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Reactive electrophile species

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The interest in reactive electrophile species (RES) stems largely from the fact that they can have powerful biological activities. RES stimulate the expression of cell survival genes as well many other genes commonly upregulated in environmental stress and pathogenesis. RES levels must be carefully controlled in healthy cells but their formation and destruction during stress is of great interest. Unlike many 'classical' signals and hormones, RES can potentially affect gene expression at all levels by chemically reacting with nucleic acids, proteins and small molecules as well as by indirectly lowering pools of cellular reductants. Recent works involving genetic approaches have begun to provide compelling evidence that, although excess RES production can lead to cell damage, lower levels of RES may modulate the expression of cell survival genes and may actually contribute to survival during severe stress.

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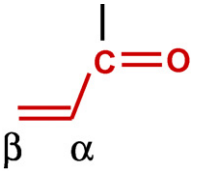
Introduction

During normal growth, uncatalyzed chemical reactions, many of which involve reactive oxygen species (ROS), create new molecular species in the cell. This process can be accelerated massively during severe stresses that are defined herein as the point at which the cell can no longer control non-enzymatic reactions. Clues as to what can happen *in vivo* have come from work *in vitro* where, for example, the non-enzymatic oxygenation of a single fatty acid species such as α-linolenic acid (ALA) can give rise to scores of new molecules. These compounds, a number of which have been observed in the cell, have diverse physicochemical properties, and some are chemically reactive. The chemical complexity of fatty acid oxygenation products is simplified by the fact that many contain simple, conserved arrangements of atoms often involving carbonyl functions adjacent to double bonds. These are α,β-unsaturated carbonyl groups:



This atom group, indicated in red in the scheme, is electrophilic (electron-accepting) and can react with electron-donor (nucleophilic) atoms common to many biological molecules. The reactivity of the group, found embedded in many stress-related molecules, depends strongly on the identity of associated atoms. In general, if one of the substituents on the β carbon is a hydrogen atom, this carbon can react with nucleophilic atoms provided by, for example, sulphydryl (–SH) groups. Compounds containing α,β-unsaturated carbonyl groups or other reactive electrophilic atom groups are termed reactive electrophile species (RES) and this group, not limited to non-enzymatic fatty acid oxygenation products, also includes some secondary metabolites, some products of haem metabolism, among others. This review focuses principally on fatty acid-derived RES, sometimes referred to as 'oxylipin RES' [1••] in plants. We draw parallels between ROS and RES signaling. We discuss the possibility that some RES may exist in latent forms in the cell and that their biological activities may be unleashed in adversity.

RES are constantly generated *in vivo*

Although many RES are derived non-enzymatically, others have a very different origin as the products of enzyme catalysis. This highlights an obvious parallel with ROS that, depending on the species, can be produced by both non-enzymatic and enzymatic routes. Well-known enzyme-derived RES include the jasmonic acid (JA) precursor oxophytodienoic acid (OPDA) [2•] as well as the volatile compound 2-(*E*)-hexenal [3]. Both of these molecules appear to be almost ubiquitous in higher plants where their increased production is clearly associated with wounding, pathogenesis and environmental stress. Among the many RES produced by non-enzymatic lipid oxidation reactions is malondialdehyde (MDA), the major small aldehyde produced in lipid oxidation in many systems [4] including plants [5], a series of related alkenals including 4-hydroxy-2-nonenal (HNE) [6,7] and cyclopentenone phytoprostanes [8]. In the case of MDA in mature Arabidopsis leaves approximately 75% of the compound originates from trienoic fatty acids and 25% must be derived from other cellular sources [5]. MDA production takes place constantly even in healthy

leaves where its levels are tightly controlled [5]. However, in conditions leading to ROS overproduction, for example photooxidation, protection systems involving the xanthophyll cycle are overwhelmed, and this can lead to lipid peroxidation and MDA generation [9]. Interestingly, lipid oxidation is also reported to increase in several mutants with defective thermotolerance [10].

