

## Showcase on Research

# What's a nice plant like you doing in a place like this? Stress, Oxidative Stress and Mitochondria

A. Harvey Millar<sup>1,2</sup> and James Whelan<sup>1</sup>

1. Department of Biochemistry, Faculty of Medicine and Dentistry, The University of Western Australia, WA 6009

2. Plant Sciences Group, Faculty of Agriculture, The University of Western Australia, WA 6009

### Environmental stress and the production of AOS

The famous plant biologist John L. Harper is quoted as saying: "Plants stand, they don't run away when you try to count them" (1). This most remarkable, or some might think unremarkable trait, of plants, underlies a fundamental aspect of plant biology. Plants, by the nature of being rooted to the spot, must cope with whatever their chosen spots bring to bear on them. Plants can sustain growth and development amidst such a wide variety of environmental fluctuations by employing flexible metabolic networks that allow dynamic changes to the prevailing conditions.

When we speak of plant stress we are not simply speaking about adaptation, for all plants adapt both structurally and metabolically in ways inconceivable to the straightjacket of mammalian cellular life. The term plant stress usually refers to conditions in which plant growth and performance are adversely affected by the environment to the extent that morphological changes and significant losses in crop yield and/or fertility occur. A key sign of such stress at a molecular level is the increased production of active oxygen species (AOS) and the subsequent accumulation of oxidative damage.

Investigating the link between plant growth under stressful environments and the endogenous production of AOS has involved a great deal of research at both the whole plant and the molecular level and a variety of mechanisms have been highlighted. AOS are directly produced in plant cells by the chemical interaction of a variety of environmental pollutants with constituents of the intracellular environment. The effects of atmospheric pollutants, such as ozone and various nitrogen oxides (NOs) on plants, are well studied and the catalysis of AOS formation by a variety of metal ions is documented in plants (2). Metabolic perturbation can also

result in AOS formation, for example, through the initiation of one-electron reductions of O<sub>2</sub> by electron transport chains of chloroplasts and mitochondria.

Such perturbations follow herbicide application, exposure to low temperature and/or high light conditions, exposure to drought, or to high salinity (3). In addition to these abiotic stresses, initiation of host defence responses to pathogen invasion results in the transient enhanced production of AOS and NOs during the so-called 'respiratory burst' of the hypersensitive response in plants (4).

Understanding the interplay of both the beneficial and the detrimental effects of AOS in plant growth and development may lead to the design of strategies for plant improvement. Plant resistance to pathogens has been successfully introduced into economically important crop species using both traditional breeding programs and genetic engineering approaches based on the gene-for-gene hypothesis of specific host-pathogen interactions.

The potential for increasing the capacity of plant lines to withstand a more diverse range of unfavourable environmental conditions without dramatic losses in yield, represents a major new initiative and a new set of challenges for the genetic manipulation of plants. Preliminary studies have shown that gains of function that alleviate oxidative stress under one unfavourable condition often provide resistance to other types of stressful environments. This suggests that induction of fundamental defence mechanisms against oxidative stress could impart a general stress resistance phenotype to the plant.

### Strategies for stress tolerance

Oxidative stress, defined as the elevation of AOS concentration and the accumulation of oxidative damage, could be alleviated by a number of approaches. Firstly, the rate of AOS production could be slowed to allow the existing antioxi-

