Defense-related proteins as families of cross-reactive plant allergens

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ABSTRACT

Higher plants produce a series of defense-related proteins to protect themselves against various stresses. Because of their stress-alleviating activities, the defense-related proteins are attracting much attention among plant breeders. However, recent studies revealed that some defense-related proteins constitute families of cross-reactive plant allergens. Their conserved structures must be responsible for the potential of cross-reactivity. In this article, the biological characteristics of the defense-related proteins, including the so-called pathogenesis-related proteins, are concisely described. Their pertinence to plant-derived food allergies is then explained in reference to the latex-fruit syndrome and the pollen-food allergy syndrome (the oral allergy syndrome: OAS). A new paring concept of complete food allergens and incomplete food allergens (non-sensitizing elicitors) becomes
important with relation to the safety of novel plants that were artificially modified to constantly express defense-related proteins. The possible contribution of such protective proteins to the growing number of immediate-type plant allergies is also discussed.

INTRODUCTION

Defense-related proteins are a series of protective proteins produced by higher plants to guard themselves against various stresses [1], and because of their stress-alleviating activities, these proteins are attracting much attention among plant breeders [2-4]. Genetically modified plants that can actively express some kinds of defense-related proteins are being developed. Less-toxic chemicals that induce plants to express anti-microbial proteins are also being examined as a novel type of agro-chemical [2,3].

On the other hand, recent studies have revealed that the defense-related proteins constitute families of cross-reactive plant allergens [5-7]. The structural conservatism of defense-related proteins most likely gives them the potential of cross-reactivity. Representative allergic disorders caused by this cross-reactivity include latex-fruit syndrome [8] and pollen-food allergy syndrome [9]. Pollen-food allergy syndrome is more often described as oral allergy syndrome (OAS) because of its characteristic oral-restricted symptoms. Some latex-allergic patients experience allergic reactions to banana, avocado, chestnut, kiwi, potato, and the like [8]. There is growing evidence that this phenomenon, the latex-fruit syndrome, is a reaction to the defense-related proteins present in both natural rubber latex and the vegetable foods [5-7]. Likewise, some pollen-allergic patients are irritated by plant-derived foods, especially by fresh fruits and vegetables [9]. The relevance of defense-related proteins to the pollen-food allergy syndrome or OAS is also becoming clear [5-7]. The latex-fruit syndrome and the pollen-food allergy syndrome are comparable disorders with respect to the causative allergens and to the syndromes’ processes, from the patients’ sensitization to the symptom manifestation.

In this article, the author attempts to describe concisely the features of defense-related proteins in higher plants following the principle of cross-reactions. The pertinence of these proteins to plant-derived food allergies is then explained in reference to the latex-fruit syndrome and the pollen-food allergy syndrome. A new concept of complete food allergens and incomplete food allergens becomes important with relation to the safety of novel plants that were intentionally modified to constantly express defense-related proteins. The possible contribution of these proteins to the growing number of immediate-type plant allergies is also discussed.

1 CROSS-REACTIVE ANTIGENS
1.1 Plant-derived pan-allergens

Immediate-type allergies are triggered by interactions of the patients' IgE antibodies with specific antigens. However, the IgE antibodies do not necessarily recognize the whole of the antigenic molecule; they usually recognize only partial structures (epitopes) exposed on the surface of antigenic molecules. Therefore, if distinct antigens contain a common epitope, there is a possibility that specific IgE antibodies cannot rigorously distinguish them. This phenomenon becomes the basic principle of the cross-reaction, which some latex- or pollen-allergic patients experience in reaction to various fresh fruits and vegetables [8-10].

Antigens that are responsible for a wide-ranging cross-reactivity are often referred to as pan-allergens. Currently, enzymes and binding proteins that are conserved in the course of evolution and produced by unrelated species of plants or animals are assumed to constitute potential pan-allergen families. The partial structures important for their specified
functions have not generally been altered with mutation, and accordingly they are evolutionarily conserved. Moreover, these structures tend to be distributed on the surface of the molecules to interact with specific substrates, ligands, and proteins. When such partial structures became an epitope for the IgE antibodies, the patient can be expected to cross-react to many unrelated species of plants or animals.

As a representative plant-derived pan-allergen, profilin has been studied extensively [11,12]. Every eukaryotic cell produces mutually homologous profilin, which is an actin-binding protein. The cross-reactivity of pollen-allergic patients to various fruits and vegetables is partially ascribed to the profilin present in both the causative pollen and in plant-derived foods [12,13]. Birch profilin and latex profilin are also formally registered as allergens, Bet v 2 and Hev b 8, respectively. Their limited contribution to pollen-food allergy syndrome (OAS) and latex-fruit syndrome is reasonably expected [13,14]. Plant proteins with calcium-binding properties also share the conserved structures required for their function. Accordingly, they are thought to form a pan-allergen family, as in the case of actin-binding profilin. Some calcium-binding proteins have indeed been verified to be cross-reactive allergens for pollen-allergic people [15]. Likewise, the structures of defense-related proteins in higher plants are conserved in the course of evolution [1,2]. Thus, these proteins are expected to have the potential for constituting pan-allergen families.

1.2 Cross-reactive carbohydrate determinant

One point that must be emphasized is that there are functional differences between a cross-reactive peptide epitope on a pan-allergen and a cross-reactive carbohydrate determinant (CCD) on a glycoprotein [16]. Peptide epitopes usually behave as multivalent antigens. They can construct a bridge-like structure with specific IgE antibodies attached to the receptor on the surface of a sensitized cell. Formation of this bridge-like structure of IgE antibodies is believed to be essential for triggering the cascade of allergic reactions [17]. In contrast, CCDs behave as monovalent antigens, except in some specific situations. They cannot form the bridge-like structure with the specific IgE antibodies attached to the receptor. This means that a CCD does not usually trigger allergic reactions, even if it was specifically recognized by the IgE antibodies of patients [18].

