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Review

Plant cellulose synthesis: CESA proteins crossing kingdoms

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ABSTRACT

Cellulose is a biopolymer of considerable economic importance. It is synthesised by the cellulose synthase complex (CSC) in species ranging from bacteria to higher plants. Enormous progress in our understanding of bacterial cellulose synthesis has come with the recent publication of both the crystal structure and biochemical characterisation of a purified complex able to synthesis cellulose *in vitro*. A model structure of a plant CESA protein suggests considerable similarity between the bacterial and plant cellulose synthesis. In this review article we will cover current knowledge of how plant CESA proteins synthesise cellulose. In particular the focus will be on the lessons learned from the recent work on the catalytic mechanism and the implications that new data on cellulose structure has for the assembly of CESA proteins into the large complex that synthesis plant cellulose microfibrils.

Contents

1. Introduction

Cellulose is most abundant biopolymer on the planet. It is a ubiquitous component of vascular plants but is also found in many lower plants including some algae, Oomcytes, tunicates as well as numerous bacterial species (Kimura and Itoh, 1995; Lin and Aronson, 1970; Romling, 2002). Mankind has utilised cellulose in daily life for millennia as it is a major component of cotton (>90%) and wood (>50%). Moreover, as we are running out of fossil fuels, attention has turned towards renewable energy sources. Cellulosic ethanol has immense potential as an alternative to fossil fuels and this has been a major driving force for recent research into the field.

There are some excellent review articles on the subject of cellulose biosynthesis (Carroll and Somerville, 2009; Endler and Persson, 2011; Li et al., 2014; Mutwil et al., 2008; Richmond and Somerville, 2000; Somerville, 2006; Somerville et al., 2004; Taylor and Turner, 2007; Wightman and Turner, 2010). In this article, we attempt to provide the reader with an up to date summary of recent developments in the field with particular focus of plant CESA proteins, their role in forming the cellulose synthase complex (CSC) and the relationship between the CSC and the basic unit of plant cellulose the microfibril (CMF).

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2. Cellulose and cellulose microfibrils

Cellulose is a polysaccharide consisting of β-1,4-linked glucose chains. These chains are then packed into highly ordered cellulose crystallites in which all chains lie in the same (parallel) direction. In plant cells, most of the cellulose occurs in the form of CMFs that are largely crystalline with some amorphous (non-crystalline) regions. Diameter of CMFs varies from species to species. The elementary fibril model for cellulose packing suggests that the higher order structure of cellulose consist of basic repeating units, the elementary fibrils. Each elementary fibril is synthesised by one CSC unit thus linking the number of chains in an elementary fibril and number of catalytic subunits or cellulose synthase (CESA) proteins in a CSC particle. Herth (1985) suggested a 36-chain model. This model has remained widely cited (Delmer, 1999), However, recent studies provide good evidence for an alternative structures. Calculations of the predicted cross sectional area of a 36-chain MF are too large to agree with recent experimental data (Jarvis, 2013). Analysis of spruce wood cellulose using both wide-angled X-ray scattering (WAXS) and small angle neutron scattering (SANS) to look at MF size, coupled with various NMR and FTIR techniques to measure the ratio of surface exposed to internal chains, are most consistent with a 24-chain MF (Fernandes et al., 2011). The authors point that while only some of the data is consistent with an 18 chain model coated with hemicellulose; the data was a good fit for 18 chain MF in which 2 adjacent MF associated along a proportion of their length. Newman et al. (2013) used mung bean microfibrils, which had earlier been shown to give excellent NMR signals, for small-angled X-ray scattering (SAXS) analysis. To ensure that no CMF aggregation occurred during preparation, other cell wall polymers were not extracted from the sample. By comparing experimental SAXS spectra with computer-simulations of MF with variable chain numbers and degree of disorder, Newman et al. (2013) showed that the data best fit an 18 chain MF, although a 24 chain MF could not be ruled out. All these observations are consistent with 18–24 chains per CMF (Fernandes et al., 2011; Kennedy et al., 2007; Newman et al., 2013; Thomas et al., 2013). Both of these studies make certain assumptions, but provide the best available experimental data to suggest that the plant MF contains 18–24 chains and not 36 as has been widely believed.

