Plant Biotechnology 22, 423–430 (2005)

Review

Trienoic fatty acids and stress responses in higher plants

Osamu Matsuda*, Koh Iba

Department of Biology, Faculty of Sciences, Kyushu University
*E-mail: omatsscb@mbox.nc.kyushu-u.ac.jp Tel & Fax: +81-92-642-2621

Received August 25, 2005; accepted August 31, 2005 (Edited by H. Fukaki)

Abstract Trienoic fatty acids (TAs) are the most abundant fatty acid species in the membrane lipids of plant cells. While serving as the major structural organizers of biomembranes on the one hand, TAs play essential roles in mediating stress signaling on the other hand. This review focuses on recent studies proposing biochemical roles for TAs in abiotic and biotic stress tolerances, and on the emerging picture of transcriptional and post-transcriptional mechanisms that act in coordination to optimize membrane TA levels under adverse environmental conditions.

Key words: ω-3 fatty acid desaturase, trienoic fatty acid.

As sessile organisms, plants cope with environmental changes by optimizing intracellular processes such as metabolism and gene expression. Biomembranes, which are the boundaries between inner and outer environments as well as between cellular compartments, provide the major sites for the perception of abiotic and biotic stresses and for the propagation of signals, leading to alleviations of deleterious stress effects. The membranes consist of glycerolipid bilayers, on which membrane-bound proteins are organized into functional lipid-protein superstructures (Marsh and Páli 2004). In addition to serving as the building blocks of membranes, lipids and their derivatives act as bioactive agents, mediating stress signaling in plant cells (Weber 2002; Wang 2004).

Consistent with such diverse functions, there is considerable diversity in the structure of glycerolipids, depending on the combination of a polar head group and the esterified fatty acids (Harwood 1980). One of the conspicuous features of the plant membrane lipids is their extremely high content of polyunsaturated fatty acids. Typically, dienoic and trienoic fatty acids (DAs and TAs, respectively) account for as much as 70% of the total fatty acids in leaf and root membrane lipids (Harwood 1980).

Rather than being a rigid "cage" of biomolecules, plant membranes have high capability to adjust their lipid and fatty acid composition in accordance with environmental conditions. For example, the content of TAs in membrane lipids changes to a certain extent in response to temperature (Iba 2002), wounding (Hamada et al. 1996; Kwon et al. 2000), and pathogen invasion (Kirsch et al. 1997; Yaeno et al. 2004). This has been observed in a wide variety of plant species, suggesting a general involvement of TAs in the adaptation to stresses.

However, molecular details regarding the mode of action of TAs in the stress responses and the stress-mediated regulation of their metabolism have just begun to be elucidated. This review focuses on TA function in environmental stress tolerances, and on transcriptional and post-transcriptional mechanisms that act in coordination to regulate the membrane TA levels.

Trienoic fatty acids and adaptation to chilling temperatures

Membrane lipids have been a focus of attention for many years as one of the factors affecting the temperature sensitivity of higher plants. Particularly, the level of saturation of the esterified fatty acids has been implicated with the functionality of biomembranes at chilling temperatures, as it is the primary determinant of the phase transition temperature of the membranes (Nishida and Murata 1996). Consistent with this point of view, saturation of the fatty acids has been found to decrease in a variety of plant species when they are exposed to low temperatures. In many cases, this is due largely to an enhanced rate of conversion of DAs (containing two *cis* double bond) to TAs (containing three *cis* double bond), a reaction catalyzed by ω-3 fatty acid desaturases (Iba 2002).

Direct tests for the relationship between the membrane

Abbreviations: DA, dienoic fatty acid; ER, endoplasmic reticulum; JA, jasmonic acid; LA, α-linolenic acid; NADPH, nicotinamide adenine dinucleotide phosphate; *Pst*, *Pseudomonas syringae* pv. *tomato*; ROS, reactive oxygen species; TA, trienoic fatty acid.
This article can be found at http://www.jspcmb.jp/

TA levels and chilling sensitivity have become feasible after *Arabidopsis* mutants with defects in this reaction were identified (Browse et al. 1986; Lemieux et al. 1990; McConn et al. 1994). ω-3 desaturases are classified into two types according to their cellular localization; one localized in the endoplasmic reticulum (ER) and the other in plastids. In *Arabidopsis*, *FAD3* encodes an ER-localized ω-3 desaturase (Arondel et al. 1992), while *FAD7* and *FAD8* encode plastidial isozymes (Iba et al. 1993; Gibson et al. 1994).

