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The Application of Antisense RNA Technology for Plant Secondary Metabolism

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식물이차대사과정에 antisense RNA기법의 응용

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요 약

유전자의 발현이 다양한 형태로 억제되는 것을 silencing이라고 한다. 유전자 발현억제 방법 중 antisense RNA는 자연 상태의 mRNA에 역상보적인 RNA 분자로서 형질전환된 세포에서 그 mRNA의 전이을 억제하는 데 이용된다. RNA분자에 대하여 상보적 염기배열을 갖는 RNA는 분자간 결합을 연결하여 RNA의 기능 발현에 억제적으로 작용한다고 생각된다. 미생물에 나타나는 유전자발현 제어기작으로서 어느 특정한 mRNA에 대하여 상보적인 RNA가 유전자 발현의 억제인자로서 작용하고 있는 예가 몇 가지 알려져 있다. 이러한 경우 antisense RNA는 mRNA 상의 전이개시 영역과 상보적 배열을 하고 있고, 전이과정을 방해한다고 추정되고 있지만 작용기작의 상세한 내용은 아직 명확하지가 않다. 한편 안티센스RNA는 임의의 표적유전자에 대하여 인위적으로 제작할 수가 있기 때문에 인위적인 유전자발현제어의 한 방법으로 이용되고 있다. 특정한 유전자에 대한 antisense RNA를, 발현하는 유전자를 인위적으로 제작하여 세포내에 도입하면 표적유전자의 발현을 특이적으로 억제·제어할 수 있는 것이 기대되어, 다양한 생물체를 대상으로 하여 많은 시도가 이루어지고 있으며 몇 가지 성공적인 보고가 있다. 그 중 식물이차대사과정에 관련 유전자를 대상으

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로 antisense RNA 기법으로 유전자의 발현억제와 이차대사산물 생산조절에 관한 연구를 이 논문에서 조사하고 정리하였다.

핵심어: 알카로이드, Antisense RNA, 플라보노이드, 리그닌, 이차대사, 유전자발현억제

Ⅰ. Introduction

Plant produces a broad variety of secondary metabolites with economical importance. These compounds are associated with important traits of the plant itself, e.g., the color or fragrance of flowers, the taste and color of food, and resistance against pests and diseases (Harborne & Tomas-Barberan, 1991).

Secondary metabolites are also used for the production of fine chemicals such as drugs, antioxidants, flavors, fragrances, dyes, insecticides, and pheromones. Interest in secondary metabolism has been rapidly increasing in plant biology for several years. Particularly, the possibilities of genetic modification have opened exciting perspectives for the exploitation of the biosynthetic capacity of plants and plant cell.

Expression of antisense genes has exploited as an applied technique in plant biotechnology for creating "metabolic engineered plants" in which the endogenous target gene is specifically suppressed. The antisense technology is used for various purposes such as silencing or ablating undesired genes. By creating "dominant negative mutants", gene function can also be assigned to a particular cloned cDNA. Despite the rapidly increasing number of successful applications of antisense genes in plants, the molecular mechanisms in which complementary RNAs down-regulate gene expression is not fully understood.

This paper describes the principles and regulatory roles of antisense RNA technology in prokaryotes and eukaryotes, specifically in plants. It also gives an update on various examples of secondary metabolically engineered plants using the expression of antisense RNAs and outlines some characteristics of the technology.

Ⅱ. Principle of the Antisense RNA Technology

Regulation by antisense RNA was first found during the study of replication of the *E. coli* plasmid ColE1. Replication of this plasmid depends on formation of an RNA primer whose precursor RNA is functional only when it assumes a unique structure during its synthesis. Interaction of a small antisense RNA to the primer precursor inhibits formation of the unique structure, and consequently replication of the plasmid. Subsequently, many additional examples of regulation by antisense RNA have been observed, such as those for DNA

replication and expression of gene functions. The ability of antisense RNA to inhibit the activity of a specific target mRNA has also led to increasing use of artificial antisense RNAs to study biological function in both prokaryotic and eukaryotic system (Green *et al.* 1986; Eguchi *et al.* 1991).

The principle of the antisense technology is based on the capability of complementary nucleic acids to form double-stranded helices. Cellular DNA usually is double-stranded, having just a partially single-stranded phase during replication and thus is not a very good target for an antisense nucleic acid. Cellular RNA normally is present only as a single-stranded molecule even if it has double-stranded domains originating from the intramolecular secondary structure.

Due to its single-stranded nature, the endogenous RNA is the preferred target for antisense technology. Specific binding of the "antisense" nucleic acid results in interference with the biological function of the "sense" RNA. Two classes of antisense nucleic acids can be used.

The complementary RNA (also named the antisense RNA) hybridizes, or forms a duplex with, the mRNA through hydrogen-bond base pairing; this inhibits translation of that specific mRNA (Fig. 1). Although the mechanism by which translation is inhibited has not yet been elucidated, it has been suggested that such duplex mRNA is either rapidly degraded, that its processing in the nucleus is impaired, or that its attachment to ribosomes and subsequent translation is blocked. In plant species, the antisense RNA can be endogenously expressed from an "antisense gene" stably transferred to the plant.

