New Phytologist

Review

Tansley insight

Breaking boundaries: exploring short- and long-distance mitochondrial signalling in plants

Authors for correspondence:
Elina Welchen
Email: ewelchen@fbcb.unl.edu.ar

Daniel H. Gonzalez
Email: dhgonza@fbcb.unl.edu.ar

Received: *29 April 2021*
Accepted: *29 June 2021*

Elina Welchen and Daniel H. Gonzalez

Facultad de Bioquímica y Ciencias Biológicas, Instituto de Agrobiotecnología del Litoral (CONICET-UNL), Cátedra de Biología Celular y Molecular, Universidad Nacional del Litoral, Santa Fe 3000, Argentina

Contents

	Summary	1
I.	Introduction	1
II.	Cell-to-cell transfer of mitochondrial information	2
III.	Long-distance signalling	4
IV.	Conclusion	6
	Acknowledgements	6
	References	6

New Phytologist (2021)
doi: 10.1111/nph.17614

Key words: cell-to-cell communication, mitochondria, mitokines, plasmodesmata, ROS wave, signalling.

Summary

Communication of mitochondria with other cell compartments is essential for the coordination of cellular functions. Mitochondria send retrograde signals through metabolites, redox changes, direct organelle contacts and protein trafficking. Accumulating evidence indicates that, in animal systems, changes in mitochondrial function also trigger responses in other, either neighbouring or distantly located, cells. Although not clearly established, there are indications that this type of communication may also be operative in plants. Grafting experiments suggested that the translocation of entire mitochondria or submitochondrial vesicles between neighbouring cells is possible in plants, as already documented in animals. Changes in mitochondrial function also regulate cell-to-cell communication via plasmodesmata and may be transmitted over long distances through plant hormones acting as mitokines to relay mitochondrial signals to distant tissues. Long-distance movement of transcripts encoding mitochondrial proteins involved in crucial aspects of metabolism and retrograde signalling was also described. Finally, changes in mitochondrial reactive species (ROS) production may affect the 'ROS wave' that triggers systemic acquired acclimation throughout the plant. In this review, we summarise available evidence suggesting that mitochondria establish sophisticated communications not only within the cell but also with neighbouring cells and distant tissues to coordinate plant growth and stress responses in a cell nonautonomous manner.

I. Introduction

Endosymbiotic acquisition of mitochondria has been a cornerstone of eukaryotic cell evolution (Lane, 2017). Modern-day mitochondria are the result of a continuous evolutionary process that adjusted organelle functions to cellular requirements and established intricate connections between mitochondria and the rest of the cell (Box 1; Welchen *et al.*, 2014). As a consequence, cells have established mechanisms to monitor mitochondrial functions and respond to mitochondrial perturbations. From a mitochondria-centric perspective, this implies that the organelle acquired the capacity to send signals to other cell compartments, mostly the

Box 1 Growth and defence: a central role of mitochondria in the administration of energy for both processes.

Due to their canonical role as energy-providing organelles, changes in mitochondria impact plant growth and development. In coordination with light, ROS, redox and hormonal pathways represented by auxins, ethylene, abscisic acid and gibberellins, mitochondrial function affects seed germination and seedling establishment (Nietzel *et al*., 2020; Jurdak *et al*., 2021), cell-cycle progression in plant meristems (Van Aken *et al*., 2007; Liu *et al*., 2019; Wang *et al*., 2019), and several other aspects of plant growth (Welchen *et al*., 2021). In addition, mitochondria play a central role in energy management for the development of plant tolerance responses during stressful situations or in the final decision towards cell death (Welchen *et al*., 2021). Stress conditions impose energy restrictions and the reorganisation of cell metabolism, therefore limiting plant growth to overcome the challenging situation. Mitochondria are involved in stress-sensing and adaptive response pathways. Mitochondrial dysfunction triggers the mitochondrial retrograde response (MRR) that alters gene expression in the nucleus and is closely interconnected to other cellular stress responses of the plant. Notably, this response is also related to changes in auxin homeostasis, possibly leading to the transmission of signals between cells. Decreased expression of certain mitochondrial metal chaperones modifies the expression of genes involved in plant responses to different stress conditions (García *et al*., 2016; Mansilla *et al*., 2019). Some mitochondrial components are also directly connected with stress alleviation as they are involved in detoxifying toxic compounds or the synthesis of antioxidants (Welchen *et al*., 2016).

