

# Cotton annexin proteins participate in the establishment of fiber cell elongation scaffold

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**C**otton plant is one of the most important economic crops in the world which supplies natural fiber for textile industry. The crucial traits of cotton fiber quality are fiber length and strength, which are mostly determined by the fiber elongation stage. Annexins are assumed to be involved in regulating fiber elongation, but direct evidences remain elusive. Recently, we have investigated the activities of fiber-specific expressed annexins AnGb5/6 and their interacted proteins in cotton. AnGb5 and 6 can interact reciprocally to generate a protein macro-raft in cell membrane. This macro-raft is probably a stabilized scaffold for Actin1 organization. The actin assembling direction and density are correlated with *AnGb6* gene expression and fiber expanding rate among three fiber length genotypes. These results suggest that annexins may act as the adaptor that linked fiber cell membrane to actin assembling. Due to the strong  $\text{Ca}^{2+}$  and lipid binding ability of annexins, these results also indicate that annexins complex may function as an intermediate to receive  $\text{Ca}^{2+}$  or lipid signals during fiber elongation.

Cotton fibers are single-cell trichomes that differentiate from the outer layer cells of seed coat.<sup>1</sup> After initiation, cotton fiber cells rapidly and continuously elongate for about 20–25 d and develop into fibers with 2–3 cm length in final.<sup>2</sup> During the repaid elongation process, fiber cells need to establish the cytoskeleton to sustain their vigorous expansive growth.

Numerous data support that actin plays an important role in the transportation of organelles and vesicles carrying

membranes components to the site of polar growing cells like root hairs and trichomes.<sup>3–6</sup> Electron microscopy and cytoplasm component analysis show that actin and microtubulin proteins are the main components of cytoskeleton in cotton fiber cell.<sup>7</sup> A large amount of F-actin bundles occur in fiber cell at the early stage of fiber elongation to keep pace with rapid fiber growth.<sup>7</sup> F-actin bundling can regulate microtubule orientation at the stages of fiber elongation and secondary cell wall biosynthesis.<sup>8</sup> Investigation of cotton actin families' expression profiles and the phenotype of *GhACT1RNAi* plants demonstrate that Actin1 has a critical function in fiber elongation.<sup>9</sup> Genetic analysis of actin binding proteins strongly supports that increased actin bundles are required for fiber expansion.<sup>10,11</sup> Although actin function in fiber elongation has been elucidated recently, there is few report to explain that who provides the domain for actin assembling during fiber elongation.

In recent study, we showed that two cotton annexins predominantly expressed in fiber are likely to provide the stabilized scaffold for Actin 1 and its subsequent proteins' recruitment during fiber elongation.<sup>12</sup> In this report, we confirmed that both AnGb5 and AnGb6 can form homo-dimers and hetero-dimers. Slightly different from AnGb5, AnGb6 specifically binds to GbACT1 on the cell membrane. Increased *AnxGb6* expression in *Arabidopsis* enhances actin aggregation at the basal of root cells, implying that there is the site for actin assembling. As confirmed by actin staining, original actin aggregation in fiber may begin