Massive non-enzymatic fatty acid oxygenation occurs in the Arabidopsis *vitamin E 2* (*vte2*) mutant, a plant lacking tocopherols and their immediate precursors [11••]. Shortly after the germination of *vte2*, there is excessive generation of MDA and phytoprostanes. This correlates with both damage to cotyledons of germinating seedlings and with strong effects on the transcriptome. In some other cases, electrophile generation may have been overlooked as a possible mechanism contributing to mutant phenotypes. The *mosaic death 1* (*mod1*) Arabidopsis mutant [12] has a spontaneous cell death phenotype because of a hypomorphic (weak) mutation in the fatty acid synthesis gene: enoyl ACP reductase (ENR). ENR metabolizes α,β-*trans* enoyl ACPs that are α,β-unsaturated carbonyl compounds. From this perspective we now speculate that enoyl-ACP mismanagement may contribute to the mutant phenotype because the compounds are electrophiles. The recent development of a method for the extraction and mass spectral analysis of RES-glutathione (GSH) adducts [1••] provides an *in vivo* fingerprint of RES production. Using this method, the production of enzyme-generated RES was found to be particularly pronounced in the hypersensitive response (HR) in tobacco leaves. Some compounds generated by non-enzymatic oxidation in lipid bilayers, however, may escape detection with this method because they may not interact with GSH. This raises a crucial point. At present, the relative importance of enzymatic RES generation in most systems (with the exception of *vte2-1* where lipid oxidation is non-enzymatic) is unknown.

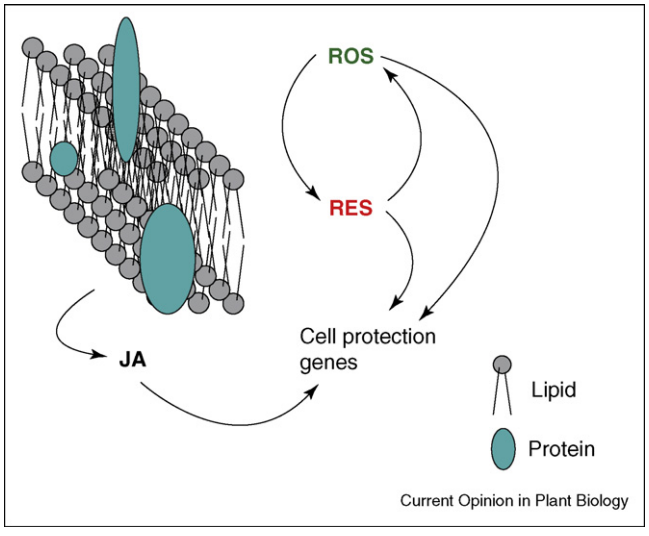
RES induce the expression of cell survival genes

Transcription is strongly affected in leaves treated with low levels of exogenous RES. Transcript levels for cell survival genes [13,14••] and pathogenesis-associated/biotic stress genes [15] as well as water stress-related and heat shock related proteins and cell wall functions [5] are affected. It is important to note that transcriptome responses induced by treatment with one particular electrophile do not necessarily indicate that this RES is the *bona fide* regulator of the genes in question and indicates only that the genes are sensitive to RES. We are now starting to understand how endogenous RES can affect transcription through the analysis of RES production and gene expression in *vte2-1* [11••]. This work implicates RES derived from non-enzymatic lipid oxidation as inducers of genes that are typically associated with pathogenesis. In this system, JA did not appear to impact the transcriptome. There is, however, circumstantial evidence suggesting that, in other systems, jasmonate perception may suppress some RES-triggered responses. Exogenous MDA stimulated the accumulation of a transcript more efficiently in plants lacking the ability to perceive JA than in wild type plants [5]. It is also known that different electrophiles activate overlapping but not identical sets of genes [5]. It is possible that a 'stress code' might exist whereby various electrophiles would be differentially generated or mobilized in response to certain types of stress thereby allowing the plant to fine-tune its survival program [5]. A summary of the relationship of RES, ROS and cell survival gene expression is shown in Figure 1.

RES, pathogenesis, defense, and cell survival

RES are implicated as signals in biotic and abiotic stresses and certainly generated and turned over in both. An early suggestion was that the liberation of RES from dying cells might activate cell protection gene expression in healthy cells and increase their survival [13]. This remains to be tested. Although there is no direct evidence that oxylipin RES are necessary to kill cells during the HR, the possibility remains that other types of RES might somehow contribute to modulating host cell collapse. What could these RES be? Among the possibilities are some haem/chlorophyll metabolites that might play roles in