The challenge is to elucidate the specific mitochondrial signal(s) controlling each aspect of these phenomena, to what extent they are only related to energy (ATP, ATP/ADP ratio) limitation or if the observed responses are also the result of the interaction with multiple hormonal, energy and stress response pathways.

Box 2 Studying cell nonautonomous mitochondrial signalling in plants.

The term cell nonautonomous refers to cases in which certain changes in a cell cause changes in other cells in which these changes did not occur. By contrast, in a cell autonomous process, changes in one cell affect only processes within the same cell. Cell nonautonomous effects require the existence of signals that originate in the effector cell (the one that suffered the change) and are transmitted to receiving cells, where they cause a response. Cell nonautonomous effects of mitochondrial changes are well documented in animals and several lines of evidence have suggested that they may also be present in plants. Studies in animals were performed after knocking-down genes encoding mitochondrial respiratory chain components in specific cell types or tissues using interfering RNAs expressed from tissue-specific promoters (Durieux *et al*., 2011; Owusu-Ansah *et al*., 2013). Alternatively, such promoters were used to express a protein that affected mitochondrial function (Berendzen *et al*., 2016). The use of tissue-specific RNA interference may be precluded by the mobile nature of small RNAs in plants. Artificial microRNAs could be used for this purpose, in combination with plant lines with reduced motility, such as mutants in *HASTY* (Brioudes *et al*., 2021). Tissue-specific gene mutation using CRISPR-TSKO (Decaestecker *et al*., 2019) would also be an alternative. In a reverse approach, complementation of mutants with tissue-specific promoters may be used to test the effect of restoring gene function in certain tissues on distant tissues or cell types, but this strategy can only be applied to nonlethal mutations. Tissue-specific expression of dominant-negative forms (i.e. aberrant or defective subunits of mitochondrial protein complexes that affect their function) could also be a valid strategy. Joining parts of plants with different genetic backgrounds through grafting is also a valuable tool. This technique has been extensively used to assess the movement of different molecules throughout the plant and may be used to analyse long-distance effects (i.e. shoot to root or *vice versa*) of mitochondrial perturbations brought about by any of the strategies described above. Similarly, obtention and analysis of genetic mosaics (Frank & Chitwood, 2016) could be useful to ascertain short-distance effects (i.e. within different sectors of an organ).

nucleus, and modify their activity accordingly. Mitochondrial dysfunction triggers in most eukaryotic organisms the mitochondrial retrograde response (MRR), often related to perturbations in reactive oxygen species (ROS) homeostasis (Van Aken, 2021), and the unfolded protein response (UPR^{mt}), brought about by perturbations in protein balance (Tran & Van Aken, 2020). The signals implicated in these responses are not completely understood, but they are likely to include metabolic and redox changes, as well as direct organelle contacts and protein trafficking in some cases (Welchen *et al*., 2021). In addition, the development of multicellularity during eukaryotic evolution necessitated the establishment of sophisticated communication mechanisms between neighbouring cells and with distant tissues. Considering this, it seems logical to assume that mitochondrial signalling functions have also evolved to communicate organelle perturbations in specific cell types to other, either neighbouring or distant, cells. Accumulating evidence indicates the existence of these cell nonautonomous pathways (Box 2) that may help to establish coordinated responses at the tissue or organismal level. In this review, we briefly summarise and discuss available evidence pointing to the occurrence of cell nonautonomous effects of changes in mitochondrial function and possible ways of communication between cells. We also mention studies on animal models that may help to guide the study of similar processes in plants.

