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Mini-Review

# The role of auxin in style development and apical-basal patterning of the *Arabidopsis thaliana* gynoecium

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**In angiosperms, the gynoecium constitutes the female reproductive organ that after fertilization develops into a fruit and in *Arabidopsis thaliana* the gynoecium is formed by the congenital fusion of two carpels. In the last few years many genes involved in female organ development have been identified and there have been several reports on the involvement of the plant hormone auxin in gynoecium patterning. An auxin gradient has been suggested to establish the apical-basal patterning of the gynoecium and recently it has been shown that elevated apical auxin levels can compensate for the loss of several style-promoting factors but that auxin is dependent on their action in apical-basal patterning. Here we discuss the role of auxin and different upstream, downstream or parallel factors in the apical-basal patterning of the gynoecium. We focus specifically on the development of style and stigma and discuss the most recent findings.**

The gynoecium is the female reproductive organ of the flower and in *A. thaliana* it consists of two congenitally fused carpels. In this species the style and stigma is formed by postgenital fusion of the apical parts of the carpels. The stigma, constituting one layer of papillar cells, mediates the adherence and germination of the pollen grains and a short solid style connects the stigma with the ovary. The ovary consists of the two fused carpels forming the valves, and the replum, which internally is connected to the septum that bisect the ovary. The most basal part of the gynoecium is the gynophore, a short stalk-like structure that connects the ovary to the base of the flower. Abaxial (outer) vs. adaxial (inner) domains and medial vs. lateral domains are specified early during development of the gynoecium primordium whereas the apical-basal polarity is specified later when the primordium grows into a tube-shaped organ.

Auxin is a plant hormone with important roles in e.g., organ patterning and cell differentiation and its profound influence on the development of the gynoecium is demonstrated by the defects caused by disturbed auxin biosynthesis, transport or signalling.[1-4] Chemical or genetical inhibition of polar auxin transport (PAT) leads to altered apical-basal patterning of the gynoecium. When PAT is inhibited, the style and stigma in the apical part, and the gynophore in the basal part of the gynoecium, elongate and the ovary size concomitantly decrease. Based on these data Nemhauser et al.,[5] suggested a model for apical-basal patterning of the gynoecium where an auxin gradient spans the gynoecial primordium. In this model high auxin levels in the apical region promote differentiation and proliferation of the style and stigma, intermediate levels specify the ovary and low levels in the basal region specify the gynophore. Inhibition of PAT is proposed to result in apical shifts in the boundaries between the different tissues because of high accumulation of auxin in the source tissues, hypothesised to be the most apical parts, and depletion of auxin in the basal tissues downstream of the transport. In agreement with this model high intensity signal of the auxin response reporter constructs DR5::GUS/GFP was detected in the apical end of developing plant organs, including the gynoecia.[6,7] Although the auxin gradient model fits well with experimental data there is, to our knowledge, no direct evidences that such a gradient exists. In a recent review Østergaard[8] suggested that another morphogen, possibly cytokinin, having a maximum at the basal part of the gynoecium, might function in parallel with auxin to specify the different apical-basal regions of the gynoecium. Future studies will hopefully shed more light on these possibilities.

Many different transcription factors regulate gynoecium development and AINTEGUMENTA (ANT), CRABS CLAW (CRC), ETTIN (ETT), JAGGED (JAG), LEUNIG (LUG), SEUSS (SEU), SPATULA (SPT) and STYLISH1 (STY1) are all required for carpel fusion and correct style and stigma development.[9-15] ETT, SPT, SEU, STY1 and LUG have all been suggested to mediate auxin related processes of the gynoecium. *ETT* encodes an auxin response factor that appears to specify abaxial fate in the gynoecium together with *KANADI* (*KAN*) genes, and *ett* mutant gynoecia have a split style, reduced ovary size and increased gynophore length.[3,5,10,16] *SPT* is ectopically expressed in *ett* gynoecia suggesting ETT to repress *SPT* activity.[17] SPT has been proposed to promote auxin signalling in the apical part of the gynoecium and in *spt* mutants the apical part or the carpels are unfused and they lack the internal transmitting tract in the septum.[5,12,17]

SEU has been suggested to promote gynoecium development by transcriptional regulation of auxin response genes together with ETT, and *seu* mutants develop gynoecia with severely distorted apical

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regions.[13,18] STY1 is a member of the SHI/STY-family of transcription factors that are expressed e.g., in the apical part of developing gynoecia, and the split style of *sty1* gynoecia is enhanced by mutations in additional family members.[15,19] Auxin levels are reduced in the *sty1 sty2* double mutant and the style phenotype can be restored by micro-application of auxin suggesting the style phenotype to be caused by reduced local auxin levels.[20,21] STY1 activates transcription of the auxin biosynthesis gene *YUC4* and we have recently shown that STY1 overexpression results in an elevated auxin biosynthesis rate.[4,20,21]