Figure 1



The relationship of ROS, RES, and jasmonate (JA) in the activation of cell survival programs. ROS produced in stress responses can strongly alter gene expression and stimulate the synthesis of jasmonates [38]. Jasmonates activate the expression of certain cell survival genes including some genes involved in ascorbate and glutathione production [39]. Heavy ROS attack on membranes leads to the oxygenation and, ultimately, fragmentation of polyunsaturated fatty acids like ALA (α-linolenic acid). This generates RES that can activate the expression of genes such as those shown in Table 1. Included in the figure is an arrow indicating the possibility that RES may stimulate the production of ROS.

lesion formation [16]. We note that among these are electrophilic structures such as that of red chlorophyll catabolite (RCC) [17,18]. Interestingly, some virulence factors produced by pathogens contain α,β-unsaturated carbonyl groups. One example is syringolin A from some *Pseudomonas syringae*. A recent transcriptome study showed that syringolin A activates cell survival gene expression in wheat and Arabidopsis [19]. We speculate that at least part of this transcription response might be because of RES activity in syringolin A.

Some plant secondary metabolites (phytoanticipines) are RES, and some RES activate the production of secondary metabolites [20]. For example, electrophilic triterpenes from Acacia have powerful activities in human cells where they suppress proinflammatory components in the immune system [21]. The role of these triterpenes in nature at the molecular level is not yet clear. Phytoalexins (disease-inducible low molecular mass antimicrobial compounds) accumulate in plant cells in response to treatment with cyclopentenone phytoprostanes [14••,22], and camalexin also accumulates above wild-type levels in seedlings of the Arabidopsis *vte2-1* mutant in which excess levels of MDA accumulate [11••]. Tobacco cells respond to OPDA as well as to cylclopentenone phytoprostanes with the accumulation of scopoletin, an antioxidant [23]. Consistent with this, tobacco cells could be protected from the toxic effects of copper sulphate by pre-treatment with exogenous cylclopentenone phytoprostanes [14••] possibly acting as electrophiles. Summarizing, there are clues that RES, at the appropriate concentrations, will actually help plant cells to survive in adversity. Tests of how their endogenous production affects plant responses to biotic and abiotic stress are needed.

The modification of metabolism by RES

RES may affect metabolism by covalently modifying enzymes and much of this evidence comes from the mitochondrion. Uncoupling protein (UCP) in plants (and animals) is a potential target for post-translational modification by HNE [24], and one of the two terminal oxidases in plant mitochondrial respiratory electron transport, alternative oxidase (Aox), is very sensitive to inhibition by this alkenal [6]. These studies and others (e.g. [25]), as well as numerous counterparts in the animal literature, suggest the possibility that RES production could affect primary metabolism and thus have far-reaching consequences for the organism. Interestingly, some of the consequences of protein modification remain largely unexplored. One can speculate that aspects of protein function other than enzyme action might be modified by RES. RES could theoretically affect the lifespans of proteins in cells. For example, lysine residues are central to protein turnover mediated by ubiquitination. Modification of lysines by RES addition might therefore affect protein lifespans in the cell. What are now needed are genetic interventions that alter RES labeling of target proteins *in planta*. This might be particularly relevant in chloroplasts where thylakoid membranes rich are in α-linolenic acid (ALA), a major source of RES.

RES affect cells in two main ways

The propensity of RES to harm plant cells and their ability to activate gene expression appear to be correlated [15], and thus any attempt to understand the damaging effects of RES on cells is valuable. RES can damage cells in at least two ways, the first of which is via indirect effects on pools of cellular reductants. The double bond in a broad range of α,β-unsaturated carbonyl compounds can be reduced by a class of NADPH-oxidoreductases currently known as alkenal reductases [26]. Vinyl ketones (and perhaps other α,β-unsaturated carbonyl compounds) can be reduced by enzymes such as flavin mononucleotide (FMN) dependent oxidoreductases including enzymes of the oxo-phytodienoic acid reducase (OPR) group [27]. Other enzymes that may intervene in the turnover of RES and other aldehydes are aldose/aldehyde reductases that use NADPH to reduce substrates such as HNE [28]. Finally, aldehyde dehydrogenases use NAD(P)+ to convert aldehydes (produced by the reduction of the double bond in some α,β-unsaturated carbonyls) into carboxylic acids, thus generating NAD(P)H [29,30]. Summarizing, RES may indirectly damage tissues as cells try to detoxify them because many routes to their elimination will cause the depletion of pools of reductants.

