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Regulation of sporopollenin synthesis for pollen wall formation in plant

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Pollen wall is the most complicated cell wall in plant. It is composed of outer exine and inner intine with the exine further divided into sexine and nexine. The composition of intine layer is generally considered to be same as a plant cell wall which is mainly composed of cellulose. Nexine layer can only be observed under transmission electron microscope. Recent investigation suggested that the major composition of nexine is arabinogalactan proteins (Jia et al., 2015). The sexine layer is composed of sporopollenin which is quite stable and resistant to degradation by enzymes and strong chemical reagents. The sporopollenin deposition determines the pollen wall pattern which is widely used for plant taxonomic classifications. After meiosis, microsporocyte divides into four microspores enwrapped in a tetrad with each microspore further develops into a mature pollen. Materials from tapetum penetrate tetrad wall to deposit on the surface of microspore at late tetrad stage. The pollen wall pattern is determined inside the tetrad (Xu et al., 2016). After microspore release, the pollen wall materials continue deposition to form decorated sexine layer and flat nexine (Zhou et al., 2015). After exine layer occurs, intine layer is gradually formed between exine and microspore plasma membrane.

Although the chemical composition of sporopollenin is not exactly known, it is assumed to consist of the heterogeneous materials derived from long-chain fatty acids, oxygenated aromatic rings and phenylpropionic acids. Several precursors are chemically cross-linked to form a rigid structure (Quilichini et al., 2015). In *Arabidopsis*, multiple genes involved in sporopollenin synthesis have been identified. Acyl-coa synthetase 5 (ACOS5) catalyzes esterification of medium and long-chain fatty acids to produce corresponding acyl-CoA ester for sporopollenin biosynthesis. *Male sterile 2* (*MS2*) encodes a fatty acyl acyl carrier protein (ACP) reductase. It converts palmiltoyl-ACP to the corresponding fatty alcohol for monomeric constituents of sporopollenin. CYP703A2 and CYP704B1 could catalyze the conversion of medium or long-chain saturated fatty acids to the corresponding monohydroxylated fatty acids. For phenylpropanoids synthesis, polyketide synthase A (PKSA) and PKSB (also named LAP6 and LAP5) catalyze the condensation of fatty acyl-CoA esters and malonyl-CoA to yield triketide and tetraketide α-pyrones in the precursor synthesis of sporopollenin. Sequentially, tetraketide α-pyrone reductase 1 (TKPR1), and TKPR2 reduce the ketone carbonyl function of tetraketide α-pyrone compounds produced by PKSA/PKSB. The pathways of these enzymes to synthesize sporopollenin precusors have been proposed (Quilichini et al., 2015).

Several transcription factors essential for tapetum development and functions have been identified in *Arabidopsis*. Dysfunctional tapetum 1 (DYT1), defective in tapetal development (TDF1) and aborted microspores (AMS) function at early stage of tapetum development. *DYT1* and *AMS* encode putative basic helix-loop-helix (bHLH) transcription factors,

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whereas *TDF1* encodes a putative R2R3 MYB transcription factor. Knockout of these genes lead to tapetal hypertrophy extending into the locule. A further member of the R2R3 MYB family, MS188 (also named AtMYB103 or MYB80), and a PHD-finger protein MS1 function at late stage of tapetum development. These genes form a genetic pathway (*DYT1-TDF1-AMS-MS188-MS1*). In this pathway, *DYT1* directly regulates the expressions of *TDF*1. The expression of *TDF1* in *dyt1* restores the expression of most pollen wall related genes suggesting pollen wall synthesis pathway is downstream of *TDF1* (Gu et al., 2014). In rice, the homologues of these transcription factors have been identified. The functions of these homologues for tapetal development and pollen wall formation are consistent with that of the regulators in *Arabidopsis*. These transcription factors also form a genetic pathway similar with that in *Arabidopsis* (Cai et al., 2015).

MS188 is an essential regulator for sexine formation as it is completely absent in *ms188*. CYP703A2 is one of the enzymes for sporopollenin synthesis (Morant et al., 2007). In *ms188*, the expression of *CYP703A2* is barely detected. Chromatin immunoprecipitation (ChiP) and electrophoretic mobility shift assays (EMSA) showed that MS188 directly associates with *CYP703A2* promoter. In tobacco leaves, MS188 can drive the expression of *CYP703A2* when co-transforming with *p35s::MS188-nos* and *pCYP703A2::CYP703A2*. These reveal that MS188 directly regulates the expression of *CYP703A2* (Xiong et al., 2016). This is in agreement with the phenotype that the sexine is absent in *ms188* mutant. In tapetum, AMS directly regulates *MS188* (Lou et al., 2014). EMSA and ChiP analysis revealed that AMS is associated with *CYP703A2* promoter (Xu et al., 2014). In plant, many MYB transcription factors have been reported to interact with bHLH transcription factors to regulate the expression of downstream genes. MS188 can also interact with AMS. Therefore, AMS and MS188 may form a complex to regulate *CYP703A2* expression (Figure 1). In *ms188*, the expression of CYP703A2 is significantly reduced. However, AMS expression is not affected in this mutant. Thus, MS188 plays an essential role in this complex to activate the expression of *CYP703A2*. In *ams*, the expression of MS188 partially restores the expression of *CYP703A2* (Xiong et al., 2016). Several bHLH transcription factors including bHLH010, bHLH089 andbHLH091 were reported to be involved in tapetum development. It is likely that AMS can be replaced by these bHLH factors to activate *CYP703A2* expression. In the glucocorticoid receptor system of *myb80*/*ms188* backgound, MS188/MYB80GR (glucocorticoid receptor) fusion protein can induce the expression of *CYP703A2*, *MS2* and *PKSA*/*LAP6* in response to dexamethasone treatment. It is likely that MS188 directly regulates the expression of several genes for sporopollenin synthesis in tapetum.

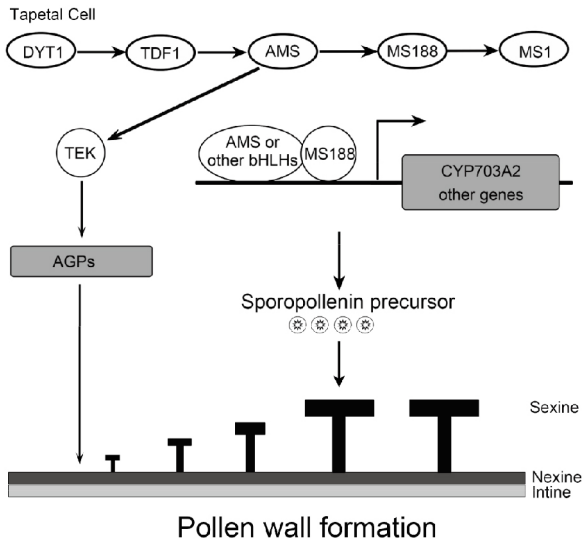

Figure 1 The gene regulation pathway in tapetum for sporopollenin synthesis and pollen wall formation. *DYT1*, *TDF1*, *AMS*, *MS188* and *MS1* are regulators in tapetum which form a genetic pathway. CYP703A2 is one of the enzymes for sporopollenin synthesis. AMS directly regulates MS188 which interacts with AMS to form a complex to regulate the expression of *CYP703A2* for sexine formation.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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