Cotton fibers constitute the most abundant renewable source of textile material. They develop from single-celled trichomes on the ovule epidermis in four overlapping stages: initiation, rapid elongation, secondary wall thickening, and maturation (Graves and Stewart, 1988; Kim and Triplett, 2001; Chen and Guan, 2011). In upland cotton (Gossypium hirsutum L.), 25–30% of ovule epidermal cells begin to differentiate into lint fibers before, or on the day of anthesis (0 days post anthesis, DPA). The differentiation of the remaining epidermal cells is initiated at approximately 4 or 5 DPA and results in the formation of fuzz fibers (Chen and Guan, 2011). Importantly, while lint fibers are valuable for the textile industry, fuzz fibers have no economic value (Fig. 1).

Numerous studies have focused on the mechanisms involved in fiber initiation leading to the suggestion that phytohormones and their signal transduction pathways play an important role at this stage, and work on the regulation of phytohormone-mediated fiber initiation can be traced back to 1971 when IAA and GA3 were demonstrated to be a critical stimulus for fiber production in vitro (Beasley, 1971). Auxin is responsible for fiber cell initiation and it has been shown that raising the content of IAA on the day of anthesis increased the number of fiber cell initials (14–19%) and reduced the number of fuzz fibers (Zhang et al., 2011). On the day of anthesis, auxin accumulates at high concentration in the initiating fiber cells, but not in other epidermal cells. The GhPIN3a protein, an auxin efflux carrier localized in the outer integument, has been proposed to mediate fiber-specific auxin accumulation, promoting fiber initiation (Zhang et al., 2017). Conversely, cytokinin (CK) inhibits fiber cell growth in cotton and is mostly required for seed development (Beasley and Ting, 1974; Jones and Schreiber, 1997; Zhao et al., 2015; Zhu et al., 2018). In their new study, Zeng et al. (2019) demonstrate that CK suppresses fiber initiation by inhibiting non-fiber-localized protein GhPIN3a and disrupting the formation of the auxin concentration gradient in fiber cells that is required for fiber initiation.

**Role of cytokinin in cotton fiber initiation**

Addition of exogenous CK significantly promotes the growth of ovules but inhibits the production of fibers (Beasley et al., 1974). However, Chen et al. (1997) reported that the level of CK in cotton fibers increased before flowering but decreased afterward, indicating that cytokinins may regulate fiber initiation. Zeng et al. (2019) have used proTCS, a cytokinin-inducible promoter, to drive the expression of GUS reporter gene for in situ observations of the changes in the level of CKs in epidermal cells. However, the epidermis is only a thin layer over the ovule, precise separation of epidermal cells from the ovules at -2, -1, and 0 DPA is very difficult and, sometimes, nearly impossible. Therefore, in situ observation of CK signal in this study was an ingenious approach to avoiding the problems inherent in the sample separation. The results demonstrated that a relatively high concentration of CK is present in the epidermis of the developing cotton ovules at -2, -1, and 0 DPA, and the level of CK declines during the subsequent development. However, addition of exogenous CK or increase of the endogenous CK concentration by inhibition of CK dehydrogenase (GhCKX3) inhibited fiber initiation, demonstrating that CK acts as a negative regulator of cotton fiber initiation.

**Relationship between CK and auxin in cotton fiber initiation**

The interaction between CK and auxin is involved in many processes of plant development (Dello Ioio et al., 2008; Muller...
Auxin transport and cytokinin antagonism

Zhang et al. (2017) documented that GhPIN3a is an auxin efflux protein and that it may function as a key regulator of fiber initiation via directing or redirecting the auxin pathway. This question was addressed in the work of Zeng et al. (2019), by demonstrating that downregulation of GhPIN3a inhibits fiber initiation and that GhPIN3a promotes the accumulation of auxin in fibers. Further analysis indicated that the GhPIN3a protein is distributed in a polar manner at the plasma membrane of non-fiber cells but is delocalized in fiber cells. Thus, GhPIN3a may participate in exporting auxin from non-fiber cells to the adjacent fiber cells, a process that would result in the accumulation of auxin in fibers. Further, a high concentration of CK inhibits the expression and polar distribution of GhPIN3a in non-fiber cells, providing a mechanism through which cytokinin antagonizes this local auxin gradient. This effect would inhibit the accumulation of auxin in cotton fiber cells and, therefore, inhibit fiber initiation. This work provides a novel insight into the interaction between fiber cells and non-fiber cells based on the differential distribution of GhPIN3a (Zeng et al., 2019).