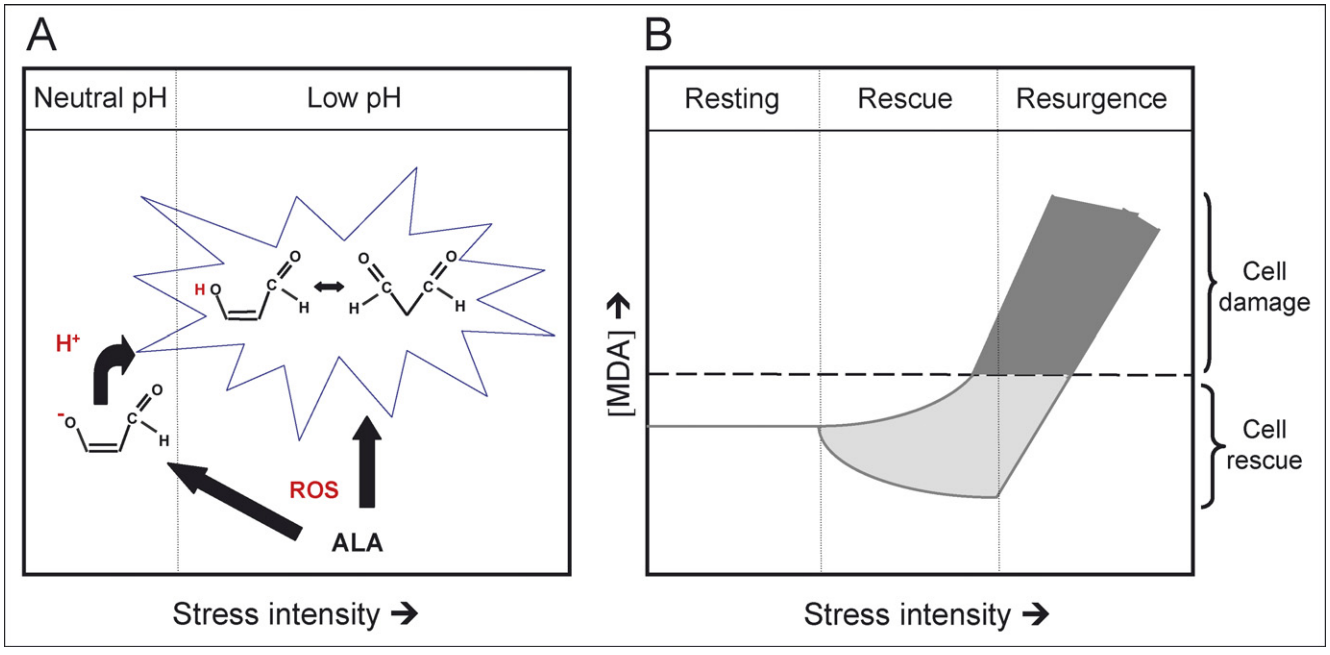
In a second mechanism, RES can modify cell contents directly because of their chemical reactivity with nucleophilic atoms. Excessive RES production, through uncontrolled oxidative reactions, corrupts the natural functions cellular constituents and leads to damage. Just how much each of these two (and other) mechanisms contribute to cell damage probably depends on the nature of the electrophile and, for some compounds, it is likely that damage is exerted to differing degrees by both mechanisms. This was provided as one possible explanation of why different exogenous electrophiles seem to trigger the activation of overlapping rather than identical groups of genes. A notable example of this is MDA, which seems to have a rather selective effect on gene expression [5].

An important corollary is that whichever mechanism results in cell damage, it will also result in the destruction of the RES. The electrophiles that are most likely to affect cellular functions including gene expression will be 'killed in action' as they act as signals. This means that, in some cases, RES might work as signals, although their levels either may remain stable or may actually fall. In a discovery that was quite unexpected at the time, unbound MDA levels were found to fall in Arabidopsis leaves infected with *Botrytis* [31]. This does not mean that MDA is irrelevant as a signal in this pathosystem. On the contrary, the fact that its levels fall probably means that MDA was impacting the physiology of the host (or pathogen) cells.

