

# ZFP5 encodes a functionally equivalent GIS protein to control trichome initiation

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The *Arabidopsis thaliana* trichome development is a model system for understanding various aspects of plant cell development and differentiation. The C2H2 zinc finger proteins GIS, GIS2, and ZFP8 play important roles in controlling trichome initiation. In our recent study, we reported that a new C2H2 zinc finger protein, ZINC FINGER PROTEIN 5 (ZFP5), controls trichome cell development through GA signaling. ZFP5 acts upstream of GIS gene family and key trichome initiation regulators, and ZFP8 is the direct target gene of ZFP5. Here we show that ZFP5 encodes a protein functionally equivalent to GIS and GIS2 in controlling trichome initiation. Furthermore, similar to GIS2, ZFP5 is not involved in trichome branching.

In plants, the C2H2 zinc finger protein (ZFP) gene family is one of the largest groups of regulatory proteins and its members play several important roles in growth and development.<sup>1,2</sup> The classical C2H2 zinc finger domain is involved in a wide range of biological activities and can bind to DNA, RNA and proteins.<sup>2,3</sup> There are 176 zinc finger proteins in *Arabidopsis thaliana*; they hence constitute the most abundant family of putative transcriptional regulators in the plant. Only 33 zinc finger proteins in *A. thaliana* are conserved in other eukaryotes.<sup>4,5</sup> C2H2 zinc finger transcription factors play an important role in plant tolerance to various environmental stresses such as drought, cold, osmotic stress, wounding and mechanical loading. To date, many sequences of C2H2 zinc finger transcription factors in relation to plant development and stress responses have been identified, including those of potato,<sup>6</sup> rice,<sup>7</sup> and tobacco.<sup>8</sup>

## ZFP5 Encodes a Protein Functionally Equivalent to that of GIS in Controlling Trichome Initiation

As we reported previously,<sup>9–11</sup> overexpression of ZFP5 leads to high levels of trichome initiation on all inflorescence organs and ectopic trichome formation on floral organs, similar to the phenotype of GIS, ZFP8, and GIS2 overexpression. In addition, overexpression of ZFP5 could restore *gis*, *gis2* and *zfp8* mutant trichome phenotypes. In order to further explore whether the coding regions of ZFP5 had similar roles of GIS and GIS2, we have done two complementation experiments. To complement the mutant phenotypes of *gis* and *gis2*, we fused the coding sequence of ZFP5 under the control of either GIS or GIS2 promoters and transfer it to *gis* or *gis2* mutants, respectively. We found that *pGIS:ZFP5* could completely restore the mutant phenotype of *gis* (Fig. 1A). Furthermore, *pGIS2:ZFP5* could

complement the less trichome phenotypes on the flowers of *gis2* (Fig. 1B). These results indicated that ZFP5 protein shares a redundant role with GIS and GIS2, and these proteins are functionally equivalent.

## ZFP5 Plays no Role in Trichome Branching

*A. thaliana* trichomes are large-branched single cells<sup>12</sup> and are considered as an excellent model system for studying plant cell development. Up to now,<sup>13</sup> many genes controlling trichome branch development have been reported.<sup>14</sup> While some of these genes control the microtubule organization,<sup>15</sup> and trichome cell branching in *A. thaliana*,<sup>12,16,17</sup> others affect the number of endoreduplication cycles and probably, as a consequence, the trichome branch number.<sup>18–21</sup> Some of these genes affect branch number without affecting endoreduplication.

As we previously reported,<sup>9</sup> GIS not only plays a key role in trichome initiation but is also involved in trichome branching. To determine whether ZFP5 affects trichome branching, we first examined the number of trichome branches on the main stem of *zfp5*, *ZFP5:Ri-2*, wild-type plants and the overexpression lines *O-1*, *O-2*. There were only one-branched and two-branched trichomes on the main stem for *zfp5* mutant, *ZFP5:Ri-2*, *O-1*, *O-2*, and the control plants (Fig. 2A). Furthermore, there were no significant differences in the percentage of one- and two-branched trichome between *zfp5* mutant, *ZFP5:Ri-2*, *O-1*, *O-2*, and the control plants (Fig. 2D). To further explore whether ZFP5 have any function in trichome branching on the rosette leaves, we investigated the differences in trichome branching on both the adaxial and the abaxial side of the 7th rosette. No significant differences among *zfp5* mutant, *ZFP5:Ri-2* and wild-type plants were observed (Fig. 2B, C, E and F). Taken together,

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**Figure 1.** *ZFP5* and *GIS*, *GIS2* have equivalent activities. (A) Trichome branching and density on the main stems of *gis* (left) and *pGIS:ZFP5:gis* (center) compared with wild-type stems (right). WT, wild-type. (B) Trichome branching and density on the flowers of *gis2* (left) and *pGIS2:ZFP5:gis2* (center) compared with wild-type stems (right). WT, wild-type.

these results indicate that *ZFP5*, similar to *GIS2*, is not required for trichome branching in *A. thaliana*.