The *fad3fad7fad8* triple mutant of *Arabidopsis*, which completely lacks ω-3 desaturase activity, contains negligible TA levels in its membrane lipids (McConn and Browse 1996). When this mutant was subjected to chilling treatment for a long period (4°C, 30 days), extensive loss of thylakoid stacking was observed in the chloroplast membranes and the quantum yield of photosynthetic electron transfer declined substantially compared with the wild type (Routaboul et al. 2000). This increased susceptibility to chilling might be related to less efficient recovery of photosystem II from chilling-induced photoinhibition (Vijayan and Browse 2002). In contrast, Kodama et al. (1994) increased the level of TAs in leaves of tobacco by heterologously expressing the *Arabidopsis FAD7* under the control of the constitutive cauliflower mosaic virus 35S promoter. The growth inhibition and chlorotic damages that were evident in the young leaves of the wild type after chilling treatment (1°C, 7 days) were significantly alleviated in the transgenic plants. Similar results were obtained with transgenic rice plants overexpressing the tobacco *FAD3* ortholog (*NtFAD3*), which accumulated high levels of TAs in the extraplastid lipids of leaf and root tissues (Shimada et al. 2000). These results provide direct proof for the requirement of TAs in the adaptation to chilling temperatures.

It appears, however, that such TA-mediated alleviation of chilling injuries is limited to certain plant species, tissues, and/or developmental stages and that it depends largely on which membrane systems are concerned. Unlike the cases described above, when *NtFAD3* was overexpressed in tobacco, no significant improvement over the wild type was detected in the transgenic plants as regards their chilling sensitivity (Hamada et al. 1998). Thus, at least in some plant species, including tobacco, plastidial ω-3 desaturases, rather than ER-type isozymes, would be promising targets for genetic engineering if it was intended to establish a membrane fatty acid composition optimally adapted to growth at chilling temperatures.

Trienoic fatty acids and adaptation to high temperatures

Despite some inconsistent results, enhanced accumulation of TAs in the membrane lipids is likely favorable for growth at chilling temperatures. On the other hand, the decreasing of the TA level commonly observed at elevated temperatures prompts us to suspect that an excess amount of TAs would be rather detrimental for growth at high temperatures.

In the study described above (Kodama et al. 1994), several transgenic lines of tobacco carrying the 35S-*FAD7* fusion gene were found to contain reduced TA levels in leaf tissues as compared with the wild type. This was due to silencing of the exogenous 35S-*FAD7* transgene followed by cosupression of the intrinsic plastidial ω-3 desaturase gene. While these transgenic plants showed no phenotypic differences compared with the wild type in the low temperature range up to the normal cultivation temperature (15 to 25°C), unambiguous differences were detected (Murakami et al. 2000). Despite a certain growth inhibition, the transgenic plants withstood both short-term (47°C, 3 days) and long-term (36°C, 60 days) exposure to high temperatures, which in the wild type caused severe chlorotic damages resulting in death at the end of the treatments. After returning to a normal cultivation temperature (25°C), the transgenic plants resumed growing and set seeds. Consistent with such visible differences, the photosynthetic activity of the transgenic plants, as evaluated by O_2 evolution, was significantly higher than that of the wild type at high temperature (40 to 45°C). Similar results were obtained with the *Arabidopsis fad7fad8* double mutant, which lacks the activity of plastidial ω-3 desaturase (Murakami et al. 2000). In this mutant, higher growth rates (Routaboul and Browse 2002; Falcone et al. 2004) and stability of the photosynthetic machinery (Kim and Portis 2005) at high temperatures have been confirmed by several labs. These results support the concept that TAs in membrane lipids need to be diminished to allow plant survival at unusually high temperatures.

Contrary to the results described above, the *fad3* mutant of *Arabidopsis*, which contains a reduced level of TAs in the extraplastid lipids, showed no phenotypic differences from the wild type, even in the temperature range in which the enhanced high temperature tolerance of the *fad7fad8* double mutant had been detected (Murakami et al. 2000). Because an excess amount of TAs had detrimental effects on photosynthesis at high temperatures, it appears rather consistent that a reduction of TAs in the chloroplast lipids, but not in the extrachloroplast lipids, made a positive contribution to the tolerance for high temperatures. On the other hand, the nearly complete depletion of TAs in the *fad3fad7fad8* triple mutant resulted in a severe inhibition of growth and photosynthesis at high temperatures (Routaboul and Browse 2002). This apparently discrepant phenotype of the mutant could not be ascribed to changes in

physicochemical membrane properties, but rather to the impairment of signaling processes mediated by TAs or their derivatives such as jasmonic acid (JA). Due to the incapability of producing JA from α-linolenic acid (LA), the most abundant species of TAs, this mutant shows pleiotropic phenotypes, ranging from male sterility (McConn and Browse 1996) to enhanced susceptibility to insect hervivory (McConn et al. 1997) and fungal infection (Staswick et al. 1998), in addition to the altered sensitivity to low and high temperatures. This exemplifies the fundamental difficulty of discriminating the mechanisms underlying effects of TAs on the sensitivity to extreme temperatures.

Nevertheless, an intriguing explanation has been presented recently for the negative effect of TAs on chloroplast function at high temperatures. Seki et al. (2005) examined the protein modification caused by peroxidized polyunsaturated fatty acids, which often results in irreversible loss of protein function, using isolated thylakoid membranes combined with a metal-catalyzed oxidation system. They found that peroxidized LA was the most effective modifier of thylakoid membrane proteins and that formation of such protein adducts was greatly promoted by an elevated reaction temperature. Identification of the predominant target proteins will help to clarify the mechanisms of heat-induced damages and the adaptive responses of plants to high environmental temperatures.