Stepping forward

The determinants of the differentiation of the ovule epidermal cells into lint fibers or fuzz fibers in cotton remain a mystery (Fig. 1). Fiber initiation is a complicated transition involving thousands of genes, and the function of a large number of them has been investigated experimentally and many indicate involvement of other hormone signaling pathways. For instance, GhDET2 and Gh14-3-3L, which are involved in Brassinolide (BR) biosynthesis and signal transduction, contribute to fiber cell initiation (Luo et al., 2007; Zhou et al., 2015) and the negative regulator of jasmonic acid (JA) signaling, GhJAZ2, inhibits fiber initiation. In the study of Zeng et al. (2019), the authors demonstrated that CK regulates fiber initiation by mediating GhPIN3a expression or distribution in non-fiber cells. Although both the non-fiber cells and fiber cells appear to have the same level of CK at the time of initiation (0 DPA) in wild-type, non-fiber cells have the GhPIN3a polar localization, but fiber cells lose the GhPIN3a polar localization (Zeng et al., 2019). Thus, the key factor controlling the loss of GhPIN3a polar localization in fiber cells remains unelucidated.

Nevertheless, the work of Zeng et al. (2019) provides novel evidence that the polar distribution of GhPIN3a in non-fiber cells constitutes a “transport pipeline” for auxin accumulation in fiber cells, which promotes fiber development. This finding points to a new beneficial strategy for cotton breeding, i.e., optimization of the auxin transport pipeline. For example, since shorter fuzz fibers are of no value for the textile industry, the pipeline transporting auxin can be directed to valuable lint fibers and away from fuzz fibers, improving the yield and quality of lint fibers. Undoubtedly, the mechanism responsible for the polar distribution of GhPIN3a still needs to be elucidated, and the factor directly regulating GhPIN3a in non-fiber cells in

Fig. 1. Phenotypic observation of lint fibers and fuzz fibers in TM-1. (A) Seed phenotype after combing of fibers. TM-1 is the Upland cotton genetic standard (Kohel et al., 1970). The seed epidermis of TM-1 had long lint fibers and short fuzz fibers. Bars, 1.0 cm. (B) Feature of the lint fibers. (C) Feature of the fuzz fibers.

and Sheen, 2008). For example, CK promotes the biosynthesis of auxin in young root and shoot tissues (Laplaze et al., 2007; Dello Ioio et al., 2008) and affects auxin transport by disrupting the PIN-dependent generation of the auxin maximum during the development of lateral roots (Laplaze et al., 2007). In cotton, auxin promotes fiber cell initiation (Zhang et al., 2011) and, in contrast to CK, the IAA concentration gradually increased in ovule epidermal cells from 0 DPA (Zeng et al., 2019). This increase in IAA concentration was measured using the GUS gene driven by an auxin-inducible promoter proDR5. These results suggest the presence of an antagonistic relationship between CK and auxin during fiber initiation (Zeng et al., 2019), and this was tested by expressing an IAA or CK biosynthetic gene (i.e., iaaM or IPT) under the control of, respectively, a cytokinin- or an auxin-inducible promoter (i.e., proTCS:iaaM and proDR5:IPT). This clever approach removed the potential antagonistic relationship over the biosynthesis of these two phytohormones. The results obtained in this experiment confirmed expectations: CKs-induced synthesis of IAA reversed the inhibition of fiber initiation imposed by CKs, and the IAA-induced formation of CK attenuated the stimulating impact of IAA. This antagonistic relationship was corroborated by analyzing the content of CK and auxin in the fiberless mutant xu142fl, whose epidermal cells contain more cytokinin and less auxin than in the wild-type plant. Thus, the study of Zeng et al. (2019) demonstrated the antagonistic relationship of CK and auxin in ovule epidermis by utilizing diverse experimental strategies, including in vitro culture of ovules, transgenic cotton, and natural mutant cotton.
CK signaling pathway must be identified. Nevertheless, the high concentration of CK in the ovule epidermis generated in the study of Zeng et al. could provide experimental direction to achieve the goal of enhancing the quality and yield of cotton lint fibers. There is no doubt that more exciting research will follow.

Competing Financial Interests
The authors declare no competing financial interests.

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Keywords: auxin, cotton, cytokinin, fiber initiation, GhPIN3a, polar auxin transport.

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