Molecular Genetics of Reproductive Biology in Orchids

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Orchids are members of the family Orchidaceae, one of the largest families of flowering plants. There are an estimated 20,000 to 25,000 orchid species, which occupy wide ranges of ecological habitats and exhibit highly specialized morphological, structural, and physiological characteristics (Dressler, 1990). In particular, the most spectacular evolution is shown in reproductive biology. The production of column (a fused structure of stamens and styles) to facilitate pollination is well documented and the co-evolution of orchid flowers and pollinators is well known (van der Pijl and Dodson, 1969). Even more significant but less well-known aspects are the early development and maturation of the pollen grains (packaged as pollinia—pollen grains bound together by viscin threads in masses for effective pollination), the post-pollination development and maturation of ovules, the synchronized timing of micro- and megagametogenesis for effective fertilization along the whole length of placenta, and the release of tens of thousands or millions of immature embryos (globular stage) in mature capsules (Raghavan and Goh, 1994). Without doubt, these various strategies, unique to orchids, contribute to the success of orchid family.

During the past decade, intensive molecular studies with the model plant Arabidopsis and with rice (*Oryza sativa*) have elucidated many gene regulation processes during development, particularly on reproductive biology of flowering. These studies would be further enhanced greatly with the complete sequencing of Arabidopsis and rice genomes and the numerous expressed sequence tag sequencing projects in a wide range of plants. In contrast, few molecular genetic investigations have so far been undertaken on floral transition and subsequent reproductive growth in orchids. This was, in some way, limited by the rather extended and complicated flowering process. The inefficient orchid transformation system also hampered the investigation of gene function and regulation in vivo. However, recent successes in in vitro thin-section techniques for micropropagation and flowering of orchids have not only shortened the orchid juvenile phase from several years to only a few months but also provided more obvious "landmark" events during development (Lakshmanan et al., 1995; Goh, 1996). Also, the improved orchid transformation system (Chia et al., 1994; Yang et al., 1999; Yu et al., 2001) would certainly facilitate studies on gene function. Progress in molecular genetics of orchids can be expected to accelerate in the near future. The unique differences in reproductive biology in orchids as compared with the normal development in Arabidopsis offer distinct advantages to study gene function and evolution in prepollination and post-pollination development.

PREPOLLINATION: FLOWER DEVELOPMENT

Our understanding of prepollination floral development in orchids at the molecular level is only at the initial stage. In some plant species, rapid progress has been made in elucidating the molecular and genetic mechanisms involved in the floral transition and subsequent flower development. In particular, a large number of MADS-box genes, which encode transcription factors containing a highly conserved DNA-binding domain, have been identified to function in subtle regulatory processes of flower development in different species (Shore and Sharrocks, 1995; Riechmann and Meyerowitz, 1997; Kyozuka et al., 2000). Using the in vitro flowering system of *Dendrobium* spp. orchids, the profile of gene expression during the transition to flowering has been examined by mRNA differential display method (Yu and Goh, 2000a). The results showed that genes involved in transcriptional regulation, cell division, and several other metabolic events are closely associated with the process of floral transition in orchids. Furthermore, four orchid members of the APETALA1/AGL9 subfamily of the MADS-box gene have been identified in *Aranda* cv Deborah and *Dendrobium* cv Madame Thong-In (Lu et al., 1993; Yu and Goh, 2000b). Study of the expression patterns of these genes indicated their important roles in the regulation of floral transition and organ identity (Yu and Goh, 2000b).

It is noteworthy that during flower development, interaction may occur between MADS-box and class 1 *knox* genes, another developmentally important class of transcription factors (Yu et al., 2000). This interesting hypothesis needs further investigation in orchids and other plant species. In cv Madame Thong-In, down-regulation of the expression of *DOH1* gene, a class 1 *knox* gene, causes multiple shoot apical meristem formation and early flowering,

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which is coupled with the early onset expression of *DOMADS1*, an orchid MADS-box gene involved in the floral transition (Yu et al., 2000). In a wild-type orchid plant, the onset of *DOMADS1* expression in the shoot apical meristem during floral transition is accompanied by a marked reduction of *DOH1* transcripts, and both kinds of transcripts are later located at the same region in the inflorescence meristem and the developing floral primordia (Yu et al., 2000). Therefore, one can reasonably envisage a possible relationship between these two different types of transcription factors during the flowering process.