Regulation of trienoic fatty acid metabolism in response to temperature

Contrary to what was predicted from the regulation of fatty acid desaturases in cyanobacteria (Los and Murata 1998), expression of most ω-3 desaturase genes isolated so far from plants has been shown to be unresponsive to temperature at the transcriptional level. Based on this observation, it has been suggested that, in higher plants, temperature-dependent changes of membrane TA levels may be mediated through the post-transcriptional regulation of ω-3 desaturase genes (Somerville 1995).

Due to its nature as a strongly hydrophobic integral membrane protein, ω-3 desaturase has long defied analysis at the post-transcriptional level. For example, recovery of ω-3 desaturase from crude membrane fractions has never been successful through the conventional biochemical methods, which had enabled characterization of the plastidial Δ9 stearoyl-acyl carrier protein desaturase localized in the soluble stromal fraction (Stumpf 1980). In addition, preparing monospecific antibodies against the ω-3 desaturase protein has been difficult, as they turned out to be cytotoxic and folded incorrectly to be immediately degraded in bacterial expression systems (Matsuda et al. unpublished data).

The first direct evidence for temperature-sensitive post-transcriptional regulation of the ω-3 desaturase was produced in wheat root tips (Horiguchi et al. 2000). Although the physiological significance of TAs in non-photosynthetic tissues is still unclear, temperature-dependent changes of the membrane TA level is commonly observed in both leaf and root tissues. As differentiated chloroplasts with stacked thylakoid membranes are absent, most fatty acids in root tissues are derived from extraplastid lipids (Lemieux et al. 1990). Thus, temperature-dependent changes of root TA levels are mostly attributable to the activity of ER-type ω-3 desaturase. The authors raised antibodies against potential extramembranous short peptide stretches of the wheat ER-type ω-3 desaturase (TaFAD3) and analyzed *TaFAD3* gene expression at both the transcriptional and post-transcriptional levels. While the transcript level remained almost unchanged irrespective of the temperature, the level of TaFAD3 proteins increased gradually at lowered temperatures. As indicated by polysomal fractionation analysis, which allows estimation of the number of ribosomes associated with specific gene transcripts, the increase of TaFAD3 proteins at low temperatures appeared due to enhanced translational efficiency of *TaFAD3* transcripts.

The temperature-sensitive post-transcriptional regulation of a plant ER-type ω-3 desaturase was reconstituted in a heterologous expression system using yeast as the host cell (Dyer et al. 2001). When the rapeseed FAD3 ortholog which carried an epitope tag for immunodetection at the amino terminus, was expressed in yeast cells under the control of the yeast-specific *GAL1-10* promoter, the FAD3 protein accumulated in a chilling-inducible manner; this was not accompanied by a concurrent change of the transcript level. Because yeast has a relatively simple fatty acid composition, lacking both DAs and TAs, and since the heterologously expressed ER-type ω-3 desaturase efficiently converted DAs supplied with the growth media into TAs, this system offers a convenient means to dissect enzymatic properties and post-transcriptional regulation of plant ER-type ω-3 desaturases.

In contrast to the metabolism of TAs in the non-photosynthetic root tissues, the plastidial FAD7 and FAD8 desaturases are major contributors for TA production in leaf tissues (McConn et al. 1994). FAD8 was originally identified as a temperature-sensitive ω-3 desaturase, based on phenotypic analyses of the *fad7* mutant (Browse et al. 1986), in which FAD8 is the only functional ω-3 desaturase in plastids. Because reduction of the leaf TA level caused by the *fad7* mutation is less pronounced at lower temperatures, it has been predicted that FAD8 activity is inducible by low temperatures. On the other hand, the temperature-dependent regulation on the transcriptional level makes *FAD8* unique among a