Lack of understanding of the functional difference between peptide epitopes and carbohydrate epitopes can bring about serious confusion in the interpretation of in vitro IgE tests, such as the radioallergosorbent test (RAST) and the enzyme-linked immunosorbent assay (ELISA) [19]. We cannot distinguish monovalent antigens from multivalent antigens using these in vitro diagnostic methods. This drawback is inherent in the in vitro IgE tests. Therefore, some degree of false-positive results must always be taken into account [20]. If a patient has IgE antibodies specific to a CCD, wide-ranging cross-reactivity is most provably detected by these tests. However, the exhibited cross-reactivity would end up as false-positive results in most cases [19]. The genuine allergenicity and cross-reactivity of antigens can be examined with a skin prick test or a histamine release test using a freshly prepared antigen solution. Monovalent antigens do not disturb the reliability of these tests. Appropriate antigen solutions are, however, essential for later tests, because most plant-derived antigens are unstable and easily lose their allergenicity [21]. When an in vitro IgE test shows suspicious allergenicity or cross-reactivity, the result should be confirmed by a skin prick test or a histamine release test.
2 DEFENSE-RELATED PROTEINS IN HIGHER PLANTS

2.1 Defense-related proteins

Higher plants have a defense system that is often likened to the immune system of animals. However, the proteins and enzymes participating in the defense system are substantially different from those in the immune system. The defensive arrangements of a plant can be divided into two categories, the static defense system and the dynamic defense system. The static defense system might be expressed as provision in peacetime against the attacks of phytopathogenic microorganisms and pests. The deposition of polyphenolic compounds that strengthen the cell walls of a plant is a good example of a peacetime provision. Storage proteins accumulated in fruits and seeds are also considered to take a role in the static defense system in addition to their preservative function. Some of the storage proteins, like 2S albumins, have been shown to exert anti-fungal activity to several pathogenic fungi [2]. Plant lectins with anti-microbial activities are also believed to assume some defensive roles [22-24].

On the other hand, the dynamic defense system might be interpreted as the inducible defense responses against various stresses. At the local site of a plant suffering from a stress, a fast and transient response called hypersensitive response (HR) is triggered. It is followed by the expression of a series of proteins with defensive properties [3,4]. Some of the defense-related proteins thus expressed contribute to the biosynthesis of low-molecular-weight plant antibiotics called phytoalexins. Others are deposited at the local cell walls to prevent the spreading of invading pathogens [1]. One group of defense-related proteins that are markedly expressed in crops after the infection of pathogenic microorganisms has been studied extensively since the beginning of this century. They are now collectively called pathogenesis-related proteins (PR proteins) [25-28].

2.2 Pathogenesis-related proteins

Pathogenesis-related proteins (PR-proteins) are defined as proteins encoded by the host plant but induced only in pathological or related situations. In the original definition [29], the inducing stimuli were confined only to the biotic stresses including various plant hormones, chitin, and chitosan. However, recent studies revealed that identical proteins are also induced by abiotic stresses such as wounding and the application of artificial chemicals [2-4,27]. Moreover, it has become evident that similar proteins are constantly expressed in particular parts of a plant, such as the root and the pollen, or in a limited growing stage of a plant. PR proteins are generally small (5 to 70 kD), stable at low pH, and resistant to the action of endogenous and exogenous proteinases. Many of them are coded by multi-gene families and expressed as a group of homologous proteins [27,29,30].

Numerous PR proteins that have been identified from various species of crops are now classified into 14 families (Table 1) [29,30]. This classification is based upon their serological or immunological relationships, sequence homologies, and characteristic enzyme activities [29]. It is worthwhile to list the properties of each family, because some of them are relevant to plant-derived food allergies [5-7].

The PR-1 family consists of 14- to 17-kD proteins with unknown biological activity. The PR-2 family occupies proteins with endo-β-1,3-glucanase activity. Most of them have a molecular weight of 25 to 35 kD. The PR-3 family includes about 30-kD proteins with endochitinase activity (class I, II, and IV chitinases) [31]. Class I chitinases commonly have an N-terminal chitin-binding domain corresponding to a major latex allergen, hevein (Hev 6.02) [31,32]. It is reported that endo-β-1,3-glucanases and endochitinases
Table 1. Recommended classification of pathogenesis-related proteins.

<table>
<thead>
<tr>
<th>Family</th>
<th>Properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR-1</td>
<td>unknown, 14-17 kD</td>
</tr>
<tr>
<td>PR-2</td>
<td>class I, II, and III endo-β-1,3-glucanases, 25-35 kD</td>
</tr>
<tr>
<td>PR-3</td>
<td>class I, II, and IV endochitinases, about 30 kD</td>
</tr>
<tr>
<td>PR-4</td>
<td>anti-fungal, win-like proteins, similar to prohevein C-terminal domain</td>
</tr>
<tr>
<td>PR-5</td>
<td>anti-fungal, thaumatin-like proteins, similar to α-amylase/trypsin inhibitors</td>
</tr>
<tr>
<td>PR-6</td>
<td>proteinase inhibitors, 6-13 kD</td>
</tr>
<tr>
<td>PR-7</td>
<td>endoproteinases, about 70 kD</td>
</tr>
<tr>
<td>PR-8</td>
<td>class III chitinases, chitinases/lysozymes, about 30 kD</td>
</tr>
<tr>
<td>PR-9</td>
<td>peroxidases, peroxidase-like proteins, about 40 kD</td>
</tr>
<tr>
<td>PR-10</td>
<td>ribonucleases, Bet α 1-related proteins, 17-18 kD</td>
</tr>
<tr>
<td>PR-11</td>
<td>endochitinase activity, 41-43 kD</td>
</tr>
<tr>
<td>PR-12</td>
<td>plant defensins, about 5 kD</td>
</tr>
<tr>
<td>PR-13</td>
<td>thionins, about 14 kD</td>
</tr>
<tr>
<td>PR-14</td>
<td>non-specific lipid transfer proteins (LTPs), 7-12 kD</td>
</tr>
</tbody>
</table>

synergistically enhance the resistance of a plant to fungal pathogens [2]. The cell walls of many fungi are degraded by these hydrolytic enzymes. Proteins having both endochitinase and lysozyme activities (class III chitinases) are now classified into the PR-8 family [31]. There is no sequence homology between an endochitinase belonging to the PR-3 family and a chitinase/lysozyme assigned to the PR-8 family. Proteins occupied in the PR-4 family are often referred to as win-like proteins. They have sequence homologies to a protein encoded by the potato win gene that is induced by wounding [33]. The C-terminal region (Hev b 6.03) of prohevein (Hev b 6.01), which is a rubber latex allergen, is also homologous to PR-4 proteins [33]. Proteins belonging to the PR-5 family are characterized by their sequence similarities to thaumatin, the sweet protein of a tropical shrub, and are often called thaumatin-like proteins. Several α-amylase/trypsin inhibitors purified from various plants also exhibit sequence homologies to PR-5 proteins; however, the inhibitory activities of PR-5 proteins have not yet been confirmed. Remarkably, sequences of some cereal and legume allergens are homologous to those of PR-5 proteins as well as α-amylase/trypsin inhibitors [6,7,27]. The PR-6 family consists of proteins with actual inhibitory activities to various proteinases. Plant proteins with endoproteinase activities are occupied in the PR-7 family, and peroxidases and peroxidase-like proteins in plants are assigned to the PR-9 family. The PR-10 family includes plant ribonucleases [34]. They are likely to be an indispensable enzyme in pollens to accomplish self-incompatibility [35,36]. The notorious major cross-reactive allergen in birch pollen, Bet α 1, is also a member of the PR-10 family [29,37,38]. The PR-12 family is assigned to plant defensins, and the PR-13 family includes thionins. Both of these proteins have compact structures with several disulfide bonds and exert anti-fungal activities [39]. Non-specific lipid transfer proteins (LTPs) are classified into the PR-14 family [30]. They also exhibit anti-fungal activities to several
pathogenic fungi [39].