The importance of being MDA

Although many different RES are likely to be produced or mobilized during pathogenesis in plants [13,15], it is often necessary to concentrate on individual molecules to learn about their provenance and activities. One such molecule is MDA. MDA is a challenging molecule to quantitate, and yet its precise measurement is vital if its proposed role in cell signaling is to be substantiated. In resting expanded Arabidopsis leaves, MDA levels are relatively high [5,31], and this is in part explicable because of the fact that at near neutral (cytosolic) pHs the hydroxyl group indicated in Figure 2 is unprotonated and the molecule has low reactivity. Part of the MDA pool in leaves is in this free form [5]. However, when MDA is exposed to lower pHs, it assumes the protonated reactive forms. Apoptosis is reported to lower cytosolic pH [32], a phenomenon that can occur in pathogenesis [33]. Lower pHs can also be generated, in chloroplasts in the lumen of the thylakoid. Even simply disrupting a cell would allow the contents of acidic compartments such as the vacuole or apoplast to alter cytoplasmic pHs favoring MDA protonation. Changes in pH may be one mechanism for 'activating' MDA. Why, then, do healthy cells permit the accumulation of such a potentially dangerous molecule? We propose that MDA is a 'latent RES', a molecule made to be mobilized in case of stress where it could function immediately to activate cell survival signaling. If this was the case, its level might, in some cases, actually fall at the onset of stress, as it would be consumed in its action as a signal. Although it is clear that MDA can be overproduced during severe stress [9], careful measurements have shown decreases in MDA levels in some pathosystems [5,31]. Figure 2 shows a model reconciling these and other data. Two important facts, already drawn from other systems [34] but often forgotten, are that, firstly, many, if not most, reports of MDA levels are based on non-specific assays. Secondly, even when specific assays are used, there is sometimes no obvious correlation between MDA levels and stress. When interpreting the

Figure 2



MDA and intracellular climate change. In healthy cells, there is a reservoir of MDA that is actively maintained and does not harm the cell because, under neutral pH conditions prevailing in the cytosol, the molecule is unreactive. The reactivity of MDA depends on pH, a parameter that can change in stress and pathogenesis. **(A)** Under normal physiological conditions (neutral cytosolic pH), MDA is a latent RES. Stress conditions cause the protonation of MDA and this unleashes its reactive potential by producing two tautomeric forms of the molecule, one of which is the highly diffusable dialdehyde form. As stress intensifies, increasing ROS production causes the uncontrolled generation of MDA from α-linolenic acid (ALA) in chloroplasts and mitochondria. A second genetically identified source(s) of MDA [5] is not indicated in the model. **(B)** Model for the roles of MDA/RES in cell protection and cell damage. Total MDA levels in healthy 'resting' tissues change when cells are stressed. As a stress occurs, the cell enters the 'rescue' phase. Here, at the onset of stress, MDA levels may either fall (because of conversion of unprotonated MDA to reactive forms and/or because of the activated metabolism of MDA) or may increase because of *de novo* production. In the rescue phase, MDA (and other RES) is useful to the cell because it signals to activate cell protection genes. As stress severity increases, however, MDA levels rise to exceed a safe threshold (indicated by a horizontal dashed line). At the point we term 'resurgence', MDA production, along with the generation of other electrophiles, damages the cell. The model reconciles careful measurements of MDA in health and disease [5,31], the proposed ability of the molecule to act as a cell survival signal [5,13] and the damaging effects of excess RES production to cells [15]. By implication, the presence of ALA, an important source of many RES, would benefit the organism until stress levels were sufficiently high, whereby excess RES/MDA production would harm the cell. At this point, it would become a disadvantage to have trienoic fatty acids.

literature, one has to be careful that MDA levels are reported from high-quality MDA-specific assays conducted with appropriate controls.

RES signaling in plants and animals

Part of the signal mechanism for electrophile perception has been elucidated in mammals. Briefly, a dimeric metalloprotein, Keap1 (Kelch-like ECH-associated protein 1), sequesters the Nrf2 (nuclear factor-erythroid 2-related factor 2) transcription factor in the cytosol and targets it for ubiquitinaton and proteasomal degradation until electrophiles bind to sulphydryl residues in Keap1. This then renders Keap1 unable to target Nrf2 for degradation, allowing its nuclear translocation to help initiate the transcription of cell protection genes [35,36,37]. Interestingly, the Arabidopsis genome does not appear to encode Nrf2 homologues, although there are genes showing similarity to Keap1. These genes could be considered as candidates in electrophile signaling in plants. The types of genes that are under the control of the Keap1/Nrf2 system in animals are shown in Table 1 and compared to MDA-responsive genes in a plant. The table illustrates three points. Firstly, many genes involved in sulphur metabolism/utilization are electrophile inducible in plants and mammals. There are strong parallels in electrophile stimulated gene expression in both types of organism; the phase II response may be conserved between plants and animals. Secondly, many genes in the table can be considered as general oxidative stress genes. At the level of signal transduction mechanisms, there may be some similarity between ROS and RES. Both families of reactive species might operate on gene expression to some extent through common mechanisms. Thirdly, some of the MDA-inducible genes in plants may not have animal orthologs and may carry out functions unique to plants. This raises another issue. Most cells in humans and mice can be killed by small changes in hydration or temperature and can be easily damaged by light. This is not always the case for plant cells that often have to function over environmental extremes. For example, RES generated in chloroplasts may function as signals for adaptive responses.

