor in processing and one that impacts the response of the fiber to dye. Even for fibers on the same seed, different amounts of nutrients will be available, and the crowding of fibers in the developing boll will result in different growth environments.

Cotton has the distinction of being, with only mechanical cleaning, quite pure cellulose, as much as 95%. Cellulose, the sugar of little cells, is a polymer of as many as 20,000 glucose residues linked β-1→4. Figure 2.1 summarizes many of the widely accepted, if incompletely understood, components and properties of the fiber. Later in this chapter, a revision is proposed. This figure is a montage, and the individual segments are not presented to a constant scale. The dimensions are for a typical upland (*G. hirsutum*) cotton fiber; other cotton species may differ considerably in fiber diameter and length. Chapter 4 discusses more of the composition of the cuticle and primary wall; it suffices for this chapter to state that the cuticle and primary wall are the locations of waxes, pectins, and other polysaccharides, as well as various sugars and metals. Substantial amounts of these components are often removed during processing, leaving the cellulose component behind. The winding layer (Fig. 2.2) is associated more with the secondary cell wall fibers; little is known about it.

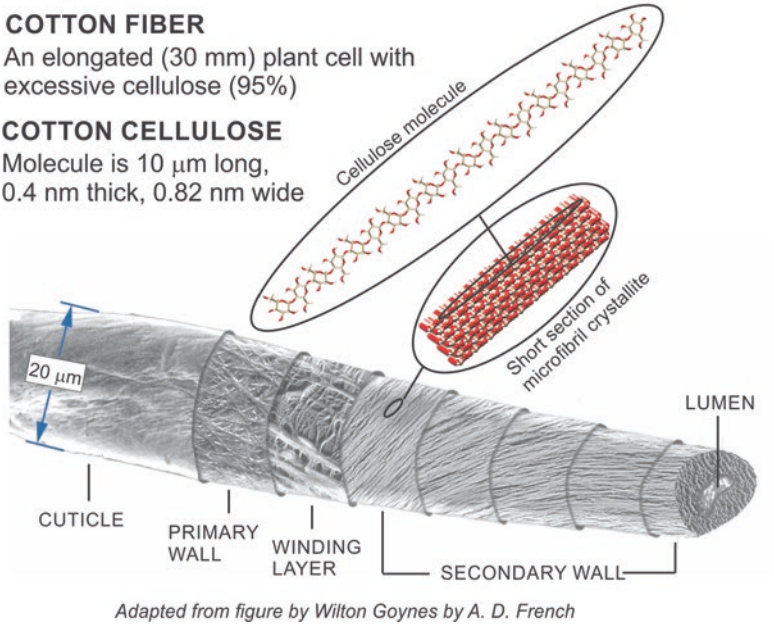

Fig. 2.1 Montage of electron micrographs, not to scale, selected and placed to resemble the different layers that compose the cotton fiber that is modified from the original figure described in Goynes (2005). In particular, the progressive change in the orientation of the microfibrils to the fiber axis as the fiber is penetrated may not be correct; see the section below on synchrotron diffraction of single fibers