One important point about the classification of PR proteins is that the species of host plants are not taken into account. The serological relationship, the sequence homology, and the enzyme activity of each protein are the criteria [29]. This means that many higher plants produce similar PR proteins irrespective of their morphological differences. The sequences of many types of defense-related proteins are relatively conserved, and this fact is not limited to PR proteins. Considering this structural conservatism, we can predict that a patient sensitized by a PR protein potentially cross-reacts to any plants containing a homologous PR protein. In reality, Bet v 1-sensitized patients often cross-react to various pollen and plant-derived foods containing structurally similar proteins (Bet v 1-related antigens) [5-7].

2.3 Defense responses of higher plants

The overall induction processes of defense-related proteins in higher plants can be summarized as follows [1,3,4,28]. The signal raised from biotic or abiotic stimuli is transmitted to the nuclei of affected cells. It induces the transcription of defense-related genes, and the produced messenger RNAs are then translated into the defense-related proteins. Some defense-related proteins, like extensins [40] and glycine-rich proteins, synergistically strengthen the cell walls of the attacked site. Peroxidases also contribute to the cell-wall enforcement [1]. Within the cytoplasm, enzymes indispensable for the biosynthesis of phytoalexins are expressed [1]. PR proteins, such as endo-β-1,3-glucanases, endochitinases, lysozymes, proteinase inhibitors, and ribonucleases, are thought to directly interact with the invading pathogens. They tend to accumulate in the vacuoles and intercellular spaces (apoplast), except for ribonucleases. A series of the glycosidases thus induced release the special oligosaccharides (elicitors) from the cell-wall structures of invading pathogens. Elicitors result in a positive feedback to the defense reactions.

Defense responses of higher plants are not restricted to the local events following the hypersensitive reaction (HR). The information about stresses is transmitted even to the cells far from the initially affected site. Then, PR proteins having anti-microbial activities are expressed in the affected and distant sites of the plant. This phenomenon might be interpreted as a plant enhancing its resistance against various stresses once it has suffered from a stress. This extensive defense response following the local reactions is usually called systemic acquired resistance (SAR) [3,4,28].

The author of this article presumed that defense-related proteins, especially water-soluble PR proteins, become important latex allergens and cross-reactive allergens in plant-derived foods (Figure 1) [41,42]. The conserved structures of defense-related proteins can rationally explain the basis for latex-fruit syndrome and pollen-food allergy syndrome (OAS). In the next section, the relevance of defense-related proteins to latex allergy and the latex-fruit syndrome is described, quoting a few of the author's experimental results.

3 DEFENSE-RELATED PROTEINS AS LATEX ALLERGENS

3.1 Latex allergy

Since the late 1980s, an immediate-type allergy caused by various natural rubber products has been reported worldwide. Natural rubber products are made from the latex of a rubber tree (Hevea brasiliensis). Therefore, the immediate-type reaction has come to be called latex allergy. The causative factor of latex allergy is plural proteins that elute from the finished products [43]. Consequently, this allergy is prevalent in people who repeatedly touch latex products, for example, healthcare
professionals and patients suffering from congenital abnormalities. The exposure route to the allergens is, however, not limited to direct contact with latex products. Lubricant powders applied on the surface of latex gloves are adsorbing latex allergens and often spread over the surrounding area. By inhaling the floating powder, people can be sensitized by the latex allergens. Asthmatic reactions can also be provoked in already sensitized patients by inhaling the allergen-adsorbing powder [44]. Moreover, automobile-tire debris contaminated by latex allergens is reported to be floating around trunk roads [45,46].

One important feature of latex allergy is patients’ cross-reactivity to various fruits, vegetables, pollen, and medical plants [47], a reaction usually called latex-fruit syndrome [8].

More than 50% of latex-allergic patients possess IgE antibodies specific to antigens from other plants [8,47,48]. Such a wide-ranging cross-reactivity is usually due to structurally related proteins that most plants have the potential to produce. However, the taxonomical dissimilarity of the causative plants has kept us from developing any concrete explanations of the cross-reactive allergens. Neither profilin nor calcium-binding proteins could fully explain the latex-fruit syndrome [14]. In the course of the efforts to specify the latex allergens, we focused our attention on this cross-reactivity. As a possible explanation of the wide-ranging cross-reactions, the author hypothesized that defense-related proteins are the latex allergens sharing common epitopes with the
cross-reactive antigens in other plants (Figure 1) [41,42].

3.2 Induction of defense-related proteins
   Rubber trees cultivated on plantations are species that have been genetically selected for economically effective latex production. During this rubber-tree breeding, a propensity to hypersensitively express defense-related proteins seems to be concurrently selected. Kush et al. investigated the latex-producing cells (laticifers) of genetically selected rubber trees and found that enzymes required for latex biosynthesis and defense-related proteins were 20 to 100 times and 10 to 50 times more abundant in the laticifers than in the leaves, respectively [49]. The tendency to over-express defense-related proteins might be inevitably selected. A rubber tree expressing a large amount of such protective proteins would be expected to resist various stresses and therefore be agriculturally valuable.

   Natural rubber latex is a milky sap exuding from the trunk of a rubber tree when it is cut spirally (tapped). After a few hours, the latex coagulates into a plug covering the wounded site and stops the flow. To obtain a large amount of the latex, rubber trees are supposed to be tapped repeatedly, which stresses the tree. In addition, ethephon is sometimes administered to the rubber trees in order to lengthen the latex flow time and shorten the period required for compensation for the lost latex. The administered ethephon is converted to ethylene in the rubber tree, which stimulates plants to produce defense-related proteins [49]. Accordingly, this ethephon application also results in over-expression of the defense-related proteins [50]. Hevein (Hev b 6.02) and an endochitinase were considerably more abundant in the latex collected from an ethephon-treated rubber tree than from a control tree [51]. Furthermore, the transcription level of a gene encoding hevein was enhanced approximately 5 times not only by ethephon administration but also by wounding or treatment with abscisic acid [52].

   The series of findings mentioned above imply that the latex collected from the rubber trees cultivated on plantations contains a large amount of defense-related proteins. If structurally conserved defense-related proteins elute from the final products and become latex allergens, we can rationally explain why some latex-allergic patients cross-react to many unrelated plants (Figure 1) [41,42]. To explore this hypothesis, the author first examined whether defense-related proteins, especially PR proteins with characteristic enzyme activities, are actually extracted from non-ammoniated latex, raw ammoniated latex, and commercially available latex gloves.

