KLUH/CYP78A5 promotes organ growth without affecting the size of the early primordium

Lena Stransfeld, Sven Eriksson, Nikolai Maria Adamski, Holger Breuninger and Michael Lenhard*†
Department of Cell and Developmental Biology; John Innes Centre; Colney Lane; Norwich, UK
†Current address: Institut für Biochemie und Biologie; Universität Potsdam; Potsdam, Germany

Mobile signals play a key role in controlling the growth of organisms. In Arabidopsis, the cytochrome P450 CYP78A5/KLUH (KLU) non-cell autonomously stimulates cell proliferation in developing organs. In a recent study, we determined the range of KLUH action, using a widely applicable system to predictably generate chimaeric plants. We showed that KLUH acts not only within individual floral organs or flowers, but that its overall activity level is integrated across an inflorescence to determine organ size. Here, we address the question at which stage of petal development KLUH acts to promote growth. We demonstrate that the size of the very young petal primordium in klu mutants is not altered, supporting the conclusion that KLUH acts during later stages of organ outgrowth and a correspondingly longer range of the presumed KLUH-dependent growth signal.

Non Cell-Autonomous Control of Arabidopsis Organ Growth by KLUH

In plants, the flower and often the whole inflorescence function as integrated structures whose component organs need to grow to their correct relative sizes to ensure reproductive success. However, very little is known at present about how this coordination of growth is achieved. Plant organ size is influenced by several phytohormones, yet their range of action in controlling growth has not been clearly established. A further growth signaling mechanism is defined by the KLUH (KLH)/CYP78A5 and the homologous CYP78A7 genes, which appear to non-cell autonomously promote plant organ growth via a novel mobile signal. To ask which developmental roles KLUH signaling plays, in particular whether it may be involved in growth coordination in flowers, we aimed to determine the range of action of the KLUH-dependent growth signal.

Long-range communication in plants has successfully been studied using mechanical grafting of scions onto genetically distinct stocks. However, mechanical grafting is limited in terms of spatial resolution and the accessible developmental stages. An alternative method to study intercellular signaling is the analysis of genetically distinct clones generated for example by Cre/loxP-mediated recombination. However, the predictable generation of specific types of clones and chimaeras has been difficult, due to the nature of the Cre-source used. To circumvent these limitations and facilitate the analysis of intercellular communication in Arabidopsis, we have combined Cre/loxP-mediated recombination with temporally and spatially controlled expression of Cre in a widely applicable system that allows for the predictable generation of chimaeric plants.

Using this system, we demonstrated that neither the final size of wild-type and klu mutant petals within the same flower, nor the final size of wild-type and mutant flowers within one inflorescence correlated with their genotypes. Instead, the petals within one flower or one inflorescence grew to sizes depending on the sum...
total of KLU activity in the respective inflorescence. By contrast, organs within an inflorescence that was chimaeric for another tested growth-control gene with largely autonomous behavior grew to sizes according to their genotype. Taken together these observations suggest that the overall activity level of KLU is integrated across an inflorescence to determine individual organ size and that growth is coordinated by a mobile KLU-dependent growth signal.

The Size of the Very Early Petal Primordium in klu Mutants is Unchanged

The comparatively long range of KLU activity beyond individual flowers that is suggested by the above results raises the question at which stage of petal development KLU acts to stimulate growth. The earlier this stage, the shorter would be the distance that the presumed growth signal has to cover to integrate growth in a flower or an inflorescence. From an analysis of dissected petals, it had been concluded that KLU acts towards the end of the proliferation phase in petals.² However, an effect on the size of the very early petal primordium at the time of its specification, called the petal anlage, could not be ruled out. The size of a floral organ anlage can be measured using the boundaries of marked sectors originating outside of the flower.³ The number of cells in the anlage is the inverse of the smallest fraction of the organ that can be occupied by a marked sector originating outside the flower.

To determine whether the petal anlage in klu mutants contains the same number of cells as in the wild type, we used the pCLV3-Alc-Cre and 35S:loxP-Stop-loxP:vYFPPer transgenes to generate YFP-positive stem-cell clones that have populated the epidermis of approximately one third of the shoot. Asterisk marks a rosette leaf with half of the epidermis expressing YFP. (C–E) Overlay of brightfield and YFP fluorescence micrographs (upper) and YFP fluorescence micrographs only (lower). (C) A mature flower from a pCLV3-Alc-Cre; 35S:loxP-Stop-loxP:vYFPPer doubly transgenic plant in a KLU wild-type background with a sector boundary running through the middle of the flower. (D) A petal of the same genotype as the plant in (C) with a sector boundary running along the midline of the organ. (E) A petal from a pCLV3-Alc-Cre; 35S:loxP-Stop-loxP:vYFPPer doubly transgenic plant in a klu-2 background with a sector boundary running along the midline of the organ. Scale bars are 1 mm in (B) and 500 µm in (C–E).

References