LUG most likely acts upstream of *STY1* and *lug-1* gynoecia are also unfused in the apical part.[9,19] The *Antirrhinum* orthologue of *LUG* affects auxin responses and the auxin response genes *ARFX15* and *ETT* have been suggested as potential downstream targets of LUG.[22,23] *JAG* acts redundantly with the closely related gene *NUBBIN* (*NUB*) in the gynoecium, and the *jag* single mutant displays only slight style defects.[14,24] The *YABBY* (*YAB*) family member *CRC* has been identified as a direct target of the carpel identity protein AGAMOUS (AG).[25,26] CRC regulates the adaxial-abaxial patterning in the gynoecium and *crc* mutants have short, wide and partially unfused gynoecia.[12,25,27] ANT is, together with LUG, necessary for the formation of all marginal tissues; replum, septum, placentae, style and stigma although *ant* single mutants are only slightly defective in the apical fusion of the gynoecia,[11,28] and is also involved in adaxial-abaxial patterning.[29]

It has been shown that *lug* mutations are epistatic to *sty1* and that *STY1* expression is reduced in *lug* mutants indicating that *LUG* acts upstream of *STY1*.[19] Overexpression of STY1 restores style development in the style mutants *lug*, *seu*, *seu lug*, *crc* and *spt,* indicating that elevated, or ectopic, activation of the STY1 regulated style development pathway can substitute for the style promotive effects not only of the upstream regulator LUG but also of CRC, SEU and SPT.[19,21] This suggest that STY1 acts downstream of all these style promoting factors or, alternatively, that STY1 acts in a parallel pathway that can compensate for the loss of CRC, SEU and SPT mediated pathways. LUG has been shown to interact with SEU and it has been suggested that LUG, SEU and ANT, together with FILAMENTOUS FLOWER (FIL), might be part of a multimeric complex involved in development of the medial domain of the gynoecium.[30,31] Furthermore, SEU has been shown to physically interact also with ETT.[18] The genetic interactions between *seu* and *lug* and also between *seu* and *sty1* are synergistic indicating that *LUG*, *SEU* and *STY1* might regulate style development in the same pathway.[13,21] *crc* may also interact syngergistically with *sty1*, suggesting that this gene also could be involved in the same pathway as *STY1*.[15] *spt* and *shi/sty* mutations show both synergistic and epistatic interactions, suggesting that they may, at least in part, act independently.[15,19] By contrast, the interactions between *sty1* and *jag* and also between *sty1* and *ant* is additive.[21] This suggests that both *JAG* and *ANT* act in different pathways than *STY1.*

Treatment with the PAT inhibitor NPA restores the stylar defects of *spt*, *sty1*, *sty1 sty2, ant*, *jag, jag nub*, *crc*, *seu* and *lug*.[5,20,21] Thus, both overexpression of STY1 and inhibition of PAT can restore style development in several different style mutants suggesting that the ability of STY1 to restore the style mutants is mediated by an increase in auxin biosynthesis at least in part mediated by *YUC4*. This also indicates that elevated apical auxin levels can compensate for the loss of a large number of style-promoting factors and that auxin acts either downstream of, or in parallel with, these. To discriminate between these two possibilities further studies needs to be made. The style defects of *sty1 sty2* plants can be restored also by application of auxin directly on the apical end of developing gynoecia, but not by spraying the whole plants with auxin, indicating that local auxin peaks mediated by auxin biosynthesis appear important for gynoecium morphogenesis and that the PAT system cannot compensate for all defects in biosynthesis.[21]

Even though the effect of inhibition of PAT on style development is similar in different style mutants, they respond differently with respect to apical-basal patterning of the gynoecium. The responses can be divided into three categories: normal (as wild type), hypersensitive and hyposensitive. The response of *ant*, *jag* and *jag nub* to inhibition of PAT is normal suggesting that the morphogenic effects of auxin distribution, or auxin distribution itself, are not affected by these mutations.[21] Auxin biosynthesis (*yuc1, yuc4*) and response mutants (*axr1, ett* and *tir1*) are hypersensitive to inhibited PAT indicating that there is a connection between auxin levels or responsiveness and sensitivity to reduced PAT.[5,21,32] Because mutations in *SEU*, *LUG*, *STY1* and *STY2* also cause hypersensitive responses these genes most likely also affect auxin signalling or homeostasis.[20,21] *crc* and *spt* are hyposensitive suggesting that mutations in both these genes results in a compensatory ability to disturbed PAT that may be linked to increased auxin responsiveness or transport.[5,21]

The response of different mutants to inhibition of PAT suggests that auxin promotes style and stigma development generally and can act independently of *ANT, CRC, JAG, LUG, SEU, SPT* and *STY1.* The morphogenic role of auxin in apical-basal patterning of the gynoecium is however dependent on several of these genes indicating a different mode of action of auxin in this process.

By controlling *YUCCA*-mediated auxin biosynthesis the *SHI/STY* genes could be responsible for the formation of a high auxin level in the apical part of the developing gynoecium, specifying the style and stigma. The upstream gene *LUG* could be one factor determining the expression domain of the *SHI/STY* genes, possibly together with *SEU*. *SPT*, and related genes, might direct differentiation of style, stigma and transmitting tract by mediating the response to the high auxin levels and/or by regulating PAT and thereby participating in the formation of the auxin gradient. The ovary size could be established by ETT responding to the intermediate auxin levels and repressing the expression of *SPT* in this region. ETT could also be involved in restricting the differentiation of style and stigma to the apical part.

It will be interesting to see if future experiments will provide more direct evidence of the existence of an auxin gradient in the developing gynoecium. The possible involvement of cytokinin and the interactions between the different factors participating in patterning the gynoecium also need to be addressed further.

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