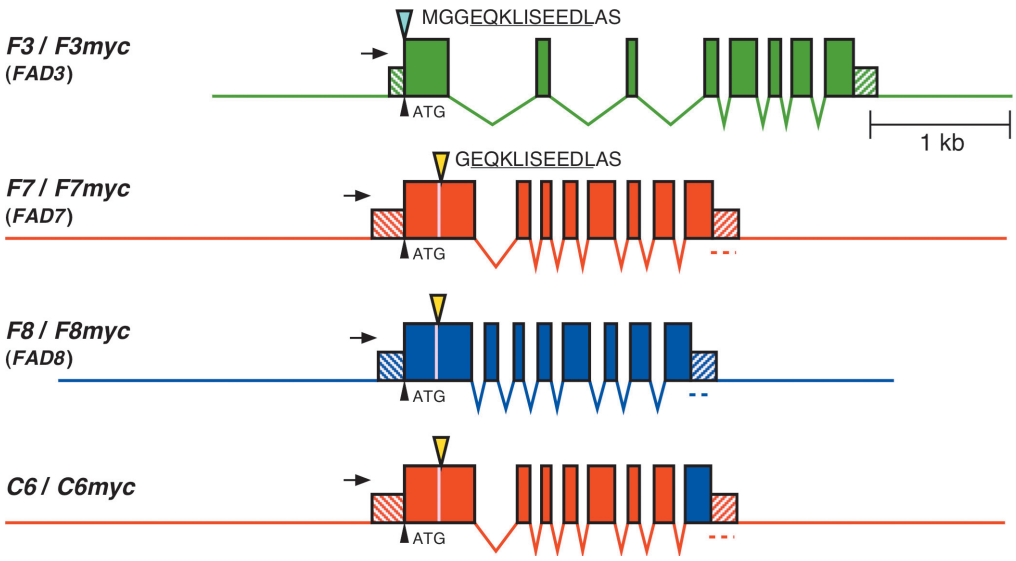

Figure 1. Structures of transgenes encoding native and epitope-tagged ω-3 desaturase isozymes and artificial FAD7–FAD8 chimeric enzymes used to complement the *Arabidopsis* mutants deficient in ω-3 desaturase activity. Lines and boxes in green, red and blue represent regions derived from endogenous genomic clones of *FAD3*, *FAD7* and *FAD8*, respectively. Solid boxes represent exons containing coding regions. Hatched boxes represent 5′- and 3′-untranslated sequences. V-shaped lines represent introns. Solid horizontal lines represent untranscribed flanking sequences (usually containing intrinsic promoters and terminators). Arrows indicate the direction of transcription. Black arrowheads with "ATG" indicate translational start sites. Pale-red vertical lines indicate nucleotide positions encoding the carboxyl-terminal residues of the FAD7 transit peptide (Glu at position 81) predicted by the ChloroP program (Emanuelsson et al. 1999), or the corresponding residue of the FAD8 protein (Glu at position 75). Reverse triangles indicate insertion sites of *c-Myc* sequences within *F3*, *F7*, *F8* and *C6* variants encoding epitope-tagged desaturases. The amino acid sequences encoded by the inserts are shown on the right with the 9E10 epitope of c-Myc protein (Evan et al. 1985) underlined. Dashed horizontal lines indicate regions used as hybridization probes for Northern blot analysis (see Figure 2). The figure is drawn to scale.

number of ω-3 desaturase genes isolated from several plant species (Gibson et al. 1994). However, even if the temperature-dependent changes of leaf TA levels in *Arabidopsis* are entirely attributable to the transcriptional regulation of *FAD8* gene expression, this mechanism cannot explain similar events occurring in other plant species in which no *FAD8* isogenes have been identified.

Because the activity of plastidial ω-3 desaturases requires electron transfer chains specific to plastid membranes (Jäger-Vottero et al. 1997; Shanklin and Cahoon 1998), the yeast expression system is not applicable to the analysis of plastidial isozymes. Neither are photosynthetic unicellular organisms, such as cyanobacteria, the hypothetical free-living ancestors of plastids, ideal heterologous hosts, as they contain high levels of polyunsaturated fatty acids and several endogenous fatty acid desaturases acting on DAs and TAs (Los and Murata 1998). Moreover, attempts to detect temperature-responsiveness of *FAD8* promoter activity, using promoter-reporter gene fusion constructs have been unsuccessful (O. Matsuda et al., unpublished data), while similar approaches produced unequivocal results regarding the developmental and hormonal regulation (Matsuda et al. 2001) and the light and wound-responsive expression (Nishiuchi et al. 1995; Nishiuchi et al. 1997) of the *FAD3* and *FAD7* genes.

To identify regulatory regions that control FAD8 desaturase activity in response to temperature, we constructed a series of *FAD7-FAD8* chimeric genes, based on the structural relatedness of the two isogenes, and introduced them into the *Arabidopsis fad7fad8* double mutant (Matsuda et al. 2005). Each chimeric gene encoded a functional plastidial ω-3 desaturase, driven by either the *FAD7* or *FAD8* promoter, and with or without an epitope tag behind the putative cleavage site of the amino-terminal plastidial transit peptide (Figure 1). In the parental *fad7fad8* double mutant, no transcripts of the mutated *FAD7* and *FAD8* are detectable (Figure 2), and the level of TAs in leaf tissues is severely reduced as compared with the wild type (Figure 3). Expression of any of the chimeric genes resulted in phenotypic complementation of the double mutant, as indicated by an accumulation of their transcripts and proteins (Figure 2) followed by an increase of the leaf TA level (Figure 3), either in a temperature-sensitive or insensitive manner. Introduction of a wild-type genomic clone of *FAD8* (*F8*) phenocopied the *fad7* mutant, showing a low temperature-specific increase of the leaf TA level, whereas introduction of a genomic *FAD7* clone (*F7*) uniformly increased the leaf TA level throughout the range of temperatures tested (15 to 27°C). One of the chimeric genes, designated *C6*, was composed mostly of *F7*, except that the coding region for the carboxyl terminus was replaced to the corresponding region of *F8* (Figure 1). Expression of this chimeric gene resulted in a low temperature-specific increase of the leaf TA level,