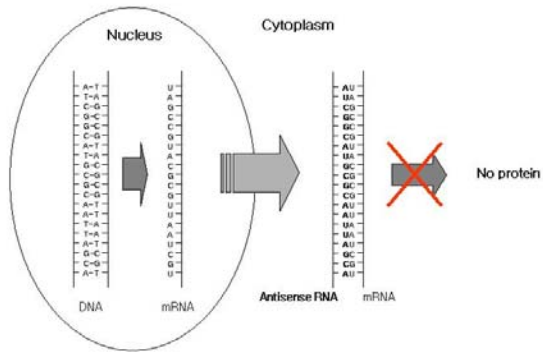

Fig. 1. The proposed mechanism of inhibition by antisense RNA

III. The Applications of Antisense RNA Methodology in Plant Secondary Metabolism

Naturally occurring and experimentally induced genetic variants are valuable assets for determining the role of particular gene product in controlling physiological processes. However, obtaining such mutants is difficult. Recently, RNA that is complementary to a specific mRNA has proven to be quite useful as a tool to prevent translation of that mRNA (van der Krol et al, 1988; Smithetal, 1988). There are some successful applications of antisense RNA technology in plant.

1. Modification of flavonoid biosynthesis: Molecular flower breeding

The flavonoid biosynthesis pathway branches off the more general phenylpropanoid pathway. Chalcone synthase (CHS), the key enzyme in flavonoid biosynthesis, is synthesized in the flower corolla, tube, and anther. CHS was the first endogenous gene in plants targeted by antisense RNA (Van der Krol *et al*, 1988).

In this experiments antisense expression of a Petunia chalcone synthase (CHS) cDNA under the control of a (constitutive viral) 35S CaMV (Cauliflower mosaic virus) promoter resulted in a dramatic change in floral pigmentation. The reduction in CHS enzyme activity resulted in fainter flower colors and pure-white flowers, which were expected since the substrates of CHS are colorless. However, changes in the pattern of flower pigmentation also occurred, and flowers with a ring or star-like phenotype were generated. Progeny plants showed the same phenotype as their parents, which demonstrated that the mutation was stable and could be transferred by sexual crossing.

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Based on the extensive investigations originally carried out in petunia (van der Krol *et al*, 1988; Napoli *et al*, 1990; Mol *et al*, 1989), The advantages of antisense applications were also shown recently in the floral industry when a variety of flower patterns was achived in Gerberahybrida (Elomaa *et al*, 1993) and a white-flowering variety in Dendranthemagrandiflora (florist's chrysanthemum) (Courtney-Gutterson *et al*, 1994).

2. Repression of lignin biosynthesis

Lignin is an integral cell wall component of all vascular plant. It plays an important role in plant cell wall, enhancing the rigidity, conferring resistance toward pathogens and mechanical stress, and enabling solute transport in the xylem. Among woody plants, the amount of lignin varies from 15 and 35% of the dry weight. Despite its biological importance in plants, lignin is an undesirable component in the pulp and paper industry because it must be moved from the wood fiber; this process consumes large quantities of energy and hazardous chemicals. Reducing the amount or changing the quality of lignin in trees would be beneficial from an economical as well as an environmental point of view. Also, lignin decreases forage crop digestibility (Baucher *et al*, 1996).

Because lignin limits the use of wood for fiber, chemical, and energy production, strategies for its down regulation are of considerable interest. We have produced transgenic aspen (*Populus remuloides* Michx) trees in which expression of a lignin biosynthetic pathway gene Pt4CL1 encoding 4-coumarate : coenzyme aligase (4CL) has been down-regulated by antisense inhibition.

Trees with suppressed Pt4CL1 expression exhibited up to a 45% reduction of lignin, but this was compensated as leaf, root, and stem growth were substantially enhanced, and structural integrity was maintained both at the cellura and whole-plant levels in the transgenic lines. Our results indicate that lignin and cellulose deposition could be regulated in a compensatory fashion, which may contribute to metabolic flexibility and a growth advantage to sustain the long-term structural integrity of woody perennials (Hu *et al*, 1999). As a result, there has been long-standing incentive to develop healthy trees that accumulate less lignin and/or more extractable lignin to facilitate pulping.

3. Modification of benzophenanthridine alkaloids

E. californica cell cultures produce several benzophenanthridine alkaloids, such as sanguinarine, chelirubine, and macarpine, with potent pharmacological activity (Facchini & Park, 2003). Antisense constructs of genes encoding two enzymes involved in benzophenanthridine alkaloid biosynthesis, the berberine bridge enzyme (BBE) and N-methylcoclaurine 3'-hydroxylase (CYP80B1), were introduced separately into California poppy cell cultures. Transformed cell lines expressing antisense-BBE or antisense-CYP80B1 constructs and displaying low levels of BBE or CYP80B1 mRNAs, respectively, showed the reduced accumulation of benzophenanthridine alkaloids compared to control cultures transformed with a β-glucuronidase gene. Pathway intermediates were not detected in any of the transformed cell lines. The suppression of benzophenanthridine alkaloid biosynthesis using BBE or CYP80B1 antisense RNA constructs also reduced the growth rate of the cultures. Two-dimensional 1H-NMR and in vivo15N-NMR spectroscopy showed no difference in the abundance of carbohydrate metabolites in the various transgenic cell lines. However, transformed cells with reduced benzophenanthridine alkaloid levels contained larger cellular pools of several amino acids including alanine, leucine, phenylalanine, threonine, and valine compared to controls. The relative abundance of tyrosine, from which benzophenanthridine alkaloids are derived, was less than two fold higher in antisense-suppressed cells relative to controls. These results show that alterations in the metabolic flux through benzophenanthridine alkaloid biosynthesis can affect the regulation of amino acid pools. These data provide new insight into the metabolic engineering of benzophenanthridine alkaloid pathways (Park *et al*, 2002).

IV. Conclusion and Perspective

Antisense RNA methodology may eventually have broad applications in both basic and applied plant biology research. We may be able to use antisense methodology to reduce expression of deleterious genes and thereby enhance overall crop productivity of remove from plants

chemicals that are hazardous to human health, such as nicotine from tobacco, caffeine from coffee or tea, or numerous carcinogenic substances from various plants.

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