Table 1

Parallels and differences in electrophile-stimulated gene expression in animals and plants

Electrophile-inducible phase II genes in vertebrates	Malondialdehyde (MDA) inducible genes in Arabidopsis
Glutathione *S*-transferases	Glutathione *S*-transferases
Glutamylcysteine ligase	Glutamylcysteine ligase
Glutathione reductase	Glutathione reductase
Glutathione conjugate exporter	Glutaredoxin family protein
Thioredoxin reductase	Thioredoxin reductase
Quinone reductase	Quinone reductase
Heme oxygenase 1	Heme oxygenase
Epoxide hydrolase	Epoxide hydrolase Peptidyl-prolyl *cis–trans* isomerase
Ferritin	Ferritin Drought response element-binding protein Embryo abundant protein-related No apical meristem (NAM) family protein

Genes stimulated by various electrophiles in animal cells are from [37]. Arabidopsis genes showing increased transcript levels in response to MDA treatment are from S Stolz and EE Farmer (unpublished data).

Conclusions

RES can act at all levels of gene expression and, like the proverbial messenger, are killed in action as they act as signals. RES generation, often thought of as an unwanted process, may actually help the organism perceive stress and respond appropriately, and this is being investigated. This, however, remains hypothetic, and direct experimental demonstrations of beneficial effects of RES production during stress are lacking. This is the next challenge. The biology of MDA, an almost ubiquitous and highly studied molecule, is particularly interesting because it may have powerful biological activity. A possibility, proposed herein, is that the cell maintains MDA as a latent RES and that stress unleashes its potential as a signal for the activation of genes of adaptative importance in cell survival. Genetic screens to unravel the mechanism of RES signaling in plants and the targeted mutation of genes thought to be important in RES responses should facilitate our understanding of their effects *in vivo*. The current speculation is that, together, jasmonates and RES play primordial and complementary roles in supporting plant life on earth. In doing so, they have a great impact on our biosphere.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Davoine C, Falletti O, Douki T, Iacazio G, Ennar N, Jean-Luc
•• Montillet J-L, Triantaphylidès C: **Adducts of oxylipin electrophiles to glutathione reflect a 13 specificity of the downstream lipoxygenase pathway in the tobacco hypersensitive response**. *Plant Physiol* 2006, **140**:1484-1493.
During the hypersensitive response in tobacco leaves, enzyme-generated RES such as 2-(*E*)-hexenal and OPDA are produced and subsequently coupled *in vivo* to glutathione (GSH). The formation of these adducts both generates new molecular forms in the cells and helps deplete glutathione pools. Interestingly, the hexenal in the GSH adduct was reduced to hexenol. The possibility that the adducts might act as signals is discussed.

2. Taki N, Sasaki-Sekimoto Y, Obayashi T, Kikuta A, Kobayashi K,
• Ainai T, Yagi K, Sakurai N, Suzuki H, Masuda T *et al.*: **12-Oxo-Phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis**. *Plant Physiol* 2005, **139**:1268-1283.
Exogenous OPDA was found to alter the expression of approximately 200 genes in Arabidopsis in a mechanism depending in part on the presence of the α,β-unsaturated carbonyl group. The expression of several of these OPDA-specific response genes (ORGs) is lower in a wounded mutant unable to synthesize OPDA than in wounded wild-type plants.

3. Matsui K: **Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism**. *Curr Opin Plant Biol* 2006, **9**:274-280.

4. Ishii T, Kumazawa S, Sakurai T, Nakayama T, Uchida K: **Mass spectroscopic characterization of protein modification by malondialdehyde**. *Chem Res Toxicol* 2006, **19**:122-129.

5. Weber H, Chételat A, Reymond P, Farmer EE: **Selective and powerful stress gene expression in Arabidopsis in response to malondialdehyde**. *Plant J* 2004, **37**:877-888.