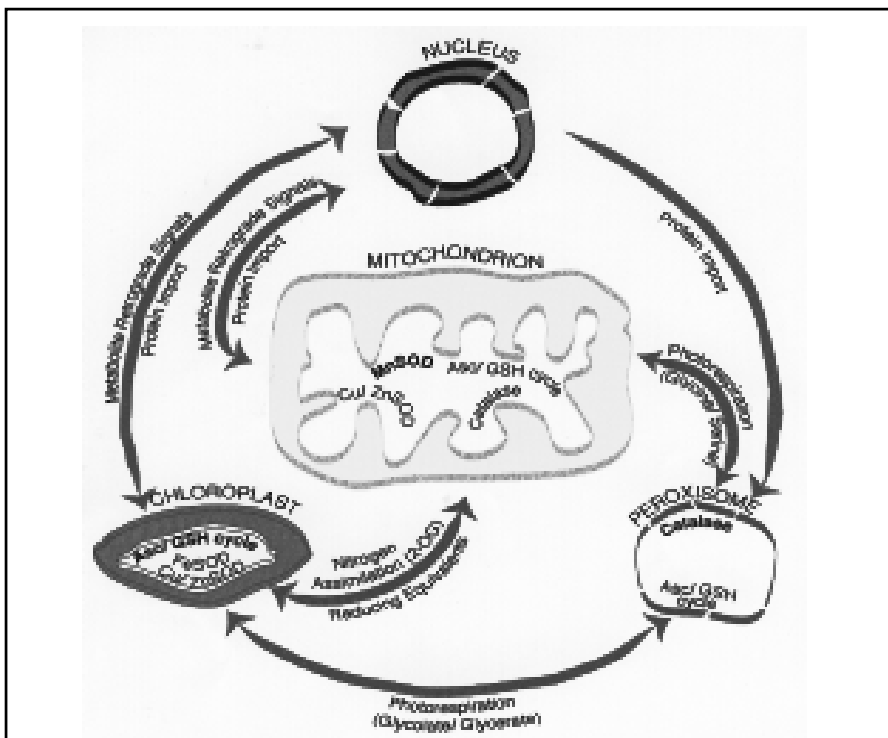
dant defences to prevent the accumulation of oxidative damage. Such an approach might involve decreasing the permeability of plants to AOS-inducing substances such as salts and free metal ions, or increasing the transport of such substances out of the plant or into sequestered stores that do not metabolically perturb the plant. Alternatively, the rate of AOS destruction could be increased in order to match the higher rate of AOS production induced by the stress conditions. Such an approach is exemplified by over-expression of antioxidant defence proteins in plants.

### What anti-oxidation defences do plant possess?

Antioxidant defence systems in plants consist of a range of enzymes and reductants that act to scavenge AOS through the interconversion of partially reduced oxygen molecules, ultimately to produce water. In this manner they avoid the danger of AOS reacting with and functionally damaging proteins, lipids or DNA (Fig. 1 next page). The cascade of reactions begins with the superoxide radical, produced by the one electron reduction of O<sub>2</sub>.

A range of superoxide dismutases (SODs) are found in plants which catalyze the dismutation of two superoxide molecules to form O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>. These SODs can be classified into three classes on the basis of their metal cofactor: copper/zinc (Cu/Zn), manganese (Mn) and iron (Fe). Plants contain all of their FeSOD and a specific Cu/ZnSOD isoform in the chloroplast; they also contain Cu/ZnSODs in the cytosol and the peroxisomes. In the mitochondrion they contain all of their MnSOD in the matrix space and also a Cu/ZnSOD in the intermembrane space. H<sub>2</sub>O<sub>2</sub> produced from superoxide is not a radical species, is not highly reactive and in itself it poses little danger to the cell. However, the reaction of H<sub>2</sub>O<sub>2</sub> with free Fe<sup>2+</sup> in the cell results in the formation of the hydroxyl radical that is the most re-

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**Fig. 1. Diagrammatic representation of the plant cell from the authors' biased point of view.**

The mitochondrion relies on the nucleus for the majority of its proteins: it is likely it sends messages back to the nucleus in so-called 'retrograde signalling' and, on a longer time-scale, some genetic material is still being transferred from the minimal mitochondrion to the nucleus in plants. Mitochondria interact with both peroxisomes and chloroplasts through the pathways of photorespiration, nitrogen assimilation and through reducing equivalent shuttles. Mutant studies clearly show there are also other means through which mitochondrial and chloroplast function are tightly linked (see text). The circuits connecting these organelles are only beginning to be elucidated and it will be of interest in the coming years to label the various signals coming from each organelles to produce a cellular and whole plant response to various stresses. Indicated are the antioxidant defence systems employed in each of these organelles and the metabolite/signal exchanges that occur between organelles in known metabolic pathways.

active of all the oxygen radicals and the most dangerous oxidant for the cell. The rapid removal of hydrogen peroxide is thus important for the cell and is undertaken by reduction of  $H_2O_2$  to water catalysed by a number of distinct enzyme systems.