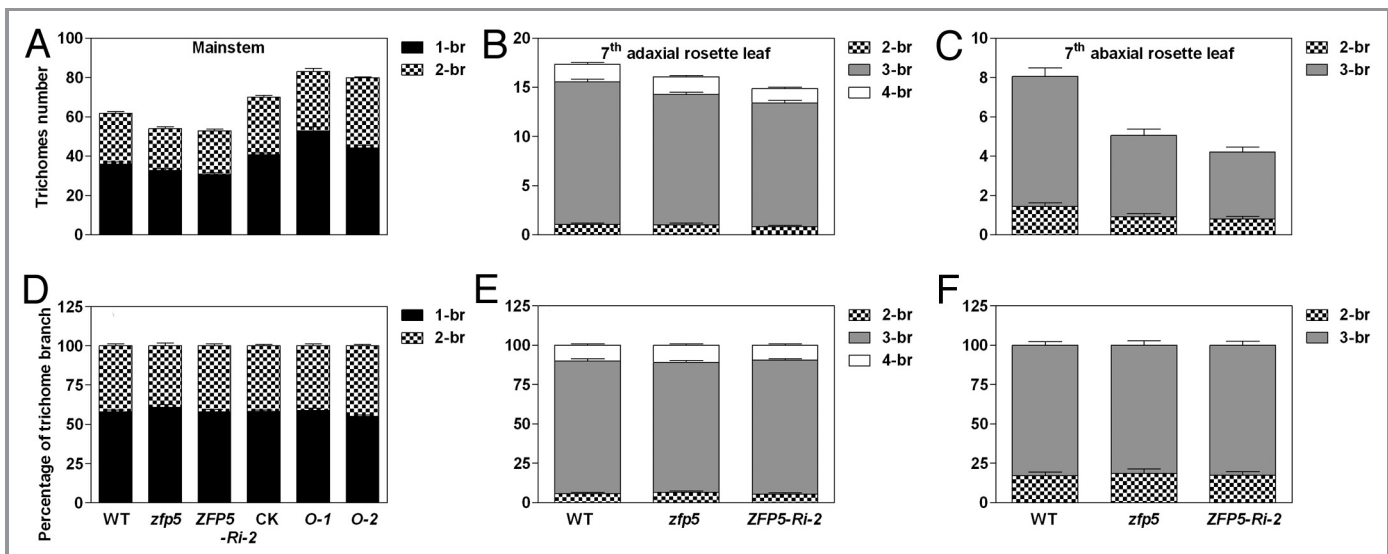
In conclusion, The *GIS*, *GIS* and *ZFP5* genes encode functionally equivalent proteins but have diverged in their response to phytohormones and in their roles during inflorescence development. *GIS* mainly controls inflorescence stem trichome initiation through GA signaling while as *GIS2* is key gene to control inflorescence flower trichome initiation by integrating GA and cytokinin signaling. *ZFP5* controls inflorescence trichome initiation through GA signaling by acting upstream of *GIS* and *GIS2*. *GIS* plays a key role in inflorescence stem branching, but *GIS2* and *ZFP5* is not involved in the regulation of trichome branching.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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**Figure 2.** The phenotype of trichome branching. (A and D) The trichome branch of the wild-type (control), *zfp5*, *ZFP5-Ri-2*, pEmpty-vector (control), *O-1* and *O-2* branching phenotype in Main stems inflorescence 1.5 cm cutting 2.0 cm counting. *O-1*, *35S:ZFP5*-overexpressing line 1; *O-2*, *35S:ZFP5*-overexpressing line 2. WT, wild-type; CK, Control; 1-br, one-branched (unbranched) trichomes; 2-br, two-branched trichomes. (B and E) The trichome branch of the wild-type (control), *zfp5* and *ZFP5-Ri-2* branching phenotype on the adaxial 7th rosette leaf trichomes. WT, wild-type; 2-br, two-branched trichomes; 3-br, three-branched trichomes; 4-br, four-branched trichomes. (C and F) The trichome branch of the wild-type (control), *zfp5* and *ZFP5-Ri-2* branching phenotype on the abaxial 7th rosette leaf trichomes. WT, wild-type; 2-br, two-branched trichomes; 3-br, three-branched trichomes.

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