3.3 Detection of defense-related enzymes
   The existence of PR proteins in a sample solution is easily deduced by simply measuring their characteristic enzyme activities. Extracts from two brands of latex gloves, raw ammoniated latex, and non-ammoniated latex were examined to determine whether they exhibit the hydrolytic enzyme activities typical of PR proteins [53]. All the extracts exhibited three kinds of hydrolytic enzyme activities, endochitinase, lysozyme, and endo-\(\beta-1,3\)-glucanase, that would directly give a plant the resistance to a wide range of pathogens [2,27]. The activity of carboxylesterase was also detected in every extract. Additionally, inhibitory activity to an alkaline proteinase was detected in the extract from the non-ammoniated latex. These results strongly suggested that PR proteins biosynthesized in a rubber tree are truly included in the latex and persist to the final products.

   By applying the extract from the raw ammoniated latex to a gel filtration chromatography, we were able to estimate the molecular weights of the endochitinase, lysozyme, and endo-\(\beta-1,3\)-glucanase to be 25 to 45 kD in
a non-denaturing condition. These hydrolytic enzymes were relatively resistant to heat denaturing. All the enzyme activities were clearly detected, even after the extract from the raw ammoniated latex had stood at 100 degrees centigrade for 20 minutes [53].

3.4 Defense-related enzymes as latex antigens

a) Latex antigen with both endochitinase activity and lysozyme activity

A basic protein having both endochitinase activity and lysozyme activity was separated from a brand of household gloves and from non-ammoniated latex [54,55]. The N-terminal sequence of a chitinase/lysozyme (29.5 kD) isolated from the non-ammoniated latex completely agreed with that of hevamines [56]. Hevamines are the chitinase/lysozyme of a rubber tree and have been recognized as playing defensive roles for the tree. The determined sequence also showed homologies to some chitinases/lysozymes found in various plant species, such as ivy, vine, cress, cucumber, adzuki bean, tobacco, and chickpea. All of these proteins probably belong to the PR-8 family.

In an ELISA experiment, the bifunctional chitinase/lysozyme isolated from non-ammoniated latex exhibited a relatively low affinity for the IgE antibodies of latex-allergic patients. Only 2 (13%) out of the 15 patients had a significant amount of the specific IgE antibodies to this antigen [55]. This result suggested the minor importance of this protein as a latex allergen. However, some of the patients exhibited clear positive responses in skin prick tests or histamine release tests to the purified chitinase/lysozyme.

b) Latex antigen with esterase activity

A carboxylesterase was separated from the raw ammoniated latex [57]. It was an acidic protein (pl 4.2 to 5.2) and estimated to have a molecular weight of 80 kD in a non-denaturing condition. By sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under a reducing condition, it was detected as the main band (45 kD) accompanying several minor bands (14, 16, 24, 25, 28, and 39 kD). The biochemical and enzymatic properties of this protein were comparable to those of hevain I, which is a serine-centered protease purified from the lutoid fraction (B-serum) of natural rubber latex [58,59]. Lutoids are derived from vacuoles where the PR proteins tend to be accumulated [60,61]. In an immunoblotting experiment, the carboxylesterase was specifically recognized by the IgE antibodies of latex-allergic patients [57].

A carboxylesterase was also separated from non-ammoniated latex [55]. This acidic protein (pl 4.6) was detected as a single band by native PAGE. However, both the main band (45 kD) and the several minor bands (28 kD and 35 to 39 kD) were concurrently detected by SDS-PAGE under a reducing condition, as in the case of the carboxylesterase separated from the ammoniated latex [57]. The N-terminus of the main protein (45 kD) was blocked. This enzyme was frequently recognized by the IgE antibodies of latex-allergic patients. In an ELISA experiment, 10 (67%) out of the 15 sera of latex-allergic individuals were shown to contain significant amounts of the specific IgE antibodies to this enzyme [55]. The allergenicity of the purified carboxylesterase was also confirmed by a skin prick test and a histamine release test.

c) Latex antigen with β-1,3-glucanase activity

Basic endo-β-1,3-glucanases (pl >10) were separated from non-ammoniated latex [55]. They were detected as three distinct bands (35, 36.5, and 38 kD) by SDS-PAGE under a reducing condition. Their N-termini were uniformly blocked. One of the fragment peptides produced by cyanogen bromide
treatment had an N-terminal sequence highly homologous to the internal sequence of a latex allergen, Hev b 2 (34 and 36 kD; pl 9.5) [62,63]. The determined sequence was also homologous to the partial sequences of endo-β-1,3-glucanases found from various plants, including tobacco, green pea, and potato. In an ELISA experiment, 6 (40%) out of the 15 sera of latex-allergic patients were revealed to contain significant amounts of the specific IgE antibodies to the endo-β-1,3-glucanase isoenzymes [55]. The actual allergenicity of this enzyme was confirmed by a skin prick test.

The author separated a basic endo-β-1,3-glucanase also from one brand of household gloves. It was substantially stable in alkaline solutions as well as in acidic solutions. In addition, the endo-β-1,3-glucanase was specifically recognized by the IgE antibodies of latex-allergic individuals.

3.5 Defense-related proteins of a rubber tree occupy an important part of latex allergens

The author of this article hypothesized that defense-related proteins in higher plants are major causes of latex allergy and accompanying cross-reactivity (Figure 1) [41,42]. Characteristic enzyme activities of a few PR proteins were detected in every extract prepared from non-ammoniated latex, raw ammoniated latex, and two brands of latex gloves, as had been expected. This result implies that latex products are generally contaminated by the defense-related proteins. Moreover, all the hydrolytic enzymes separated from a brand of household gloves, ammoniated latex, or non-ammoniated latex were more or less recognized by the specific IgE antibodies of latex-allergic people. These results support the initial hypothesis.

A partial sequence of a basic endo-β-1,3-glucanase isoenzyme separated from non-ammoniated latex was highly similar to the reported sequence of a latex allergen (Hev b 2) [62]. In addition, the N-termini of the separated enzymes were uniformly blocked [55] as in the case of Hev b 2 [63]. These results indicate that the separated endo-β-1,3-glucanases are identical or at least closely related to this allergen. Hev b 2 was first documented by a group at the Rubber Research Institute of Malaysia [63]. They also mentioned that Hev b 2 possessed endo-β-1,3-glucanase activity. However, they have not described why they expected this particular enzyme activity of Hev b 2. The author separated endo-β-1,3-glucanases from natural rubber latex to verify the hypothesis on latex allergens (Figure 1), because many proteins with this activity take defensive roles in plants [25-28,64]. The fact that the separated isoenzymes were closely related to the independently identified latex allergen supports the correctness of the author’s initial hypothesis.