Catalase converts two molecules of  $H_2O_2$  to  $O_2$  and  $2H_2O$ . Catalase is primarily located in the peroxisome where it functions to remove  $H_2O_2$  produced by the soluble enzymes xanthine oxidase and glycolate oxidase, and by the membrane-bound NADH oxidase in the peroxisomal electron transport chain. Catalase is also found in much smaller amounts in plant mitochondria.

Ascorbate peroxidase reduces  $H_2O_2$  to form  $H_2O$  with the concomitant oxidation of ascorbate. The ascorbate is regenerated by the ascorbate/glutathione cycle. This system is utilised in both the chloroplast and the mitochondrion to combat  $H_2O_2$  derived from their respec-

tive electron transport chains. Lipid membranes are highly susceptible to peroxidative damage and contain a variety of redox chemicals capable of breaking the chain of free radical cascades initiated by the peroxidation of unsaturated fatty acids by  $OH^\cdot$ . These antioxidant chemicals include  $\alpha$ -tocopherol,  $\beta$ -carotene, plastoquinones and ubiquinones, which are found differentially located in chloroplast, mitochondrial and peroxisomal membranes.

Several attempts have been made to manipulate antioxidant defences with the hope of increasing plant resistance to abiotic stress. Over-expression of a tobacco MnSOD in the chloroplast or the mitochondria in alfalfa clearly enhanced yield and plant survival under drought conditions (5). Similar overexpression of MnSOD in tobacco dramatically protected leaves from oxidative injury induced by elevated ozone concentrations (6).

Overexpression of FeSOD in

chloroplasts of tobacco also protected cell membranes from methyl viologen-induced peroxidative damage in leaf disc experiments, but had little effect on the plant's response to low temperature or salt stress (7). However, these beneficial effects may not be the direct result of SOD defence during stress. Rather, the enhanced  $H_2O_2$  production by these SODs, which could occur even before application of a stress, may indirectly activate disease resistance genes that prepare the transgenic plants for an environmental assault (8; see also Andersson *et al.*, this issue).

### Compartmentation of anti-oxidation defences

From this account of the antioxidant defences in plants, it is clear that a key ingredient for the successful protection of plant cells from oxidative damage is the compartmentation of antioxidant defences. A variety of reasons can be postulated to explain the importance of this compartmentation.

Firstly, there is a need for spatially localised action against AOS to prevent their spread and to minimise the time available for their initiation of free radical chains of reaction. For example, if catalase was the only enzyme that scavenged  $H_2O_2$  and it was all in the peroxisome, then significant damage would occur to chloroplasts and mitochondria due to formation of  $OH^\cdot$  before the  $H_2O_2$  reached the peroxisome.

Secondly, there are clearly different types of defences and varying quantities of each type in different compartments depending on the rate and type of metabolism that occurs. The varying response of antioxidant defence in different compartments to an imposed stress has been highlighted in a variety of plants (9,10). Such studies suggest that any real resistance on a whole plant level, or even a whole cell level, to environments inducing oxidative stress must involve an 'integrated enhancement' of antioxidant systems both within and between compartments (11).

One possible means of generating such an integrated response is to equip each compartment with all of the required defence systems to combat such insults.

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### Stress, Oxidative Stress and Mitochondria (contin.)