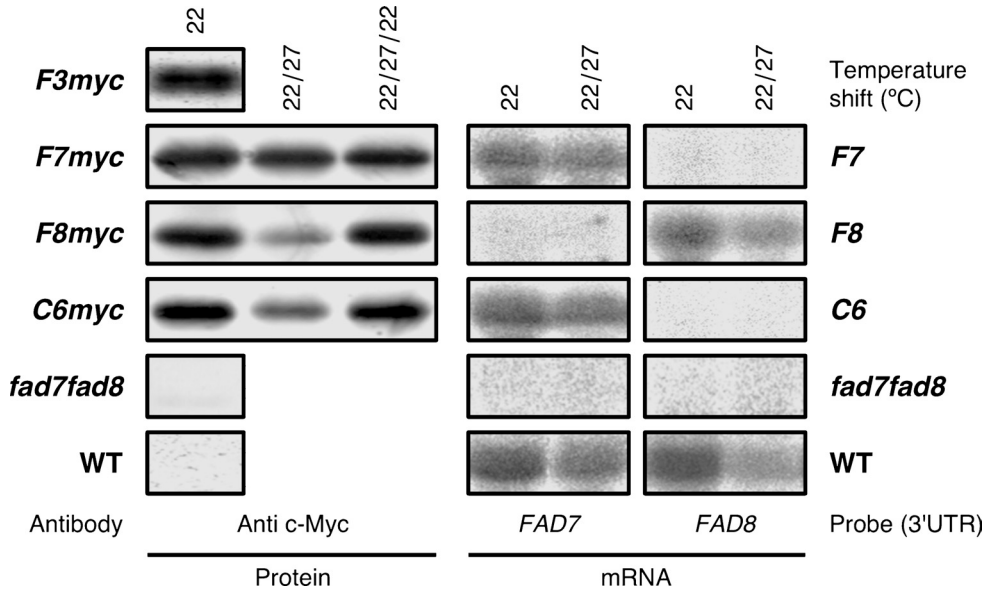

Figure 2. Detection of transcriptional and translational products of ω-3 desaturase genes in transgenic *Arabidopsis* plants. *F3myc* was introduced into the *fad3* mutant and its translational products accumulated in the shoot apical meristem were detected by Western blot analysis using anti-c-Myc antibody. The rest of the transgenes were introduced into the *fad7fad8* double mutant, and temperature-dependent accumulation of their transcripts and proteins in total aerial tissues was evaluated by Northern blot analysis using gene-specific hybridization probes (see Figure 1) and Western blot analysis using anti-c-Myc antibody, respectively. Prior to the analyses, the indicated transgenic, mutant and wild-type (WT) plants, initially grown at 22°C for 14 days, were subjected to different temperature-shift protocols as follows: 22 (control), kept at 22°C for 2 days; 22/27, kept at 22°C for 1 day and shifted to 27°C for 1 day; 22/27/22, shifted to 27°C for 1 day and reincubated at 22°C for 1 day. UTR: untranslated region.

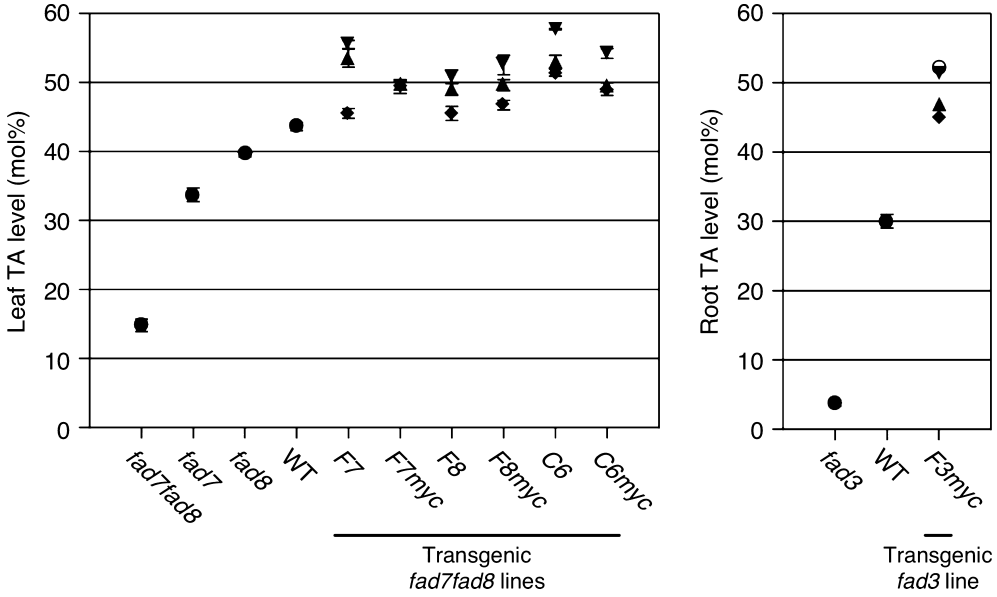

Figure 3. Complementation of reduced TA levels in *Arabidopsis* mutants deficient in ω-3 desaturase activity by functional expression of epitope-tagged desaturases. Plants of the indicated mutants and transgenic lines carrying each construct in Figure 1 (2–4 lines for each construct) were grown at 22°C for 16 days, and young rosette leaves or total root tissues were subjected to fatty acid analysis (Kodama et al. 1994). Values represent mean±SD of at least six independent measurements for each line. Deviations of less than 1% are not shown for clarity. WT: wild type.

and the amount of epitope-tagged C6 proteins increased correspondingly at lower temperatures (Figure 2). In contrast, the transcript level of *C6*, similar to that of *F7*, remained almost unchanged irrespective of the temperature (Figure 2). Analysis of the decay of epitope-tagged products after inhibiting protein synthesis by application of cycloheximide revealed that the FAD8-derived carboxyl terminus acts in an autoregulatory fashion to destabilize the protein at high temperature. These results suggest that, while the temperature-sensitive expression of the FAD8 desaturase is regulated at both the transcriptional and post-translational levels,

the response of its enzymatic activity to temperature depends mainly on the regulation at the post-translational level.