6. Winger AM, Millar AH, Day DA: **Sensitivity of plant mitochondria terminal oxidases to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE)**. *Biochem J* 2005, **387**:865-870.

7. Schneider C, Boeglin WE, Yin H, Ste DF, Hachey DL, Porter NA, Brash AR: **Synthesis of dihydroperoxides of linoleic and linolenic acids and studies on their transformation to 4-hydroperoxynonenal**. *Lipids* 2005, **40**:1155-1162.

8. Mueller MJ: **Archetype signals in plants: the phytoprostanes**. *Curr Opin Plant Biol* 2004, **7**:441-448.

9. Havaux M, Eymery F, Porfirova S, Rey P, Dörmann P: **Vitamin E protects against photoinhibition and photooxidative stress in *Arabidopsis thaliana***. *Plant Cell* 2005, **17**:3451-3469.

10. Larkindale J, Hall JD, Knight MR, Vierling E: **Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance**. *Plant Physiol* 2005, **138**:882-897.

11. Sattler SE, Mené-Saffrane L, Farmer EE, Krischke M, Mueller MJ,
•• DellaPenna D: **Non-enzymatic lipid peroxidation reprograms gene expression and activates defense markers in tocopherol deficient mutants**. *Plant Cell* 2007.
This paper provides a wealth of new information on non-enzymatic lipid oxidation in the seedlings of tocopherol deficient mutants. The overproduction of phytoprostanes and MDA is associated with tissue damage, camalexin production, and the expression of genes commonly activated in pathogenesis. The paper shows that non-enzymatic fatty acid oxygenation activates biotic stress gene expression. The work also indicates that excess RES production can damage cells as predicted previously [15].

12. Mou Z, He Y, Dai Y, Liu X, Li J: **Deficiency in fatty acid synthase leads to premature cell death and dramatic alterations in plant morphology**. *Plant Cell* 2000, **12**:405-418.

13. Vollenweider S, Weber H, Stolz S, Chételat A, Farmer EE: **Fatty acid ketodienes and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased *Arabidopsis* leaves**. *Plant J* 2000, **24**:467-476.

14. Loeffler C, Berger S, Guy A, Durand T, Bringmann G, Dreyer M, von
•• Rad U, Durner J, Mueller MJ: **B1-phytoprostanes trigger plant defense and detoxification responses**. *Plant Physiol* 2005, **137**:328-340.
Phytoprostanes B_1 (PPB_1) are produced along with many other phytoprostanes in oxidative stress. This paper reports the biological activity of exogenous PPB_1 isomers that trigger stress and secondary metabolite-related gene expression in Arabidopsis cultures. Furthermore, PPB_1 pretreatment of tobacco cells increases their resistance to copper sulphate. The authors leave open the question of which structural features of the molecule promote these responses.

15. Alméras E, Stolz S, Vollenweider S, Reymond P, Mène-Saffrané L, Farmer EE: **Reactive electrophile species activate defense gene expression in Arabidopsis**. *Plant J* 2003, **34**:205-216.

16. Yao N, Greenberg JT: ***Arabidopsis* ACCELERATED CELL DEATH2 modulates programmed cell death**. *Plant Cell* 2006, **18**:397-411.

17. Mach JM, Castillo AR, Hoogstraten R, Greenberg JT: **The *Arabidopsis*-accelerated cell death gene *ACD2* encodes red chlorophyll catabolite reductase and suppresses the spread of disease symptoms**. *Proc Natl Acad Sci U S A* 2001, **98**:771-776.

18. Wüthrich KL, Bovet L, Hunziker PE, Donnison IS, Hörtensteiner S: **Molecular cloning, functional expression and characterisation of RCC reductase involved in chlorophyll catabolism**. *Plant J* 2000, **21**:189-198.

19. Michel K, Abderhalden O, Bruggmann R, Dudler R: **Trancriptional changes in powdery mildew infected wheat and Arabidopsis leaves undergoing syringolin-triggered hypersensitive cell death at infection sites**. *Plant Mol Biol* 2006, **62**:561-578.

20. Farmer EE: **Surface-to-air signals**. *Nature* 2001, **411**:854-856.

21. Haridas V, Hanausek M, Nishimura G, Soehnge H, Gaikwad A, Narog M, Spears E, Zoltaszek R, Walaszek Z, Gutterman J: **Triterpenoid electrophiles (avicins) activate the innate stress response by redox regulation of a gene battery**. *J Clin Invest* 2004, **113**:65-73.