This can be achieved by expressing organelle-specific forms of each enzyme, but may also involve sending the same enzyme to two or more compartments. Recent reports of dual targeting of proteins to chloroplasts and mitochondria indicates that the plant itself employs such a strategy; for example, amino acyl tRNA synthetases and glutathione reductase are targeted to both the chloroplast and the mitochondrion. It is likely that this list will expand and anti-oxidant defence systems will no doubt be an important subset of dual targeted enzymes.

#### Mitochondrial anti-oxidant defences

Much of our understanding of oxidative stress and antioxidant defences in plants comes from research undertaken on the chloroplast and its photosynthetic function. However, it is now clear that mitochondrial function is essential for the maintenance of photosynthesis and also for the continuation of plant cellular life. Only one report of a homoplasmic mutation exists for a mitochondrial encoded gene in plants despite extensive efforts to generate such mutations.

Mutation of plant mitochondrial genomes cause the well described cytoplasmic male sterility while mutations of nuclear encoded mitochondrial genes may result in developmental abnormalities. The *non-chromosomal striped* (NCS) plant mutants have lesions in mitochondrial encoded genes which greatly affect chloroplast function (12).

The converse is also true: in the white leaves of *albostrians* barley, which lacks a plastid translational apparatus, elevation of both the mitochondrial genome copy number and mitochondrially encoded transcripts occurred in leaves but notably not in roots (13).

Obviously mitochondria and chloroplasts interact genetically with each other and the nucleus. Except for a small number of proteins encoded by mitochondrial and chloroplast DNA (< 5%), the remaining proteins are encoded in the nucleus and are post-translationally targeted specifically to their respective organelle. Metabolic pathways also link the organelles through the exchange of metabolites pro-

duced during photorespiration, nitrogen assimilation and sugar metabolism, Retrograde signals from the mitochondrion to the nucleus serve to coordinate mitochondrial and cytosolic protein synthesis (Fig. 1). How such signals between organelles are integrated into the response of the plant to environmental stress remains a challenging question.

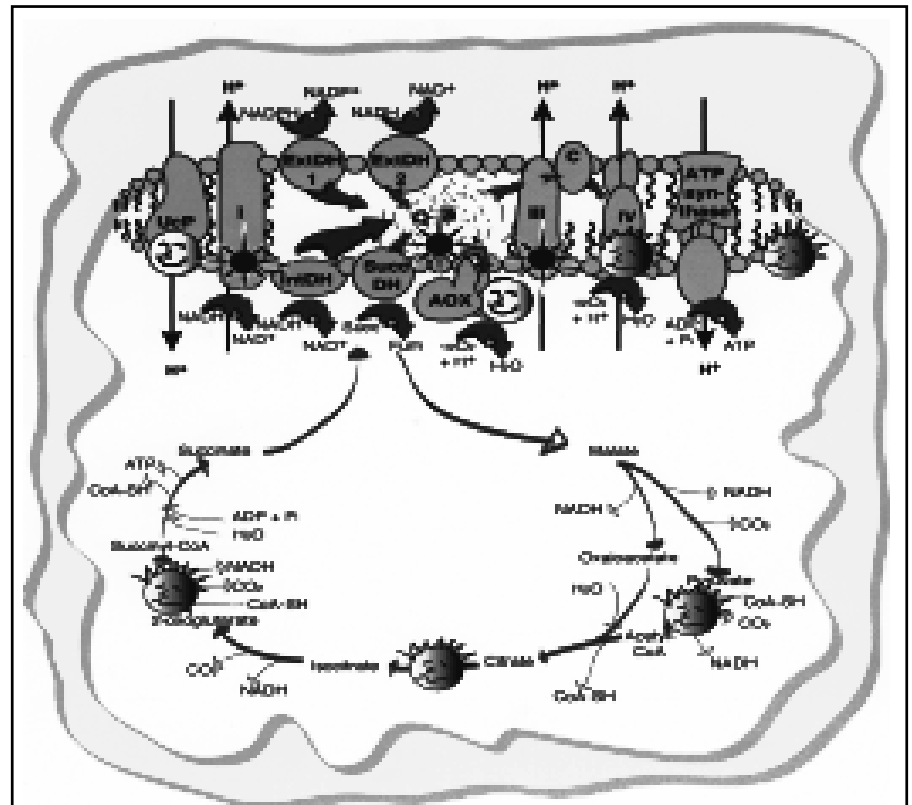
In mammalian systems, oxidative stress invoked by mitochondrial AOS production is thought to be linked to symptoms of disease and cancer, and to aging and eventually cell death. The high mutation rate of mitochondrial genomes in mammals has long been linked to oxidative damage.