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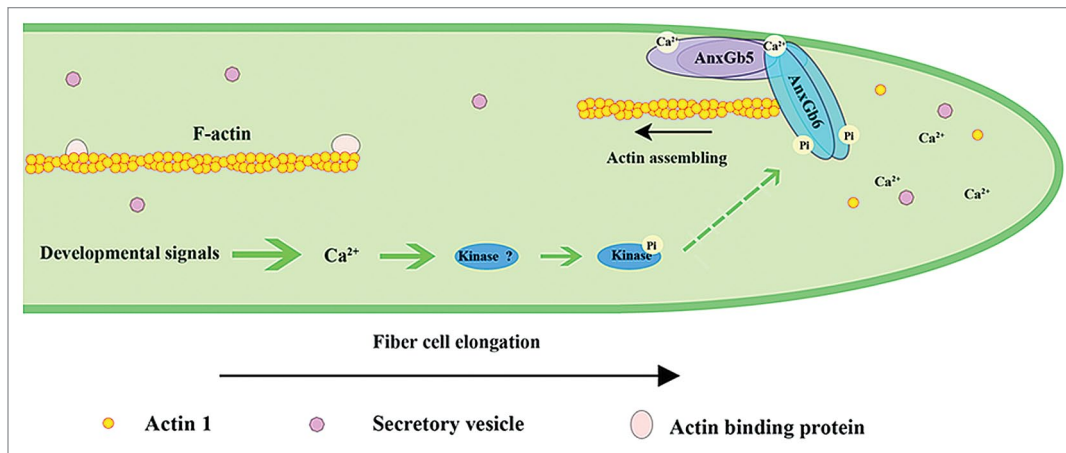
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**Figure 1.** A proposed model illustrating potential functions of annexins during cotton fiber elongation. High concentration of  $\text{Ca}^{2+}$  ions induce the phosphorylation of unknown CDPK kinase upon stimulated by developing signals. CDPKs then modify annexins at post-translational level; AnxGb5 homo-dimers are localized on the cell membrane of fiber tip, and provide a macro-raft for AnGb6 homo-dimers and their interactions with GbAct1. This protein complex helps F-actin assembling, cytoskeleton organization and vesicle transportation.

from the tip of fiber cell, and is consisted with fiber expanding direction. Decrease AnxGb5 and AnxGb6 transcriptional and post-transcriptional expression in cotton mutant result in shorter fiber length in cotton.<sup>13</sup> According to the model of depicting different annexins' functions in animal cell, we deduce that the roles of AnxGb5, 6 are different during fiber elongation.<sup>14</sup> AnxGb5 homo-dimers that are localized in the fiber cell membrane provide the formation of macro-rafts. These macro-rafts are the places where AnGb6 interacts with GbAct1 for F-actin organization (Fig. 1).

Elongation fiber cell has only primary cell wall without secondary cell wall. Fiber primary cell walls contain significantly higher amounts of pectin than ovule cells. Biosynthesis of pectin precursors like UDP-L-rhamnose, UDP-D-galacturonic acid is proved to be important for cotton fiber initiation and elongation.<sup>15</sup> Suppressed actin gene expression blocks transporting CesA or pectin precursor to the growing regions, directed expanding of tip-growing cell and fiber elongation.<sup>9,16,17</sup> *AnxGb6* gene expression in three cotton genotypes with different fiber length is correlated with actin density and actin organization. Based on above data together, we deduce that *AnxGb6* expression affects fiber elongation possibly by regulating the formation of actin bundle and pectin precursor deposition.

The annexins are a multi-gene family of calcium-dependent or independent phospholipids binding proteins, which can participate in signaling network and membrane trafficking during cell expanding.<sup>18</sup> In animal, annexin regulates F-actin cytoskeleton rearrangement by reversible protein phosphorylation.<sup>19</sup> In rice, annexin protein Os05 g31750 can interact with 4 kinases including Ste20-like kinase and SPK-3 kinase Os01 g64970.<sup>20</sup> A cotton annexin like protein, phosphorylated by a unknown  $\text{Ca}^{2+}$ -dependent kinase, modulates the activity and/or localization of callose synthase during fiber elongation.<sup>21</sup> McCDPK1 from *Mesembryanthemum crystallinum* is involved in vesicle-mediated trafficking along the actin-filament of the cytoskeleton.<sup>22</sup> AnxGb6 is predicted to have several phosphorylation sites like Ser<sup>113</sup> and Ser<sup>169</sup> by searching phosphorylation site on ExPASy (<http://www.expasy.org/>). These studies lead to the hypothesis that AnxGb6 may be phosphorylated by unknown CDPK and functions as a signal transducer in fiber elongation.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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