II. Cell-to-cell transfer of mitochondrial information

Mitochondria interact with the endoplasmic reticulum (ER), peroxisomes and chloroplasts, exchanging ROS, enzymes and metabolites (Fig. 1) (Jaipargas *et al*., 2015; Oikawa *et al*., 2021). Responses to alterations in the organelle microenvironment are frequently revealed as changes in shape and the formation of elastic tubular extensions from the organelle body (Mathur, 2021). These thin tubular extensions, or matrixules, are observed as a consequence of mitochondria–ER interactions that regulate organellar dynamics in response to hypoxia, light and the energetic state (Jaipargas *et al*., 2015). The biological significance of many physical mitochondria–ER interactions remains to be demonstrated in plants. The Arabidopsis MITOCHONDRIAL RHO

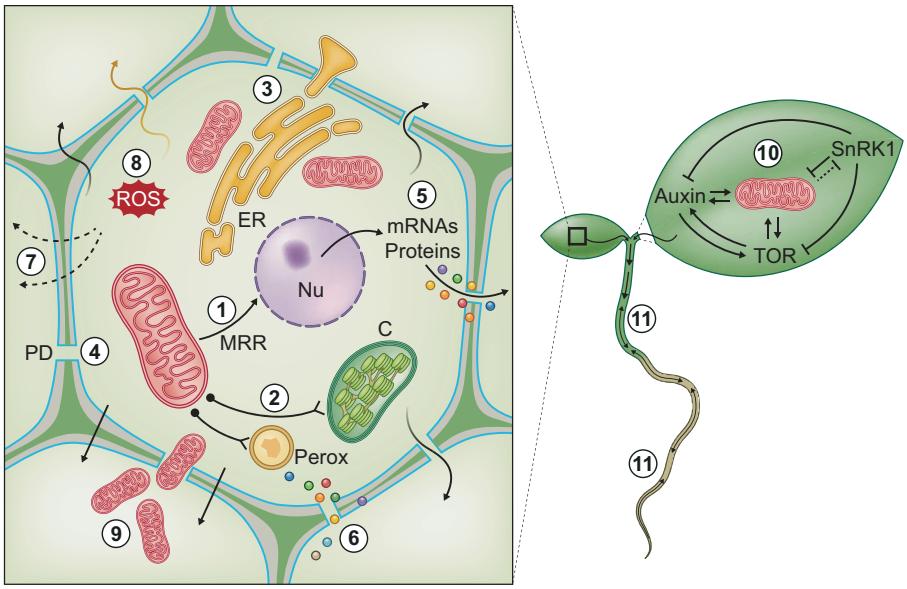

Fig. 1 Short-distance signalling: mitochondria establish multiple intracellular interactions to coordinate energy, growth and stress responses. These include: (1) retrograde signals that induce mitochondria retrograde responses (MRR) in the nucleus (Nu); (2) interactions with chloroplasts (C) and peroxisomes (Perox) to exchange information in the form of reactive oxygen species (ROS) and other molecules; and (3) functional interactions with the endoplasmic reticulum (ER), important for triggering the MRR. Signals may also be used to communicate the 'mitochondrial state' to neighbouring cells, either through the transmission of interactions with the ER through desmotubules (3) or by regulation of plasmodesmata (PD) function (4). Proteins and mRNAs (5), metabolites (6) or unknown factors (7) can transmit mitochondrial signals through PD. ROS are also able to transmit redox signals to nearby cells (8). The intercellular migration of submitochondrial vesicles or entire mitochondria was also reported (9). Mitochondria also share information with hormonal and energy signalling pathways (10). Part of these signals may also be used to transmit information about the 'mitochondrial state' to distant parts of the plant (11). Solid or dotted lines represent known or unknown signals transmitted within and outside the cell. Dashed arrows represent signals sent between cellular compartments migrating outside. Blunt-ended arrows represent the crosstalk signals established between mitochondria, chloroplasts (C) and peroxisomes (Perox).

GTPASE 2 (MIRO2) when expressed in tobacco cells tethers mitochondria to the ER, controlling mitochondrial dynamics and size (White *et al.*, 2020). It was proposed that these interactions would be important to modulate plant energetic metabolism, as well as responses to pathogen attack. It is also noteworthy that two transcription factors that mediate the MRR are tethered to the ER membrane and released by proteolysis under conditions of mitochondrial dysfunction (De Clercq *et al.*, 2013; Ng *et al.*, 2013). The nature of the signal involved in this process is not known, but the transmission of either the signal or the released factor to adjacent cells remains a possibility. In addition, plant mitochondrial metabolism produces ROS and changes in the amount or nature of these species produce signals that modify cellular functions (Van Aken, 2021). ROS signals can be transmitted through contact sites between different organelles and from cell to cell through the apoplast and plasmodesmata (PD) (Considine & Foyer, 2020; Fichman *et al.*, 2021). Changes in mitochondrial ROS production in one cell may then trigger coordinated responses in adjacent cells (Fig. 1).