In contrast to their conservative cousins in animals, plant mitochondrial genomes are larger, do not display higher oxidation induced mutation rates than the nuclear genome (but in fact lower) and, it

would appear, are still quite happily transferring genetic material to the nucleus (14). Recently, mitochondria have been placed at the centre of apoptosis research following the discovery that these organelles can initiate the process via an AOS and protease signal cascade resulting in programmed cell death via cytochrome c release.

In addition to AOS, oxidative damage of mammalian cells is enhanced by the production of active nitrogen species (ANS), notably (NO). This gaseous secondary messenger induces cyclic-GMP and Ca<sup>2+</sup> cascades and is also a potent inhibitor of mitochondrial cytochrome c oxidase (COX). The latter inhibition leads to enhanced mitochondrial AOS production that initiates reaction of NO and O<sub>2</sub>- to form OH<sup>•</sup> and peroxynitrite (ONOO<sup>-</sup>).

In contrast to the mammalian system,



**Fig. 2. Detailed view of the engine room of respiration in plant mitochondria.** Highlighted are the known sites of AOS production (star), the sites of AOS attack (sad face), and some intriguing pre-oxidant defence systems that may be employed to limit damage (happy face). The diagram depicts the mitochondrial electron transport chain and the TCA cycle. Complexes I to IV, along with the ATP synthase, represent the classical components of the respiratory chain. Additional components found in plants, although not unique to plant mitochondria, are the uncoupling protein (UcP), external NADH and NAD(P)H dehydrogenase (ExtDH1 and ExtDH2), internal NADH dehydrogenase (IntDH) and the alternative oxidase (AOX).

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### Stress, Oxidative Stress and Mitochondria (contin.)

little systematic work has been undertaken on the effect of oxidative stress on plant mitochondrial function. Important plant mitochondrial enzymes are known to be damaged by AOS while the expression of other mitochondrial proteins involved in the electron transport chain, membrane transport systems and the protein folding and assembly apparatus, are enhanced by oxidative stress.

presence of NO in plants was virtually unknown in the literature just four years ago, but it is now clear that NO is synthesised, causes induction of defence systems and is an integral component of secondary messenger cascades in plants (4). An important difference in plant mitochondrial is that while COX is potently inhibited by NO, the secondary terminal oxidase of plant mitochondria, the alternative oxidase (AOX), is unaffected (15) (Fig. 2). The presence of AOX also means that if cytochrome *c* is released from mitochondria during apoptosis in plants, respiratory electron transport can continue. AOX, along with the uncoupling protein (UCP), are believed to act as electron transport uncouplers, allowing the free-wheeling of respiration and the concomitant decrease in ubiquinone redox status which greatly limits the potential for one electron reductions of O<sub>2</sub> by the semi-ubiquinol radical.

While little is known about the transcriptional or post-translational regulation of UCP in plants, AOX is known to be activated when respiratory substrate supply is at its maximum (leading to high reduced ubiquinone) and *de novo* synthesis of the oxidase is induced by endogenous production or exogenous application of AOS (16,17). AOX and UCP have the potential to be pre-oxidative defences, lowering AOS generation under normal metabolic conditions and preventing runaway AOS formation in the presence of NO (Fig. 2).