3. The CSC

CSCs were visualised by electron microscopy as early as 1972 (Dobberst and Kiermaye, 1972). The complexes were described as globular particles transported to the plasma membrane in "flat vesicles". These particles were first implicated in cellulose biosynthesis in the green alga *Micrasterias denticulata*. Brown and Montezinos (1976) frequently found CSCs attached to the end of CMFs and therefore named them terminal complexes (TCs). TCs were mainly seen as linearly ordered particles until Mueller and Brown (1980) described a rosette-shaped structure associated with TCs in freeze-fractured samples of higher plants. These hexameric rosette particles consisted of six lobes with 6-fold symmetry. Rosettes were subsequently described from several different species and associated with CMF (Chapman and Staehelin, 1985; Giddings et al., 1980; Haigler and Brown, 1986; Herth and Weber, 1984; Hogetsu, 1983; Juniper et al., 1981; Schneider and Herth, 1986). Analysis of the temperature sensitive *radially swelling 1* (*rsw1*) mutant provided direct evidence for the involvement of rosettes in cellulose synthesis. At the restrictive temperature, *rsw1* plants exhibit decreased cellulose which is correlated with a loss of rosettes from the plasma membrane (Arioli et al., 1998).

One important observation worth noting is that, while there is good evidence to link rosettes with cellulose synthesis, there are many systems, such as cotton fibres, in which rosettes have not been observed. This might partly reflect an absence of experimentation, but it is also possible that there are circumstances in which plants make different kinds of cellulose that maybe synthesised by enzyme complexes other than rosettes (see below). Furthermore, while cellulose is largely made at the plasma membrane, in some circumstances, such as cell plate formation, cellulose appears to be made within the tubulo-vesicular membrane network during cell plate formation and precedes formation of the new plasma (Miart et al., 2014).

4. Cellulose synthase activity

Because of the biological and economic importance of cellulose, attempts have been made to purify the intact complex from plants and achieve *in vitro* cellulose biosynthesis. This has proved extremely difficult to achieve. One of the main problems arises from the fact that the detergent-solubilised protein extracts from plants frequently synthesise both β-1,4 glucan (cellulose) and very large amounts of β-1,3 glucan (callose), and it has proved difficult to separate the two activities. A report from Lai-Kee-Him et al. (2002) demonstrated clear cellulose synthase activity in solubilised extracts; however, this activity was lost as soon as purification was attempted.

While purifying a functional CSC from plants remains problematic, some useful information can be gained from recent breakthroughs in the study of cellulose synthase activity in bacteria. Bacterial cellulose synthesis (Bcs) genes were first characterised more than 20 years ago (Saxena et al., 1990; Wong et al., 1990). The molecular biology of Bcs proteins has previously been reviewed by Romling (2002). Essentially, the Bcs operon encodes at least 3 proteins. BcsA (also known as celA, acsAB) is an integral membrane protein that contains the catalytic domain and forms a trans-membrane (TM) pore across the inner membrane. BcsB (also known as celB) is a periplasmic protein anchored to the plasma membrane (PM) via a single TM domain. BcsZ (also known as celC) has been shown to encode a cellulase which is required for cellulose biosynthesis. Other bacterial genes implicated in cellulose biosynthesis include BcsC, BcsD, Ccp and CelDE.

The demonstration that bacterial proteins (BcsA and BcsB) are sufficient for *in vitro* cellulose biosynthesis when heterologously expressed in *Escherichia coli* has brought real clarity to this area (Omadjela et al., 2013). The authors were able to demonstrate a requirement for UDP-glucose as a substrate, but no requirement for any lipid linked intermediates as has been shown for some glycosyltransferases (Matthysse et al., 1995). The purified complex of BscA/B was able to generate chains with a degree of polymerisation (DP) of 200–300 and at a rate that might be up to 10× of that observed in plants. This supports an idea that the assembly of individual chains into a higher order structure maybe the rate limiting step for cellulose synthesis in plants. Since no other energy source was added to the assay, this work also demonstrates that the energy to drive the growing cellulose chain through the membrane pore must come from polymerisation of UDP-glucose.

Cellulose synthesis from purified BscA/B also exhibits no requirement for a primer for chain initiation (Omadjela et al., 2013). An early report that a sitosterol glucoside (SG) is required as a primer for cellulose synthesis (Peng et al., 2002) has been hard to unambiguously substantiate. Several sterol mutants are cellulose deficient, but there is no correlation between sitosterol levels and cellulose content (Schrick et al., 2004). Sitosterol glucosides are synthesised by UDP-glucose: sterol glycosyltransferase that is encoded by two genes in Arabidopsis. A double mutant caused by insertion in both of these genes exhibited dramatically decreased SG levels and a variety of phenotypic abnormalities,

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