In addition, mitochondria and chloroplasts regulate cell-to-cell communication via PD, probably through changes in the redox state (Stonebloom *et al.*, 2009) and organellar signals control PD formation (Ganusova *et al.*, 2020; Dmitrieva *et al.*, 2021). These findings have contributed to the hypothesis of organelle–nucleus–PD signalling (ONPS), critical for controlling plant development in response to environmental signals and stresses (Azim & Burch-Smith, 2020). The authors postulated that acquisition of the ONPS was instrumental for the evolution of plants to allow the coordination of responses to different cues. In this sense, it was recently demonstrated that an increase in ATP and NADPH levels regulates PD closure (Dmitrieva *et al.*, 2021). Therefore, changes in mitochondrial metabolism may affect plant functions in a cell nonautonomous manner through the regulation of PD transport. Mitochondria and PD may also be linked through the activity of the TARGET OF RAPAMYCIN (TOR) energy signalling pathway (Brunkard, 2020). TOR regulates cell-to-cell transport through PD and mitochondrial energy production is required for TOR activation.

Extracellular vesicles (EVs) carrying mitochondrial components or even entire mitochondria have been observed in animals (Liu *et al.*, 2020). EVs are formed under physiological conditions and after oxidative stress and it was proposed that they participate in cell-to-cell metabolic regulation and the modulation of immune responses. For instance, intercellular transfer of mitochondria has been shown to restore respiratory capacity in deficient cancer or neuronal cells (Hayakawa *et al.*, 2016; Dong *et al.*, 2017). In addition, cellular injury releases mitochondrial *N*-formyl peptides and mitochondrial DNA (mtDNA) into circulation with functionally important immune consequences (Zhang *et al.*, 2010). Release of mtDNA into the extracellular milieu also activates multiple inflammatory pathways (Riley & Tait, 2020). Recent evidence has indicated that selective mitochondrial proteins are incorporated into EVs under physiological conditions and that this process depends on the formation of mitochondria-derived vesicles

(MDVs), involved in intracellular transfer of mitochondrial components to other cellular locations (Todkar *et al.*, 2021). Notably, MDVs with oxidised mitochondrial components do not form EVs and are instead targeted to lysosomes for degradation, possibly as a mechanism to prevent the transfer of damaged proteins outside the cell (Todkar *et al.*, 2021).

Mitochondrial vesicle-like structures have also been observed during dark-induced leaf senescence in Arabidopsis, but transfer of these vesicles between cells was not demonstrated (Yamashita *et al.*, 2016). Cell-to-cell movement of entire mitochondria was observed in grafting experiments with two tobacco species, an alloplasmic male-sterile *N. tabacum* variety and fertile *N. sylvestris* (Gurdon *et al.*, 2016). In this case, mobility was assessed by restoration of fertility by the transmission of fertile mitochondrial genome from *N. sylvestris* to *N. tabacum* in plants obtained after culturing tissue from the graft zone. This showed that mitochondrial transmission between cells is possible, but the frequency of these events is difficult to assess. Using grafting experiments, Hertle *et al.* (2021) also detected highly motile amoeboid-like plastid structures moving through connective pores established between neighbouring cells as a possible mechanism for horizontal genome transfer. In this study, mitochondria were also frequently observed within the pores, but the transfer of individual mitochondria between cells could not be assessed due to the highly dynamic nature of these organelles (Fig. 1). In summary, the intercellular transfer of vesicles containing mitochondrial components in plants, as observed in animals, remains a possibility, but clear evidence about the existence of these vesicles and their possible roles is lacking.