The N-terminal sequence of the chitinase/lysozyme purified from non-ammoniated latex coincided with those of hevamines [55]. Hevamines are defense-related proteins of a rubber tree that would be classified into the PR-8 family [56]. A database search for proteins having a homologous sequence listed several proteins with the same activity but coming from diverse species. This result indicates that chitinases/lysozymes have the potential to form a plant pan-allergen family, although hevamines are reported to be a minor latex allergen [43]. The low affinity of this antigen for the IgE antibodies of latex-allergic patients was also shown in the author’s in vitro experiments [55].

The major protein (45 kD; SDS-PAGE) constructing another latex allergen with serine-centered carboxylesterase activity was N-terminally blocked [55]. Beezhold et al. already reported a latex allergen (Hev b 7) having a similar molecular weight (46 kD; SDS-PAGE) [48,65,66]. The carboxylesterase
activity of recombinant Hev b 7 was later demonstrated [67]. However, there is a meaningful disagreement. Beezhold et al. reported that the N-terminus of Hev b 7 was not blocked [65]. They also mentioned the sequence similarity of Hev b 7 to patatin [48], which is a major storage protein in potato tubers. Patatin is now registered as an important potato allergen (Sol t 1), and it exhibits hydrolytic enzyme activities, such as the presence of lipid acyl hydrolase and esterase [68], for which a serine residue plays a vital role as in the case of the latex carboxylesterase [57,69]. The exemplified inhibitory activity of patatin toward the growth of invertebrate pests indicates the defensive function of this enzyme [69]. On the other hand, Subroto et al. documented an N-terminally blocked latex protein (43 kD; SDS-PAGE) an internal sequence of which was homologous to that of patatin [70]. Taken together, it is reasonable to consider that Hev b 7 and patatin are encoded by multi-gene families. Consequently, Hev b 7 should exist as isoallergens in natural rubber latex with blocked or unblocked N-termini [65,67].

Several other latex allergens officially registered to date also have some connection with the defense responses of a rubber tree. Alenius et al. [71,72] and Baur et al. [73,74] reported that hevein (Hev b 6.02) and its precursor (prohevein or hevein preprotein; Hev b 6.01) were the most important latex allergens (hevein-related allergens). Hevein has anti-fungal activities to some pathogenic fungi [75]. Some defense-related proteins, like class I endochitinases and wheat germ agglutinin, contain a hevein-like domain [31,32]. In addition, the C-terminal domain (Hev b 6.03) of prohevein shows sequence similarities to PR-4 proteins (win-like proteins) [27,33]. These facts indicate that hevein-related allergens would take defensive roles in a rubber tree. On the other hand, Posch et al. reported that latex antigens recognized by the IgE antibodies of latex-allergic patients had the N-terminal sequence homologies to class II endochitinases or superoxide dismutases [76]. These antigens could also be defense-related proteins of a rubber tree. Table 2 shows the officially registered latex allergens and their

<table>
<thead>
<tr>
<th>Name</th>
<th>Trivial name</th>
<th>MW (kD)</th>
<th>Predicted physiological roles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hev b 1</td>
<td>rubber elongation factor</td>
<td>58</td>
<td>rubber biosynthesis</td>
</tr>
<tr>
<td>Hev b 2</td>
<td>β-1,3-glucanases</td>
<td>34, 36</td>
<td>defense-related protein</td>
</tr>
<tr>
<td>Hev b 3</td>
<td>small rubber-particle protein</td>
<td>24</td>
<td>rubber biosynthesis</td>
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<tr>
<td>Hev b 4</td>
<td>microhelix component</td>
<td>100-115</td>
<td>defense-related protein?</td>
</tr>
<tr>
<td>Hev b 5</td>
<td>acidic latex protein</td>
<td>16</td>
<td>?</td>
</tr>
<tr>
<td>Hev b 6.01</td>
<td>prohevein, hevein preprotein</td>
<td>20</td>
<td>defense-related protein</td>
</tr>
<tr>
<td>Hev b 6.02</td>
<td>hevein</td>
<td>4.7</td>
<td>(latex coagulation)</td>
</tr>
<tr>
<td>Hev b 6.03</td>
<td>prohevein C-terminal domain</td>
<td>14</td>
<td>defense-related protein, inhibitor of rubber biosynthesis</td>
</tr>
<tr>
<td>Hev b 7</td>
<td>patatin-like proteins</td>
<td>46</td>
<td>structural protein, etc.</td>
</tr>
<tr>
<td>Hev b 8</td>
<td>latex profilin</td>
<td>14</td>
<td>?</td>
</tr>
<tr>
<td>Hev b 9</td>
<td>latex enolase</td>
<td>51</td>
<td>?</td>
</tr>
<tr>
<td>Hev b 10</td>
<td>Mn-superoxide dismutase</td>
<td>26</td>
<td>?</td>
</tr>
</tbody>
</table>
probable physiological functions. From this table, it is evident that defense-related proteins from a rubber tree occupy an important part of the latex allergens.

4 DEFENSE-RELATED PROTEINS AS PLANT PAN-ALLERGENS
4.1 Defense-related proteins as a cause of latex-fruit syndrome

Some latex-allergic patients experience generalized symptoms or OAS when they ingest fresh fruits and vegetables [8,47,48]. If defense-related proteins in higher plants become cross-reactive allergens, we can reasonably understand this seemingly curious cross-reactivity. The primary structures of defense-related proteins are conserved in the course of evolution; namely, many kinds of plants express serologically related proteins. If a person has been sensitized by a defense-related protein from a rubber tree, many kinds of fruits and vegetables containing a homologous protein become potentially allergenic for the patient (Figure 1). As described in the previous section, it is becoming clear that the defense-related proteins of a rubber tree occupy an important part of the latex allergens [6,7,43,77].

It must be emphasized that hevein (4.7 kD; Hev b 6.02) and prohevein (Hev b 6.01) were verified to be the important latex allergens. Small domains highly homologous to the sequence of hevein are commonly involved at the N-termini of class I endochitinases [31,32,78]. Some plant-derived lectins with chitin-binding properties, like wheat germ agglutinin, also contain hevein-related domains [32,78]. Because of these conserved hevein-related structures, patients previously sensitized by latex hevein or prohevein are predicted to potentially cross-react to fruits and vegetables containing a class I endochitinase or a lectin with a hevein-related domain [43,79].

Akasawa et al. purified an avocado allergen that was recognized by the IgE antibodies of latex-allergic individuals. The allergen's partial sequence was highly homologous to those of class I endochitinases. In a later study, they determined the entire DNA sequence of an important cross-reactive avocado allergen and showed that it corresponded to a class I endochitinase [80]. Mikkola et al. reported that both the N-terminal and the internal sequences of a cross-reactive banana allergen were highly homologous to those of class I endochitinases [81]. The cross-reactive allergen in chestnut was also a class I endochitinase [82]. Furthermore, Beezhold et al. demonstrated that several plant lectins, including wheat germ agglutinin, were specifically recognized by the IgE antibodies of individuals who were allergic to hevein [83]. All of these results support the idea that the conserved hevein-related structures are an important source of common epitopes responsible for the latex-fruit syndrome [84-88].