The extensive molecular and genetic studies of Arabidopsis and rice genomes as well as physiological responses have described detailed regulatory networks of flower development including several pathways leading to the onset of flowering, and the initiation and formation of floral meristem and organs. These studies will undoubtedly play directional roles in future elucidation of basic mechanisms of flower development in orchids. On the other hand, unique and different developmental programs may be present in orchids due to the highly evolved floral structures, which are being investigated to contribute to our understanding of the molecular events regulating the general floral development in flowering plants. For example, in contrast to the homologs in Arabidopsis, the isolated orchid MADS-box genes, *DOMADS2* and *DOMADS3*, have shown novel expression patterns in the shoot apical meristem during floral transition (Yu and Goh, 2000b).

It should be noted that, although floral organ identity (ABC) genes have been well studied in Arabidopsis and rice, no such homologs have been isolated from orchid. The development of column, which involves whorl 3 and whorl 4, would be one of the most interesting subjects to elucidate the evolution and interaction of *B* and *C* genes. Indeed, clarification of the early development of pollinia with respect to other floral organs would provide insights on regulation of microspore genesis.

PREPOLLINATION: PIGMENTATION

Compared with Arabidopsis and rice, orchid is ideal for the study of floral coloration because of its enormous variation in color. Some investigations have shed light on the clarification of genes affecting orchid flower pigmentation, most of which encode enzymes relevant to the flavonoid pathway (Liew et al., 1998; Johnson et al., 1999). These genes include *dihydroflavonol 4-reductase* (*DFR*), *chalcone synthetase*, *flavanone 3-hydroxylase*, and *Phe ammonia-lyase*. However, molecular and genetic studies of these genes are too limited to figure out the fundamental factors regulating the floral coloration of orchids, although the transgenic approach has been adopted for the study of *Cymbidium hybrida DFR* in a heterologous petunia (*Petunia hybrida*) system (Johnson et al., 1999). Orchid flowers are striking for their specific patterns of colors in sepals, petals, and the modified dorsal petals (lips). These may be discrete spots, streaks, or blotches rather than flushes or shades of different intensity. The patterns on the lips of some species are even more strikingly contrasting to serve as the landing platform for insect pollinators. It follows that the regulation of pigmentation is refined to specific cells of the different floral organs, and the expression of genes involved in flavonoid synthesis may be just the initial steps in the complex regulation of pigmentation. Indeed, *DFR* was shown to be expressed in the white petal tissues of *Bromheadia finlaysoniana* (Liew et al., 1998). To understand the development of color patterns in flowers, it will be also important to identify additional regulatory genes in the flavonoid pathway. Further investigations of their regulatory mechanisms and pathways will provide strategies toward genetic engineering of color in orchid flower, which is now possible with efficient and reliable transformation systems (Chia et al., 1994; Yang et al., 1999; Yu et al., 2001).

POST-POLLINATION: OVULE DEVELOPMENT

In most of flowering plants, the ovules are mature, and the egg cells are ready to be fertilized at anthesis. In contrast, ovule development is triggered by pollination in orchids. This makes orchids attractive systems for the investigation of ovule initiation and subsequent development. Molecular aspects of ovule development have been investigated in *Phalaenopsis* spp. orchids (O'Neill et al., 1993; Zhang and O'Neill, 1993; Nadeau et al., 1996). These studies led to the isolation and characterization of a series of orchid genes associated with ovule differentiation, which provided insights into the function of their homologs in Arabidopsis development (Lu et al., 1996; Porat et al., 1998). For example, the *Phalaenopsis O39* gene is a member of a new class of plant homeobox transcription factors designated HD-GL2 (Lu et al., 1996; Nadeau et al., 1996). It is expressed in the ovule from primordium formation at early stages to various late stages of ovule differentiation, suggesting its possible role as an important regulator involved in the ovule tissue initiation and development. Subsequently, the Arabidopsis homolog of *O39*, *ATML1*, has been identified in an Arabidopsis floral bud cDNA library using the *O39* probe (Lu et al., 1996). The *ATML1* transcript is located in the L1 layer of the meristem during embryonic pattern formation and throughout shoot development, which indicates that *ATML1* may participate in the meristem patterning from the earliest stages of embryogenesis to the later stages of shoot development.