With a set of transgenic plants expressing different epitope-tagged variant of the three ω-3 desaturase isozymes now available (Figures 1–3), it will be feasible to investigate further their differential regulation on the transcriptional to post-translational levels. In particular, comparative analyses of FAD7 and FAD8 desaturases with regard to their enzymatic properties, spatiotemporal expression and suborganellar localization, will provide new insights into the adaptive mechanism of plants to adverse temperatures.

Trienoic fatty acids and defense responses to biotic stresses

As described above, TAs not only function as the determinants of membrane properties, but also play a role as the precursors of JA and related oxylipins engaged in a wide range of signaling processes (Weber 2002). While these signaling compounds are reminiscent of prostaglandins in mammalian systems, the bioactivity and function of each of their biosynthetic intermediates are largely unexplored. Recently, it has been suggested that chloroplast membrane-localized TAs themselves, rather than their major oxygenated derivatives, play an essential role in activating defense responses against microbial pathogen invasion (Yaeno et al. 2004).

Challenge with avirulent pathogens induces transient production of reactive oxygen species (ROS) in plant cells, which is known as oxidative burst, acting as the trigger for subsequent acquisition of disease resistance (Wojtaszek 1997). Ozone induces a series of cellular responses reminiscent of those activated during plant-pathogen interaction (Rao et al. 2000). Consistently, when the leaves of wild-type *Arabidopsis* (ecotype Columbia) were exposed to ozone (250 p.p.b., 6 h) or inoculated with an avirulent pathogen, *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 (*avrRpm1*) (10^6 cfu ml^{-1}, 12 h), an enhanced accumulation of superoxide (O_2^-) and its dismutated product, hydrogen peroxide (H_2O_2), was detected, which was followed by hypersensitive cell death in the treated tissues. While similar results were obtained with the *fad3* mutant, accumulation of ROS as well as subsequent cell death were attenuated in the *fad7fad8* double mutant. Because ozone-induced O_2^- formation was prevented when wild-type leaves were pretreated with diphenylene iodonium, an inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Levine et al. 1994), it was hypothesized that TAs in the chloroplast lipids might have a competence to activate this ROS-forming enzyme. Actually, the activity of NADPH oxidase, which is assumed to be localized in the plasma membrane (Keller et al. 1998) was induced by TAs, especially by LA, *in vitro*, but not significantly by other fatty acid species and their derivatives, such as DAs, JA and arachidonic acid. However, there remained the question of how TAs in chloroplast lipids can act as signals activating an enzyme localized in a different cellular compartment. This riddle was resolved by an analysis of the fatty acid composition in individual lipid classes, which revealed that, upon exposure to ozone or inoculation with *Pst* DC3000 (*avrRpm1*), TAs specific to chloroplast galactolipids are rapidly liberated from the chloroplast membranes and, at least in part, incorporated into the extrachloroplast phospholipids (Yaeno et al. 2004). In agreement with the phenotype of hypersensitive cell death, the *fad7fad8* double mutant showed reduced resistance to the avirulent *Pst* strains, DC3000 (*avrRpm1*) and DC3000 (*avrRpt2*), suggesting that TAs in the chloroplast lipids are involved in a signaling cascade downstream of the *Resistance*/*Avirulence*-gene recognition event (Thordal-Christensen 2003).

Based on these results, it appears that TAs in the chloroplast lipids play essential roles in a range of adaptive responses, affecting both abiotic and biotic stress resistance, and that membrane rearrangements induced by these stresses are more dynamic than previously thought. To make the best use of these responses in improving the adaptability of plants to environmental stresses, a better understanding of the crosstalk between signaling processes triggered by different sets of environmental cues will be required.

Concluding remarks

The extremely high TA contents of plant membrane lipids attract interest in plant-specific physiological processes and in the mechanisms by which their metabolism is regulated to cope with a changing environment. Generally, investigations into physiological processes that take place in membranes, in which strongly hydrophobic membrane proteins and functional protein-protein and/or lipid-protein complexes interact, are inherently difficult. In this respect, the availability of a set of *Arabidopsis* mutants with defects in TA production and the genes encoding ω-3 desaturases has provided a means to overcome technical difficulties. Recent studies have demonstrated the involvement of TAs in the adaptation to abiotic and biotic stresses. It is now becoming clear that the metabolism of TAs, which depends largely on the activity of ω-3 desaturases, is regulated at several steps of gene expression from the transcriptional to the post-translational levels. The transgenic lines of *Arabidopsis* described here were established to elucidate tissue-specific transcriptional and post-transcriptional expression of the three ω-3 desaturase genes. Further dissection of the enzymatic

properties and regulatory mechanisms of the ω-3 desaturase isozymes will greatly broaden our knowledge of fundamental adaptive strategies of plants in response to diverse environmental stresses.