Besides the hevein and prohevein, the C-terminal domain (Hev b 6.03) of prohevein and the patatin-like latex allergen (Hev b 7) are expected to bring about cross-reactivity in patients already sensitized by them. The sequence of Hev b 6.03 is homologous to those of proteins belonging to the PR-4 family [33,83]. On the other hand, the sequence of Hev b 7 is similar to that of the major potato allergen, patatin (Sol t 1) [48,89]. Seppälä et al. mentioned the significant cross-reactivity between Hev b 7 and Sol t 1 [90]. In contrast, Sowka et al. reported that there was no cross-reactivity between patatin and four kinds of recombinant Hev b 7 isoforms [91]. Both patatin and Hev b 7 are most likely encoded by multigene families and expressed as isoforms [65-67]. Hev b 7 isoforms (heavins) [58,59] are present not only in the cytoplasmic fraction (C-serum) but also in the lutoid fraction (B-serum) [92,93]. Some of them may also be post-translationally modified. All the recombinant Hev b 7 isoforms reported by Sowka et al. were proposed to localize in the cytoplasm
Defense-related proteins as pan-allergens

(C-serum) from the lack of the leader peptides [91]. Because natural hevains exist in a few compartments and in diverse molecular forms [58,59], the cross-reactivity of Hev b 7 and patatin should be further studied.

4.2 Defense-related proteins as a cause of pollen-food allergy syndrome

Some people suffering from tree or grass pollen also become allergic to fresh fruits and vegetables with time [9]. Most of the causative foods for these patients are not notorious for their allergenicity; in fact, the number of patients who complain about allergies solely to these foods is small. Moreover, many pollen-allergic patients can ingest cooked plant-derived foods without any troubles. This type of food allergy concomitant with pollinosis is often referred to as pollen-food allergy syndrome [9], but it is more generally called oral allergy syndrome (OAS) from its typical conditions. As in the case of latex-fruit syndrome, it is becoming clear that part of the pollen-food allergy syndrome can be ascribed to the cross-reactivity of defense-related proteins in pollen and in vegetable foods [5-7].

Representative cases of pollen-food allergy syndrome can be seen in patients allergic to birch pollen. More than 70% of birch-pollen allergic patients are reported to have the IgE antibodies recognizing the antigens of fruits and vegetables [94]. Many of the patients actually experience the oral symptoms when they ingest fresh apple, celery, cherry, carrot, and so on. Some of these cross-reactions are due to profilin and calcium-binding proteins [13,15]. Isoflavone reductase, which is an enzyme used for phytoalexin biosynthesis, also functions as a minor cross-reactive allergen [95,96]. However, the most important cross-reactive allergens are Bet v 1-related proteins. Bet v 1 is a major allergen in birch pollen and a member of the PR-10 family with ribonuclease activity [34,36-38]. It has been reported that the expression level of Bet v 1 was heightened by infection of pathogens [97], wounding [98], or by treatment with heavy metals [99], ozone, or nitric oxide. These facts suggest the defense-related function of Bet v 1 [38,100]. The irritating fruits and vegetables for birch pollen-allergic patients often contain Bet v 1-related antigens, for instance, Mal d 1 (apple) [101], Api g 1 (celery) [102], Pru av 1 (cherry) [103], and Dau c 1 (carrot) [104]. Once people have been sensitized by Bet v 1 through inhalation of birch pollen, they are predicted to cross-react to plant-derived foods containing a Bet v 1-related antigen [10,94,105]. Remarkably, Mal d 1 is mentioned to be unstable to enzymatic degradation. That is to say, sensitization is hardly established by simply ingesting Mal d 1. Pollen-food allergy syndrome is in fact considered a phenomenon coming after pollinosis as described in section 5 (Figure 2) [10,94,105-107].

Besides birch pollen, pollen from some other species of trees contains Bet v 1-related antigens, for instance, Aln g 1 (alder), Cor a 1 (hazel), Car b 1 (horsembeam), Que a 1 (white oak), and so on. The patients sensitized by these allergens through inhalation of the pollen are also expected to cross-react to fresh fruits and vegetables containing the Bet v 1-related antigens [94]. Moreover, patients with birch pollinosis would react to the other kinds of pollen containing the Bet v 1-related allergens, and vice versa. However, it is very difficult to assume that a patient with pollen-food allergy syndrome was first sensitized by ingesting fruits or vegetables and then became allergic to pollen [94,106]. Bet v 1 has been shown to contain almost all the epitopes present in the cross-reactive food allergens, but the reverse was not true [107].

The repertoire of cross-reactive foods and pollen is generally inclined to become larger as the pollinosis becomes more serious. Horikawa et al. have explained this phenomenon by the concept of epitope spreading [108]. The se-
Figure 2. Traditional food allergy (left) and food allergy based upon the cross-reactivity of antigens (right). Digestible food proteins hardly establish per-oral sensitization. However, there is a possibility that allergic symptoms are provoked in already sensitized patients by digestible food proteins based upon their cross-reactivity to the seemingly independent sensitizers.

quences of cross-reactive allergens are highly homologous to each other but are not the same. Therefore, the community of their epitopes is also valuable. At the initial stage of pollinosis, a patient has the IgE antibodies specific to the limited number of the common epitopes on the pollen allergen. Accordingly, cross-reactivity is seen only to the confined foods and pollen containing an antigen with such epitopes. As the pollinosis becomes more serious, the patient develops the IgE antibodies against most of the common epitopes on the pollen allergen. The range of cross-reactivity is consequently spread to various foods and pollen containing an antigen with at least one of the common epitopes on the pollen allergen.

The spread from three-dimensional IgE epitopes to sequential IgE epitopes is also probable.

4.3 Defense-related proteins as food allergens

Although the number is not so large, some people experience allergic reactions to various fruits and vegetables accompanying neither latex allergy nor pollinosis. The symptoms are not confined to the oral cavity in most cases but are often extended to systemic reactions. One of the cross-reactive allergens responsible for this type of fruit and vegetable allergy is non-specific lipid transfer proteins (LTPs) [109,110]. These proteins were initially pre-
dicted to function as a carrier protein for lipids. However, this property was denied by later studies. They are now considered one of the defense-related proteins in higher plants (PR-14 family) because of their growth-inhibiting activities to some pathogenic fungi [30,39,111]. LTPs are reported to be stable in simulated gastric fluid (SGF) [112] and therefore can be per-oral sensitizers as described in the next section (Figure 2) [110]. Their stability in the presence of heat and the SGF are most probably the result of their compact structures (around 9 kD) with several disulfide bonds [39,111]. Hevein domains in class I endochitinases have a similarly compact structure accompanying several disulfide bonds [32,39,78], but their molecular weights (around 4.7 kD) are significantly lower than those of LTPs.

