General Discussion

In cucumber plants, it is suggested that the sex expression is regulated by the levels of endogenous ethylene and gibberellin. However, the regulatory mechanisms of sex expression by endogenous hormones have remained to be clarified.

As described in Part I, I identified the cDNA (CS-ACS2) for ACC synthase that is the key enzyme in the pathway of ethylene biosynthesis from the apices of monoecious cucumber plants. Both the timing and the levels of expression of the *CS-ACS2* transcript were correlated with the development of female flowers at the nodes of cucumber cultivars. Furthermore, I showed that the *CS-ACS2* was expressed at floral buds that would develop into female flowers. From these results, the level of the *CS-ACS2* transcript at floral buds is considered to regulate the sex expression in cucumber plants *via* ethylene production.

In Part II, I demonstrated the correlation between the expression of *CS-ACS1/CS-ACS1G*and *CS-ACS2* transcripts at the apex and the sex phenotypes. Only the transcript of *CS-ACS2* was detected at the apices of monoecious line. This and above results suggest that the sex expression of monoecious line is regulated by the level of *CS-ACS2* mRNA. On the other hand, both the transcripts of *CS-ACS1/CS-ACS1G* and *CS-ACS2* were expressed at the apices of isogenic gynoecious line. Female sex expression in cucumber plants is regulated by the partially dominant allele at the *F* locus. The expression of *CS-ACS1/CS-ACS1G* transcript was gynoecious-specific, and increased as the dosage of dominant alleles at the *F* locus increased from one to two. Since the *CS-ACS1G* gene that is a gynoecious-specific ACC synthase gene is closely linked to the *F* locus (Trebitsh *et al.* 1997), the transcript of *CS-ACS1/CS-ACS1G* detected at the apices of gynoecious line with the use of CS-ACS1 probe is considered

to be the transcript of *CS-ACS1G*. These results support the idea that the *CS-ACS1G* gene is corresponded to the *F* gene. In addition to the expression of *CS-ACS1G* transcript, the level of transcript of *CS-ACS2* was higher at the apices of gynoecious lines than at those of monoecious lines. Since the *CS-ACS2* gene was ethylene-inducible gene, it seems likely that the expression of *CS-ACS1G* transcript at apices of the gynoecious line hastens the timing of expression of the *CS-ACS2* transcript and increases the levels of expression of the transcript *via* ethylene production at the apices compared to the isogenic monoecious line.

Many studies suggest that gibberellin have an opposite effect of ethylene on sex expression and that gibberellin promotes the development of male flowers. Yin and Quinn (1995) showed that the effect of ethylene was the inhibition of the development of stamens and the effect of inhibitor of ethylene action was the inhibition of the development of a pistil in flower of cucumber plants and suggested that the sex of flowers of cucumber plants is primarily regulated by the level of ethylene. They speculated that gibberellin acts more upstream than ethylene on the regulation of sex expression in cucumber plants. In Part II, I showed that the application of gibberellin induced the development of male flowers following decrease in the level of *CS-ACS2* transcript at the apices of monoecious line. Based on the finding, the effect of gibberellin on sex expression is interpreted as that gibberellin suppresses the expression of *CS-ACS2* transcript, which in turn, decreases ethylene production. The decreased ethylene level inhibits the development of a pistil, as a result, and initiates a development of male flowers. It is considered that the level of endogenous gibberellin acts on the regulation of sex expression as one of factors that regulate the level of *CS-ACS2* transcript.

From the results reported in the thesis, I propose a working hypothesis concerning the regulatory mechanisms of sex expression in