Acknowledgements

This work was supported by Research for the Future from the Japan Society for the Promotion of Science.

References

Arondel V, Lemieux B, Hwang I, Gibson S, Goodman HM, Somerville CR (1992) Map-based cloning of a gene controlling omega-3 fatty acid desaturation in *Arabidopsis*. *Science* 258: 1353–1355

Browse J, McCourt P, Somerville C (1986) A mutant of *Arabidopsis* deficient in $C_{18:3}$ and $C_{16:3}$ leaf lipids. *Plant Physiol* 81: 859–864

Dyer JM, Chapital DC, Cary JW, Pepperman AB (2001) Chilling-sensitive, post-transcriptional regulation of a plant fatty acid desaturase expressed in yeast. *Biochem Biophys Res Commun* 282: 1019–1025

Emanuelsson O, Nielsen H, von Heijne G. (1999) ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. *Protein Sci* 8: 978–984

Evan GI, Lewis GK, Ramsay G, Bishop JM (1985) Isolation of monoclonal antibodies specific for human c-myc proto-oncogene product. *Mol Cell Biol* 5: 3610–3616

Falcone DL, Ogas JP, Somerville CR (2004) Regulation of membrane fatty acid composition by temperature in mutants of *Arabidopsis* with alterations in membrane lipid composition. *BMC Plant Biol* 4: 17

Gibson S, Arondel V, Iba K, Somerville C (1994) Cloning of a temperature-regulated gene encoding a chloroplast ω-3 desaturase from *Arabidopsis thaliana*. *Plant Physiol* 106: 1615–1621

Hamada T, Nishiuchi T, Kodama H, Nishimura M, Iba K (1996) cDNA cloning of a wounding-inducible gene encoding a plastid ω-3 fatty acid desaturase from tobacco. *Plant Cell Physiol* 37: 606–611

Hamada T, Kodama H, Takeshita K, Utsumi H, Iba K (1998) Characterization of transgenic tobacco with an increased α-linolenic acid level. *Plant Physiol* 118: 591–598

Harwood JL (1980) Plant acyl lipids: structure, distribution, and analysis. In: Stumpf PK, Conn EE (eds) *The Biochemistry of Plants: A Comprehensive Treatise*. Vol 4, Academic Press, New York, pp 1–55

Horiguchi G, Fuse T, Kawakami N, Kodama H, Iba K (2000) Temperature-dependent translational regulation of the ER ω-3 fatty acid desaturase gene in wheat root tips. *Plant J* 24: 805–813

Iba K, Gibson S, Nishiuchi T, Fuse T, Nishimura M, Arondel V, Hugly S, Somerville C (1993) A gene encoding a chloroplast ω-3 fatty acid desaturase complements alterations in fatty acid desaturation and chloroplast copy number of the *fad7* mutant of *Arabidopsis thaliana*. *J Biol Chem* 268: 24099–24105

Iba K (2002) Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. *Annu Rev Plant Biol* 53: 225–245

Jäger-Vottero P, Dorne A-J, Jordanov J, Douce R, Joyard J (1997) Redox chains in chloroplast envelope membranes: spectroscopic evidence for the presence of electron carriers, including iron-sulfur centers. *Proc Natl Acad Sci USA* 94: 1597–1602

Keller T, Damude HG, Werner D, Doerner P, Dixon RA, Lamb C (1998) A plant homolog of the neutrophil NADPH oxidase $gp91^{phox}$ subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs. *Plant Cell* 10: 255–266

Kim K, Portis AR Jr (2005) Temperature dependence of photosynthesis in *Arabidopsis* plants with modifications in Rubisco activase and membrane fluidity. *Plant Cell Physiol* 46: 522–530

Kirsch C, Takamiya-Wik M, Reinold S, Hahlbrock K, Somssich IE (1997) Rapid, transient, and highly localized induction of plastidial ω-3 fatty acid desaturase mRNA at fungal infection sites in *Petroselinum crispum*. *Proc Natl Acad Sci USA* 94: 2079–2084

Kodama H, Hamada T, Horiguchi G, Nishimura M, Iba K (1994) Genetic enhancement of cold tolerance by expression of a gene for chloroplast ω-3 fatty acid desaturase in transgenic tobacco. *Plant Physiol* 105: 601–605

Kwon JH, Lee YM, An CS (2000) cDNA cloning of chloroplast ω-3 fatty acid desaturase from *Capsicum annuum* and its expression upon wounding. *Mol Cells* 10: 493–497

Lemieux B, Miquel M, Somerville C, Browse J (1990) Mutants of *Arabidopsis* with alterations in seed lipid fatty acid composition. *Theor Appl Genet* 80: 234–240

