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(植物分子育種分野担当: 第33回)

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## Distinguished Professor Teh-hui Kao

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### S-RNase-Based Self-Incompatibility in *Petunia*: a Complex Mechanism of Self/Non-Self Recognition between Pollen and Pistil

Self-incompatibility allows the pistil to reject self-pollen to prevent inbreeding and accept non-self pollen for outcrossing. Over the past three decades, my lab has been using *Petunia inflata* as a model to study the type of self-incompatibility that has so far been found in the Solanaceae and two other families. Self/non-self recognition is regulated by the highly polymorphic *S*-locus (e.g., >30 *S*-haplotypes have been reported in *Petunia*). Matching of the *S*-haplotype carried by the pollen and one of the *S*-haplotypes present in the pistil results in inhibition of self-pollen tube growth in the upper segment of the pistil. The *S*-locus houses the *S-RNase* gene for pistil specificity<sup>1)</sup> and multiple *S*-locus *F*-box (*SLF*) genes for pollen specificity<sup>2, 3)</sup>. We have used pollen transcriptome analysis to identify a total of 17 *SLF* genes in *S*<sub>2</sub>-haplotype and *S*<sub>3</sub>-haplotype<sup>4)</sup>, and used co-immunoprecipitation and mass spectrometry to show that all these *SLF* proteins are assembled into similar SCF complexes, which contain a conventional Rbx1 (named PiRBX1), a pollen-specific Cullin1 (named PiCUL1-P) and a pollen-specific Skp1-like protein (named PiSSK1)<sup>5)</sup>. According to the collaborative non-self recognition model<sup>3)</sup>, for a given *S*-haplotype, each SCF complex interacts with a subset of non-self *S*-RNases, determined by the *SLF* protein it contains, to mediate their ubiquitination and degradation via the 26S proteasome. A complete suite of SCF complexes is required to detoxify all non-self *S*-RNases to allow cross-compatible pollinations. We have used a transgenic functional assay<sup>2)</sup> to determine the interaction relationships of *SLF* proteins of *S*<sub>2</sub>-haplotype and *S*<sub>3</sub>-haplotype with 11 *S*-RNases. So, far 137 interaction relationships have been determined. We have used a chimeric gene approach to identify candidate amino acids responsible for differential interactions between *SLF* proteins and *S*-RNases<sup>6)</sup>. We have used CRISPR/Cas9 genome editing to examine the roles of *S*<sub>2</sub>-*SLF1*, *PiCUL1-P* and *PiSSK1*<sup>7)</sup>, and the results fully support the collaborative non-self recognition model and the interaction relationships between *SLF* proteins and *S*-RNases determined by the genetic approach.

#### References

- 1) Lee H-S, Huang S, Kao T-h (1994). S-proteins control rejection of incompatible pollen in *Petunia inflata*. *Nature* 367: 560-563
- 2) Sijacic P, Wang X, Skirpan AL, Wang Y, Dowd, PE, McCubbin AG, Kao T-h (2004). Identification of the pollen determinant of S-RNase-mediated self-incompatibility. *Nature* 429: 302-305
- 3) Kubo K-I, Entani T, Takara A, Wang AJ, Fields AM, Hua Z, Toyoda M, Kawashima S-i, Ando T, Isogai, Kao T-h, Takayama S (2010). Collaborative non-self-recognition in S-RNase-based self-incompatibility. *Science* 330: 796-799
- 4) Williams JS, Der, JP, dePamphilis CW, Kao T-h (2014). Transcriptome analysis reveals the same 17 *S*-locus *F*-box genes in two haplotypes of the self-incompatibility locus of *Petunia inflata*. *Plant Cell* 26: 2873-2888
- 5) Li S, Sun P, Williams JS, Kao T-h (2014). Identification of the self-incompatibility locus F-box protein-containing complex in *Petunia inflata*. *Plant Reprod* 27: 31-45
- 6) Wu L, Williams JS, Wang N, Khatri WA, San Román D, Kao T-h (2017). Use of domain-swapping to identify candidate amino acids involved in differential interactions between two allelic variants of type-1 *S*-locus F-box protein and *S*<sub>3</sub>-RNase in *Petunia inflata*. *Plant Cell Physiol* doi.org/10.1093/pcp/pcx176
- 7) Sun L, Kao T-h (2017). CRISPR/Cas9-mediated knockout of *PiSSK1* reveals essential role of *S*-locus F-box protein-containing SCF complex in recognition of non-self *S*-RNases during cross-compatible pollination in self-incompatible *Petunia inflata*. *Plant Reprod* doi.org/10.1007/s00497-017-0314-1

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