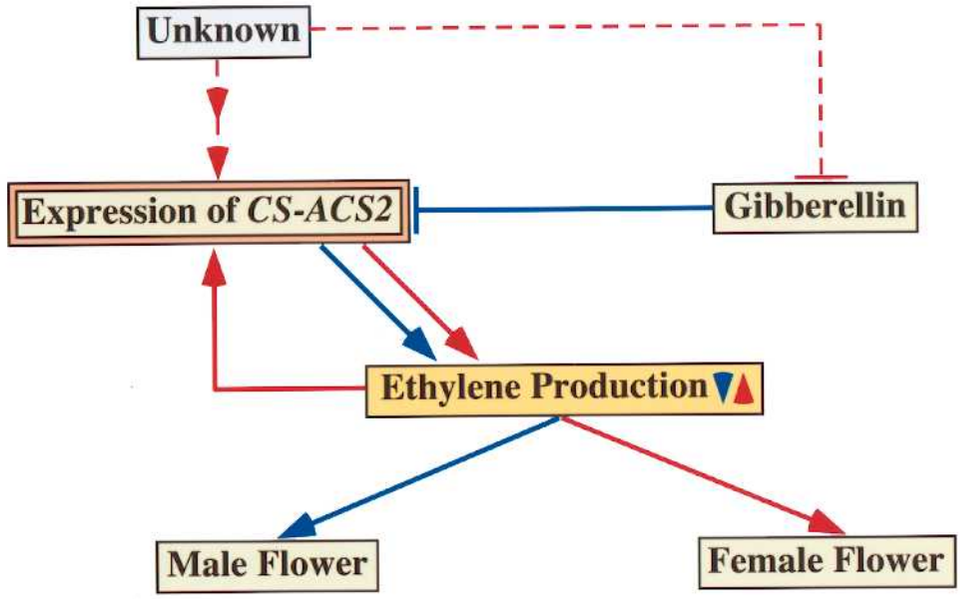
monoecious and gynoecious cucumber plants (Fig. III-1). In monoecious cucumber plants (Fig. III-1a), sex expression is regulated by the level of *CS-ACS2* transcript at floral buds and the expression of *CS-ACS2* is regulated developmentally by unknown factors. The level of gibberellin seems to be one of factors that regulate the level of *CS-ACS2* transcript. The high level of gibberellin decreases in the level of ethylene *via* suppression of the expression of *CS-ACS2* transcript at floral bud. As the results, floral buds develop into male flowers. Once the expression of *CS-ACS2* mRNA is induced, the expression of *CS-ACS2* transcript is subject to positive feedback regulation *via* ethylene production. In consequence, the increased level of ethylene induces the development of female flower. On the other hand, gynoecious cucumber plants (Fig. III-b) possess the *CS-ACS1G* gene that is expressed at the apices of gynoecious cucumber line from early stage of development. The expression of *CS-ACS1G* transcript increases ethylene synthesis and ethylene induces the expression of *CS-ACS2* mRNA at all floral buds leading to enhanced ethylene production. The increased level of ethylene initiates a developmental process of the formation of female flowers.

In this study, I characterized the genes for ACC synthase that regulate the sex expression in flower buds at the apices of cucumber plants, and suggested that the regulation of the levels of ethylene plays an important role in the regulation of sex expression in cucumber plants. This is the first report of the identification of genes involved in the biosynthesis of plant hormone that regulates the sex expression in unisexual plants. From the results reported in this thesis, it is considered that the sex of flower buds at the apex of monoecious cucumber plants is determined by the levels of expression of *CS-ACS2* transcript and the difference in the levels of expression produces two types of flower (male and female) along a stem. Further studies on the characterization of *CS-ACS1G* and on the

regulatory mechanisms of the expression of *CS-ACS2* by GA_4 will clarify the regulatory mechanisms of sex expression by plant hormones in other plant species. For example, the sex of dioecious *Mercurialis annua* that is evolved from monoecious plants is determined by three independently segregating genes, *A1*, *B1*, and *B2* (Louis 1989, Durand and Durand 1991). Either an *A1* dominate gene together with recessive alleles of *b* genes or an *a1* recessive allele together with dominant *B* alleles induce femaleness. Male determination requires complementary gene action, *i.e.* the presence of a dominant *A1* allele together with one additional dominant *B* allele. The degree of maleness is determined by the *B1-B2* genotype. On the other hand, high levels of endogenous cytokinin, *trans*-zeatin, in *Mercurialis annua* appear to be correlated with induction of floral primordia to carpels (Dauphin-Guerin *et al.* 1980) and the zeatin nucleotide, rather than the free base, accumulates in the apices of male plants (Dauphin-Guerin *et al.* 1980, Louis *et al.* 1990). The qualitative and quantitative variation of zeatin in males and females has been shown to be under genetic regulation by the sex determination genes. In this thesis, I showed that the expression of *CS-ACS1G* increased in a proportion of female flowers and that cucumber plants which possess the *CS-ACS1G* changed sex phenotype from monoecious to gynoecious. From these results, it is considered that the sex in *Mercurialis annua* is brought about by modifications of the biosynthetic pathway of *trans*-zeatin and that the sex determining genes may be involved in the production of *trans*-zeatin and other plant hormones.

Figure III-1. A working hypothesis of the regulatory mechanisms of sex expression in cucumber plants. (a) Monoecious plants. (b) Gynoecious plants. Blue lines show the pathway to the expression of a male flower. Red lines show the pathway to the expression of a female flower. Upward and downward arrowheads described in "Ethylene Production" indicate the increase and decrease in ethylene production, respectively. Lines with " ⊢" indicate the inhibition.

(a) Monoecious



(B) Gynoecious