The critical role of ethylene in ovary maturation and ovule differentiation in orchids has been extensively investigated by both physiological and molec-

ular methods (O'Neill et al., 1993; Nadeau et al., 1996). Most of these studies were focused on two components, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase, both of which are key enzymes in the ethylene biosynthetic pathway. Although evidence has been presented for the coordinated regulation of ACC synthase and ACC oxidase gene expression in the ovary (O'Neill et al., 1993), the molecular events triggered by pollen-pistil interactions leading to these gene expressions are not well understood. Furthermore, the signaling pathway following ethylene perception is still not clarified during post-pollination gynoecium changes in orchids.

POST-POLLINATION: PERIANTH SENESCENCE/DEVELOPMENT

Perianth senescence induced by pollination in orchids is a typical post-pollination symptom with rapid sepal and petal wilting and pigment loss. Successful pollination signals the completion of a job well done by the perianth display, and the resources are then redirected for ovule development and subsequent embryogenesis after fertilization. The physiological and molecular mechanisms of pollination-induced senescence have been studied in several orchid species, such as *Phalaenopsis* and *Dendrobium*, especially in terms of both ethylene sensitivity and production. The enhanced sensitivity to ethylene following pollination is the initial event triggering an increase in ethylene production and the consequent physiological changes of flower (Porat et al., 1995). Although molecular evidence is hitherto far from resolving pollen-derived signals, the identification of some putative sensitivity factors such as GTP-binding protein (Porat et al., 1994), short-chain saturated fatty acids (Halevy et al., 1996), and auxin (Zhang and O'Neill, 1993) has shed light on the elucidation of the mechanisms under the regulation of ethylene sensitivity.

Increase in ethylene production is another early event induced by pollination, which occurs after a heightened sensitivity to ethylene in orchid flowers. In *Phalaenopsis* spp., despite the production of abundant ethylene in the perianth up to 72 h after pollination, the accumulation of ACC synthase is not detectable in this tissue (O'Neill et al., 1993). However, the ACC oxidase expression is up-regulated in the petals and sepals about 48 h after pollination in parallel with the onset of perianth senescence. It is generally accepted that both ACC synthase and ACC oxidase, positively regulated by ethylene, function in a feedback loop, leading to the increased ethylene production (O'Neill et al., 1993).

At the other extreme, in some *Phalaenopsis* species (subdivision stauroglottis), the sepals and petals turn green and photosynthetic following successful pollination. These organs become leaf-like and provide photosynthates for the developing ovules/ovary and the embryos subsequent to fertilization over an extended period of many months until the capsule is mature. The molecular genetics for this transformation of the perianth from an energy sink to an energy source during post-pollination development is another marvelous opportunity to study the interaction (suppression?) of ABC genes.

The reproductive biology in orchids is still lagging behind in terms of molecular genetics. Information on the genomes of Arabidopsis and rice, together with large collections of expressed sequence tag, is providing new strategies for addressing the universal knowledge of plant systems in a more integrated perspective. Application of this knowledge through the common language of nucleotide sequences will allow the assignation of potential functions to the corresponding genes in orchids, and thus partially direct further characterization of these candidates involved in the important developmental processes. On the other hand, gene cloning in orchids, as compared with similar work in other plant species, will also contribute to studies of the functions of specific genes, which are yet to be or have not been completely determined in Arabidopsis or rice. As another kind of flowering plants, orchids demonstrate some special characteristics and offer unique advantages in the study of certain important developmental programs, such as floral coloration, ovule development, and perianth senescence/development. Future concentration of efforts on one orchid species, which is easily transformable with relatively detailed genetic map, will promise an exciting future for orchidology with a fusion of classical plant physiology and modern molecular genetics.

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