Cross-reactive allergens in cereals and legumes were found to correspond to 2S albumins [6,7,113]. Although their primal function is preservation of nutrition, some of them were confirmed to have anti-fungal activities [2]. In addition, several plant allergens, those found from the mountain cedar (Jun a 3) [114], paprika [115], cherry [116], and bell pepper [117], exhibited sequence homologies to PR-5 proteins [118,119]. These proteins are also regarded as members of cross-reactive plant allergens [6,7].

5 FOOD ALLERGY BASED UPON CROSS-REACTIVITY
5.1 Complete food allergens
It has been broadly believed that food proteins responsible for immediate-type allergies have common characteristics, including stability in the presence of heat and digestive enzyme degradation. Truly, the major allergens in the notorious foodstuffs, milk, egg, peanut, soybean, etc., are stable in the presence of heat and not easily digested by either simulated gastric fluid (SGF) or simulated intestinal fluid (SIF) [120,121]. Nevertheless, many food proteins responsible for latex-fruit syndrome and pollen-food allergy syndrome (OAS) are generally unstable in the presence of heat or enzymatic digestion [21,122]. The author of this article demonstrated that most IgE-reactive proteins extracted from fresh fruits and vegetables were degraded by the SGF (US Pharmacopoeia) within a few minutes [123]. Vieths et al. [124] and Wigotzki et al. [125] also pointed out the instability of hazelnut allergens to enzymatic degradation. Besides the digestibility, it is well known that many pollen-allergic patients suffering from OAS to fresh fruits and vegetables can eat cooked foods without serious troubles. It has also been reported that cross-reactive class I endochitinases in fresh vegetable foods completely lost their allergenicity for latex-allergic patients following a simple heat treatment [126]. Moreover, the numbers of patients who are allergic only to fresh fruits or vegetables are indeed small [88]. From these findings, we can predict that the food proteins responsible for latex-fruit syndrome or pollen-food allergy syndrome (OAS) have different properties from those of traditional food allergens.

With traditional food allergies, only exceptional food proteins that are not degraded under cooking conditions and by the digestive enzymes are assumed to become allergens [120,127]. After arriving at the intestine without substantial fragmentation, they are adsorbed and recognized by the immune system, completing the sensitization of a person. If he or she ingests the same antigen after the establishment of sensitization, it would be adsorbed again from the intestine and bring about generalized symptoms. In this whole process (class 1 food allergy) [6], the identical antigen is relevant to both the sensitization stage and the elicitation stage of allergic reactions (Figure 2). Such food antigens having not only the symptom-provoking ability but also the sensitizing ability are often called complete food allergens [128].
5.2 Incomplete food allergens

In contrast to persons suffering from traditional food allergies (class I food allergies), patients suffering from latex-fruit syndrome or pollen-food allergy syndrome (OAS) are most likely sensitized through direct contact with latex products or inhaling allergen-adsorbing powder or pollen [44,94,107]. The sensitized people are then thought to experience allergic episodes when they ingest fresh fruits and vegetables containing cross-reactive antigens as well as when they are exposed to the sensitizing antigens again [81,106,122]. In this whole process (class 2 food allergy) [6], the antigens relevant to the sensitization stage and to the symptom provoking stage are not identical. The antigens in fruits or vegetables are responsible only for the symptom elicitation. Accordingly, they do not need to be resistant to heat and digestive enzymes [129]. These properties of food proteins are a prerequisite for becoming a per-oral sensitizer [120,128]. Food antigens having only the symptom-provoking ability in people who have already been sensitized are often called incomplete food allergens [128]. Incomplete food allergens are understood to induce allergic reactions based upon their cross-reactivity to the inhalant, contact, or per-oral sensitizers (Figure 2).

Considering the pertinence of incomplete food allergens to latex-fruit syndrome and pollen-food allergy syndrome, we can explain why the major symptoms tend to be restricted

<table>
<thead>
<tr>
<th>Non-allergens</th>
<th>Profilin ~14kD</th>
<th>Hevein ~4.7kD</th>
<th>LTPs ~9kD</th>
<th>Traditional food allergens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Citrus</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tree</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>±</td>
</tr>
<tr>
<td>Nuts</td>
<td>−</td>
<td>+(OAS)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Per-oral allergy</td>
<td>−</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Diverse allergenicity of food proteins. To display per-oral sensitizing ability, both the resistance to digestion and the minimum molecular weight, probably around 9 kD, would be necessary. The hevein domain of a class I endochitinase and profilin most likely correspond to incomplete food allergens (non-sensitizing elicitors). Drawings show the features of each group.
to the oral cavity (OAS). Incomplete food allergens are not resistant to the digestive enzymes. Therefore, they are predicted to rapidly lose the antigenicity in the digestive tract, even though they may be recognized by the immune system at the oral cavity. The lability of incomplete food allergens also clarifies why fresh fruits and vegetables are highly allergenic while cooked foods retain little allergenicity [21,126].

The marked difference between latex-fruit syndrome and pollen-food allergy syndrome is the severity of allergic reactions provoked by fresh foods. Most vegetable food allergies concomitant with pollinosis are restricted to oral symptoms (OAS). In contrast, latex-fruit syndrome is not confined to oral symptoms; it sometimes extends to generalized urticaria and anaphylaxis. The author examined the stability of hevein (Hev b 6.02) that shares the important epitopes with the cross-reactive vegetable allergens (class I endochitinases) [80-88]. Hevein was stable in the SGF (US Pharmacopeia) as complete food allergens were [123]. On the other hand, class I endochitinases in vegetable foods were presumed to be digested into small fragments, leaving the stable hevein domains [88,123]. After being adsorbed from the intestine, the hevein domains can cause generalized symptoms like those occurring with complete food allergens. However, few patients complain of allergic disorders only to vegetable foods containing class I endochitinases, such as avocado, banana, and chestnut [88]. Considering these facts, it is reasonable to speculate that the hevein domains (about 4.7 kD) are too small to per-orally sensitize a person (Figure 3). Nevertheless, they seem to be recognized by the abdominal immune system of a person who has already been sensitized by hevein or prohevein and bring about the generalized symptoms [81,88]. The molecular weight of LTPs (around 9 kD) might correspond to the lower limit of food proteins (complete food allergens) that can sensitize people per-orally (Figure 3).

6 INDUCTION OF ALLERGENIC PROTEINS IN PLANTS

6.1 Possible allergen induction by stresses

It is becoming increasingly clear that many of the plant-derived allergens assume defensive functions in higher plants [5-7]. Some defense-related proteins are accumulated in the storage organs, and others are expressed by various stresses. Defense-related proteins in a storage organ generally change in quantity and quality following organ maturity [118]. On the other hand, the stressful stimuli that induce plants to produce defense-related proteins are various, including chemicals [49-52], heavy metals [99], microbes [97], air pollutants like ozone [130], ultraviolet rays, salt, drought, submersion, and so on [3,4,28]. Considering these variable facts, we can imagine that allergens in individual plants are changeable rather than constant depending on their maturity, circumstances, and species.