22. Thoma I, Loefler C, Sinha AK, Gupta M, Krischke M, Steffan B, Roitsch T, Mueller MJ: **Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants**. *Plant J* 2003, **34**:363-375.

23. Iqbal M, Evans P, Liedó A, Verdaguer X, Pericàs MA, Riera A, Löffler C, Sinha AK, Müller MJ: **Total synthesis and biological activity of 13,14-dehydro-12-oxo-phytodienoic acids (Deoxy-J_1-Phytoprostanes)**. *Chem Bio Chem* 2005, **6**:276-280.

24. Smith AMO, Ratcliffe RG, Sweetlove LJ: **Activation and function of mitochondrial uncoupling protein in plants**. *J Biol Chem* 2004, **279**:51944-51952.

25. Millar AH, Leaver CJ: **The cytotoxic lipid peroxidation product, 4-hydroxynonenal, specifically inhibits decarboxylating dehydrogenases in the matrix of plant mitochondria**. *FEBS Lett* 2000, **481**:117-121.

26. Mano J, Belles-Boix E, Babiychuk E, Inze D, Torii Y, Hiraoka E, Takimoto K, Slooten L, Asada K, Kushnir S: **Protection against photoxidative injury of tobacco leaves by 2-alkenal reductase**. *Plant Physiol* 2005, **139**:1773-1783.

27. Breithaupt C, Strassner J, Breitinger U, Huber R, Macheroux P, Schaller A, Clausen T: **X-ray structure of 12-oxophytodienoate reductase 1 provides structural insight into substrate binding and specificity within the family of OYE**. *Structure* 2001, **9**:419-429.

28. Oberschall A, Deák M, Török K, Saa L, Vass I, Kovács I, Fehér A, Dudits D, Horváth GV: **A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses**. *Plant J* 2000, **24**:437-446.

29. Sunkar R, Bartels D, Kirch H-H: **Overexpression of a stress-inducible aldehyde dehydrogenase gene from *Arabidopsis thaliana* in transgenic plants improves stress tolerance**. *Plant J* 2003, **35**:452-464.

30. Kotchoni SO, Kuhns C, Ditzer A, Kirch H-H, Bartels D: **Over-expression of different aldehyde dehydrogenase genes in *Arabidopsis thaliana* confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress**. *Plant Cell Environ* 2006, **29**:1033-1048.

31. Muckenschnabel I, Goodman BA, Williamson B, Lyon GD, Deighton N: **Infection of leaves of *Arabidopsis thaliana* by *Botrytis cinerea*: changes in ascorbic acid, free radicals and lipid peroxidation products**. *J Exp Bot* 2002, **53**:207-214.

32. Matsuyama S, Reed JC: **Mitochondria-dependent apoptosis and cellular pH regulation**. *Cell Death Differ* 2000, **7**:1155-1165.

33. Sakano K: **Metabolic regulation of pH in plant cells: role of cytoplasmic pH in defense reaction and secondary metabolism**. *Int Rev Cytol* 2001, **206**:1-44.

34. Janero DR: **Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury**. *Free Radic Biol Med* 1990, **9**:515-540.

35. Satoh T, Okamoto S-I, Cui J, Watanabe Y, Furuta K, Suzuki M, Tohyama K, Lipton SA: **Activation of the**

Keap1/Nrf2 pathway for neuroprotection by electrophilic phase II inducers. *Proc Natl Acad Sci U S A* 2006, **103**:768-773.

36. Dinkova-Kostova AT, Holtzclaw WD, Kensler TW: **The role of Keap1 in cellular protective responses**. *Chem Res Toxicol* 2005, **18**:1779-1791.

37. Holtzclaw WD, Dinkova-Kostova AT, Talalay P: **Protection against electrophile and oxidative stress by induction of phase 2 genes: the quest for the elusive sensor that responds to inducers**. *Adv Enzyme Regul* 2004, **44**:335-367.

38. Orozco-Cardenas ML, Narvaez-Vasquez J, Ryan CA: **Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate**. *Plant Cell* 2001, **13**:179-191.

39. Sasaki-Sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N, Susuki H, Hirai MY, Noji M, Saito K *et al.*: **Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in Arabidopsis**. *Plant J* 2005, **44**:653-668.