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Mutation and Complementation of a Cellulose Synthase (CesA) Gene

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Cellulose is a carbohydrate polymer that is composed of repeating glucose subunits. Being the most abundant organic compound in the biosphere and comprising a large percentage of all plant biomass, cellulose is extremely plentiful and has a significant role in nature. Cellulose is present in plant cell walls, in commercial products such as those made from wood or cotton, and is of interest to the biofuel industry as a potential alternative fuel source. Although indigestible by humans, cellulose is nutritionally valuable, serving as a dietary fiber. Because of its ubiquity and importance in many areas, studying cellulose will prove to be useful.

Understanding its production mechanisms would lead to the ability to control cellulose synthesis and composition. Furthermore, since the cellulose producing machinery differs amongst all types of plants, learning more about cellulose production could increase our understanding of the evolution of certain plants and the cellulose producing proteins. Cellulose fibers are synthesized by a Cellulose Synthesizing Complex (CSC). Each CSC is comprised of hypothetically 36 cellulose synthase proteins (CesA proteins), which are arranged in a six-lobed "rosette" structure. Therefore, a CSC is made up of six lobes, each of which is composed of six CesA proteins/subunits. However, the organization of the subunits is undetermined, so for example, whether there is a specific, ordered arrangement to the positioning of the subunits, what the ratios of the subunits are, or which subunits associate with like or unlike subunits is unknown.

The objectives of this project are to create a "knockout" mutation in the gene that encodes the CesA4 protein in the moss Physcomitrella patens, and to subsequently determine whether CesA4 mutants are phenotypically distinct from the wild-type organism. Mutation of P. patens will be achieved by knocking out CesA4. P. patens is a model organism because it spends a large portion of its life cycle in a haploid phase where the organism has only one set of chromosomes, and thus one set of genes. This, along with P. patens' high rate of homologous recombination makes knocking out and integrating genes into its genome feasible. In conclusion, the purpose of this project is to further the understanding of the role of the CesA proteins, specifically CesA4, and of the synthesis of cellulose.

Keywords: cellulose, CesA, CSC, Knockout, Moss, Mutation, Physcomitrella patens, Synthase.