Because numerous chemicals and air pollutants induce defense-related proteins, environmental pollution is suspected to play a part in the increasing trend of people suffering from plant allergies. It has been reported that the higher the concentration of ozone in the atmosphere the larger the amount of group 5 allergens in rye grass (Lolium perenne) pollen, for instance [131]. Hänninen et al. also documented notable experimental results [132]. They treated a turnip (Brassica rapa) with salicylic acid or ethephon in combination with wounding it to find out whether stimulating the defense system of a plant results in increased allergen content. After these treatments, approximately 10 times the amount of IgE-reactive antigens were expressed in the turnip. One of the allergens thus induced had a sequence homologous to those of PR-4 proteins. Similarly, Sánchez-Monge et al. reported that class I endochitinases were significantly expressed by ethylene treatment [126]. On the
other hand, researchers are experimenting with less-toxic substances that induce plants to produce defense-related proteins as a novel type of agrochemical [3,4]. Such an agrochemical must be safer in the view of acute toxicity, but it might increase the allergen content of a plant. Further studies are urgently required to clarify whether various pollutants and chemicals actually augment the amount of allergenic proteins in a plant.

6.2 Possible allergen induction by breeding

Defense-related proteins ordinarily confer on a plant resistance to a wide range of pathogens and harsh growing conditions [133]. Consequently, plant species that tend to express a large quantity of defense-related proteins are expected to withstand various stresses and therefore be agriculturally valuable [2-4,133,134]. Part of the conventional plant breeding has aimed to produce stress-resistant species. However, is there any possibility that an allergen-producing propensity is unexpectedly selected during the course of such plant breeding? There are some reports describing the species-dependent variability of allergen content in apple, bell pepper, and rice [117,118]. The rubber trees cultivated on plantations have also been genetically selected for economically effective latex production [49,51]. To date, no studies have addressed the question of whether the allergen content in crops is increased by conventional plant breeding.

Stress-resistant crops are also being developed with biotechnological procedures [2-4,133,134]. Genes encoding defense-related proteins are transferred from one plant to another and constantly expressed to make the transgenic plant be increasingly resistant to various stresses. Class I endochitinases are regarded as a promising protein for producing pathogen-resistant varieties because of their anti-fungal activities [2,31,133]. If an endochitinase and an endo-β-1,3-glucanase are co-expressed, their anti-fungal activities are enhanced synergistically. Some novel crops transformed to constantly express an endochitinase with or without co-expression of an endo-β-1,3-glucanase have already been reported [2,133,135]. These genetically modified plants showed resistances to various sorts of pathogenic fungi, as expected. Other defense-related proteins, such as LTPs and 2S albumins with anti-fungal activities, are also being artificially expressed in crops [2-4,133,134].

However, these manipulations may increase the allergen content of the novel crop. The pathogen-resistant tomato variety produced by transferring a gene encoding proteahevein (Hev b 6.01) to an ordinal tomato is a surprising example [136]. Class I endochitinases with an N-terminal hevein domain were also demonstrated to be an important cross-reactive allergen for latex-allergic patients [80-88]. In addition, endo-β-1,3-glucanases of a rubber tree are officially registered as a latex allergen (Hev b 2) [63]. Furthermore, some LTPs and 2S albumins were verified to be cross-reactive plant allergens [5-7,109,113]. These facts suggest that a genetically modified plant with stress-resistant properties could be more allergenic than the original untransformed plant.

Accordingly, in order not to lose the public trusts in genetically modified organisms, novel transgenic plants must be carefully evaluated for their allergenicity before they are put on the market as foodstuffs. One should remember the vital point that many defense-related proteins become incomplete food allergens based upon their cross-reactivity to homologous inhalant or contact sensitizers. As mentioned in the previous section, incomplete food allergens are usually not stable in the presence of heat or digestive enzymes [122-125,128]. We cannot fully anticipate the allergenicity of a food protein based upon digestion tests alone. The allergenicity of a
newly expressed protein needs to be collectively evaluated based upon not only its stability in the presence of heat and digestive enzymes but also its homology to other allergens [137], structural conservatism, reactivity to the IgE antibodies of appropriate patients, and so on [17,138-140].

PERSPECTIVES

It has long been understood that there are specific allergens for each sensitization route, such as ingestion, inhalation, and contact, and that they cause allergic symptoms independently. This concept seems to derive from a presupposition that a patient is previously sensitized by an allergen and the symptoms are provoked by the second exposure to the same allergen. However, the latex-fruit syndrome and the pollen-food allergy syndrome (OAS) are now ascribed to the cross-reactivity between sensitizing allergens and incomplete food allergens. Because cross-reactivity is the sole prerequisite for an incomplete food allergen, exposure routes to the sensitizer can be different from ingestion. The emerging concept of immediate-type allergies based upon the cross-reactivity might give rise to a discussion about the definition of allergens. Which type of antigens should be called allergens, sensitizing antigens or symptom-eliciting antigens? Anyway, we must carefully evaluate whether an IgE-reactive protein is relevant only to the sensitization process, only to the symptom-provoking process, to both, or to neither.

The partial or the entire sequences of many proteinous allergens have been elucidated, whereas for a long time researchers believed that only certain special proteins become allergens. However, neither common sequences nor characteristics are extracted from the official list of proteinous allergens. This fact implies that most proteins can become an allergen if the specific conditions are favorable, for example, exposure route, amount, frequency, and cross-reactivity. Until about 10 years ago, representative food allergens were restricted to proteins stable in the presence of heat and digestion. This is probably due to the condition that the major exposure route to proteinous allergens had been restricted to ingestion. If other sensitization routes, such as inhalation of allergen-adsorbing powder or pollen and direct contact with latex products, are established by any reason, many food proteins having cross-reactivity to the sensitizers can newly become non-sensitizing elicitors (incomplete food allergens). The increasing episodes of allergies to fresh fruits and vegetables in adults are interpreted as a concomitant phenomenon with the prevalence of pollinosis.

Several defense-related proteins have been revealed to form plant pan-allergen families. These proteins are potentially induced by various stresses and plant breeding. Moreover, the exposure routes to sensitizers are expanding into inhalation and direct contact in addition to ingestion. The increasing incidence of allergic disorders has been pointed out for several decades, but the genuine reason is still obscure. The changes in the susceptibility of human beings have been well documented; however, the quantitative increase and qualitative change of allergenic proteins as well as the expansion of exposure routes to sensitizers may be other factors that have been overlooked.

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