More direct antioxidant defence components that scavenge AOS in plant mitochondria include MnSOD and Cu/Zn SODs, ascorbate peroxidase, catalase, glutathione and ascorbate. The ascorbate/glutathione cycle is supported by glutathione reductase which is also found in chloroplasts and appears to be dual tar-

geted. Recently it was shown that the final step of ascorbate synthesis in plants occurs on the inner mitochondrial membrane, further indicating that mitochondrial function is vital for cellular antioxidant defence (18).

Despite scavenging of AOS by the antioxidant defences noted above, there is the potential for continued oxidative damage through the AOS that escape to initiate free radical chains of reaction in the lipid environment of the membrane. Lipid peroxidation in the mitochondrial membrane limits respiratory function by inhibiting a number of lipoyl containing enzymes involved in the TCA cycle as well as glycine decarboxylase which is an important component of the photorespiratory cycle in leaves (19) (Fig. 2).

It is important to identify mechanisms of lipid aldehyde destruction in the mitochondrial membranes. Cross-linking of proteins via disulphide bridges can also be induced by AOS attack and can modify enzyme activities. In this context, mitochondrial-specific thioredoxins have the potential to modify the redox state of such proteins and protect against oxidative damage (20).

#### Taking an integrated approach?

Identifying intracellular sites that are susceptible to oxidative damage by AOS and elucidating the strategies employed for maintenance of metabolism during AOS production, is essential for the design of transgenic strategies to maintain the rate of plant growth, and thus crop yields, in the face of the oxidative stress induced by both biotic and abiotic challenges.

In this endeavour, protection of the whole plant is paramount, so that all compartments of plants that produce AOS and suffer oxidative damage must be considered to arrive at an integrated understanding of antioxidant defences. In this context it is also important that the signal transduction pathways which coordinate antioxidant responses during environmental stress are elucidated.

At UWA we are currently undertaking research on the functions of the pre-oxidative defence proteins AOX and UCP, identifying other antioxidant defence

pathways in mitochondria using a proteomic approach, and dissecting the signalling between mitochondria and the rest of the cell during plant stress.

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Fig. 1

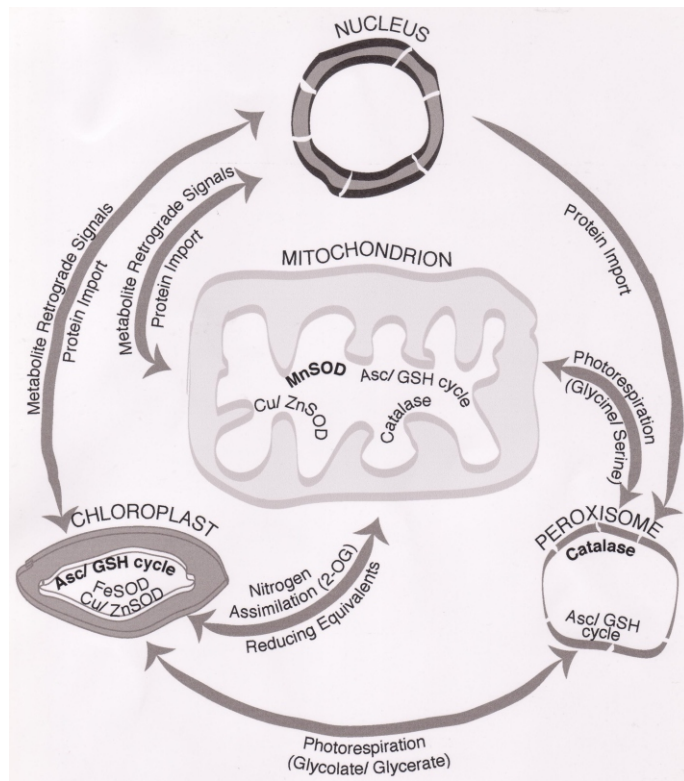


Fig. 2