Levine A, Tenhaken R, Dixon R, Lamb C (1994) H_2O_2 from the oxidative burst orchestrates the plant hypersensitive disease resistance response. *Cell* 79: 583–593

Los DA, Murata N (1998) Structure and expression of fatty acid desaturases. *Biochim Biophys Acta* 1394: 3–15

Marsh D, Páli T (2004) The protein-lipid interface: perspectives from magnetic resonance and crystal structures. *Biochim Biophys Acta* 1666: 118–141

Matsuda O, Watanabe C, Iba K (2001) Hormonal regulation of tissue-specific ectopic expression of an *Arabidopsis* endoplasmic reticulum-type ω-3 fatty acid desaturase (*FAD3*) gene. *Planta* 213: 833–840

Matsuda O, Sakamoto H, Hashimoto T, Iba K (2005) A temperature-sensitive mechanism that regulates post-translational stability of a plastidial ω-3 fatty acid desaturase (FAD8) in *Arabidopsis* leaf tissues. *J Biol Chem* 280: 3597–3604

McConn M, Hugly S, Browse J, Somerville C (1994) A mutation at the *fad8* locus of *Arabidopsis* identifies a second chloroplast ω-3 desaturase. *Plant Physiol* 106: 1609–1614

McConn M, Browse J (1996) The critical requirement for linolenic acid is pollen development, not photosynthesis, in an Arabidopsis mutant. *Plant Cell* 8: 403–416

McConn M, Creelman RA, Bell E, Mullet JE, Browse J (1997) Jasmonate is essential for insect defense in *Arabidopsis*. *Proc Natl Acad Sci USA* 94: 5473–5477

Murakami Y, Tsuyama M, Kobayashi Y, Kodama H, Iba K (2000) Trienoic fatty acids and plant tolerance of high temperature. *Science* 287: 476–479

Nishida I, Murata N (1996) Chilling sensitivity in plants and cyanobacteria: the crucial contribution of membrane lipids. *Annu Rev Plant Physiol Plant Mol Biol* 47: 541–568

Nishiuchi T, Nakamura T, Abe T, Kodama H, Nishimura M, Iba K (1995) Tissue-specific and light-responsive regulation of the promoter region of the *Arabidopsis thaliana* chloroplast ω-3

fatty acid desaturase gene (*FAD7*). *Plant Mol Biol* 29: 599–609

Nishiuchi T, Hamada T, Kodama H, Iba K (1997) Wounding changes the spatial expression pattern of the *Arabidopsis* plastid ω-3 fatty acid desaturase gene (*FAD7*) through different signal transduction pathways. *Plant Cell* 9: 1701–1712

Rao MV, Koch JR, Davis KR (2000) Ozone: a tool for probing programmed cell death in plants. *Plant Mol Biol* 44: 345–358

Routaboul JM, Fischer SF, Browse J (2000) Trienoic fatty acids are required to maintain chloroplast function at low temperatures. *Plant Physiol* 124: 1697–1705

Routaboul JM, Browse J (2002) Trienoic fatty acids and plant tolerance of temperature. *OCL-Ol Corps Gras Li* 9: 43–47

Seki K, Yamauchi Y, Tanaka K, Wakiuchi N, Sugimoto Y (2005) Peroxidation of linolenic acid triggered by heat and reactive oxygen species causes protein modification in chloroplasts. *Plant Cell Physiol* 46: s78

Shanklin J, Cahoon EB (1998) Desaturation and related modifications of fatty acids. *Annu Rev Plant Physiol Plant Mol Biol* 49: 611–641

Shimada T, Wakita Y, Otani M, Iba K (2000) Modification of fatty acid composition in rice plants by transformation with a tobacco microsomal ω-3 fatty acid desaturase gene (*NtFAD3*). *Plant Biotech* 17: 43–48

Somerville C (1995) Direct tests of the role of membrane lipid composition in low-temperature-induced photoinhibition and chilling sensitivity in plants and cyanobacteria. *Proc Natl Acad Sci USA* 92: 6215–6218

Staswick PE, Yuen GY, Lehman CC (1998) Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. *Plant J* 15: 747–754

Stumpf PK (1980) Biosynthesis of saturated and unsaturated fatty acids. In: Stumpf PK, Conn EE (eds) *The Biochemistry of Plants: A Comprehensive Treatise.* Vol 4, Academic Press, New York, pp 177–204

Thordal-Christensen H (2003) Fresh insights into processes of nonhost resistance. *Curr Opin Plant Biol* 6: 351–357

Vijayan P, Browse J (2002) Photoinhibition in mutants of *Arabidopsis* deficient in thylakoid unsaturation. *Plant Physiol* 129: 876–885

Wang X (2004) Lipid signaling. *Curr Opin Plant Biol* 7: 329–336

Weber H (2002) Fatty acid-derived signals in plants. *Trends Plant Sci* 7: 217–224

Wojtaszek P (1997) Oxidative burst: an early plant response to pathogen infection. *Biochem J* 322: 681–692

Yaeno T, Matsuda O, Iba K (2004) Role of chloroplast trienoic fatty acids in plant disease defense responses. *Plant J* 40: 931–941