III. Long-distance signalling

Hormones and energy-regulating pathways

It has been well established that modifications in mitochondrial function affect the activity of several hormonal pathways (Berkowitz *et al.*, 2016; Welchen *et al.*, 2021, and references therein). Even if this may be distinct from the general concept of long-distance signaling, it is evident that changes in mitochondrial activity may be transmitted over long distances by modifying these hormonal and associated energy-regulating pathways. Most explored is the connection between mitochondrial activity and auxins, in which changes in mitochondrial activity affect plant growth through changes in auxin levels and/or responses (Ohbayashi *et al.*, 2019; Gras *et al.*, 2020). It is highly suggestive that three REGULATORS OF AOX1a (RAO) proteins are auxin transporters located in the ER membrane (Ivanova *et al.*, 2014), a place where auxin-mediated responses are regulated (Middleton *et al.*, 2018). Mitochondria and auxin may also be connected through the activity of energy sensing pathways. The catalytic subunit of the stress and energy sensor SUCROSE NON-FERMENTING1 (Snf1)-RELATED PROTEIN KINASE 1 (SnRK1) dynamically localises between the nucleus and the ER (Blanco *et al.*, 2019) and affects both mitochondrial activity and auxin-mediated pathways (Simon *et al.*, 2018). SnRK1 antagonises the TOR pathway, which in turn is regulated by auxins and mitochondrial activity (Brunkard, 2020) (Fig. 1). These complex interactions establish a scenario that places mitochondria as pivots between auxins and growth and stress regulatory kinases (Box 1).

Perturbation of mitochondrial proteostasis triggers the UPR^{mt} that involves changes in ethylene signalling, in addition to auxin, and effects on plant development and ageing (Wang & Auwerx, 2017). Mitochondrial dysfunction also triggers an ethylene-like response during early seedling growth in darkness (Merendino *et al.*, 2020). Notably, this response and the UPR^{mt} are mediated by the ER-anchored transcription factor ANAC017 (Kacprzak *et al.*, 2020) that participates in the classical retrograde signalling pathway.

A change in the amount of the mitochondrial electron transport chain (mETC) component CYTOCHROME C (CYT*c*) was also shown in Arabidopsis to alter the levels of gibberellins and, through this, the rate of vegetative growth and starch accumulation (Racca *et al.*, 2018). It is highly likely that changes in hormone distribution brought about by mitochondrial alterations act cell nonautonomously to regulate plant growth. Although there has been no clear evidence that CYT*c* is involved in early events that trigger programmed cell death in plants, CYT*c* release from mitochondria as a consequence of oxidative damage was recently connected to this event (Elena-Real *et al.*, 2020; Matilla, 2021). Due to its reduced size, about 12 kDa, there is a possibility that CYT*c* also translocates to adjacent cells through PD when released from mitochondria, although this has not been experimentally demonstrated.

Many pieces of evidence also exist connecting mitochondrial proteins to resistance against pathogen infection and biotic stress, and on the role of mitochondria in modifying salicylic acid (SA) levels or responses (Colombatti *et al.*, 2014; Belt *et al.*, 2017; Mencia *et al.*, 2020). SA is a main player in the build-up of the systemic acquired resistance observed in plants after infection with biotrophic pathogens. Changes in mitochondrial activity at the site of pathogen infection may then affect systemic responses to pathogen challenge. There have been several examples of pathogen effectors that are targeted to mitochondria to affect the immune response of the plant (Colombatti *et al.*, 2014; Sperschneider *et al.*, 2017).

In animal models, cell nonautonomous effects of mitochondrial perturbations have been 'elegantly' described (Box 2). Durieux *et al.* (2011) showed that the knockdown of components of the mETC in specific cells increased organismal longevity (Fig. 2a). Similarly, a mechanism based on the specific translational inhibition of the CYT*c* in germline cells that causes a lifespan extension has been described (Lan *et al.*, 2019). In both cases, this was related to the induction of the UPR^{mt} in distant tissues and the release of systemic signals generally termed mitokines, from cells with dysfunctional mitochondria (Fig. 2). Similarly, perturbation of protein homeostasis causes the UPR^{mt} response in plants that is associated with the activation of hormonal signals (Wang & Auwerx, 2017). Whether perturbations in specific tissues trigger responses in distant tissues was not evaluated in this case. Available evidence has indicated that certain hormones, such as auxin, ethylene and SA, affect mitochondrial metabolism, MRR and ROS production (Ivanova *et al.*, 2014; Belt *et al.*, 2017; Wang & Auwerx, 2017; Mencia *et al.*, 2020; Jurdak *et al.*, 2021). As these hormones also respond to mitochondrial perturbations, they may

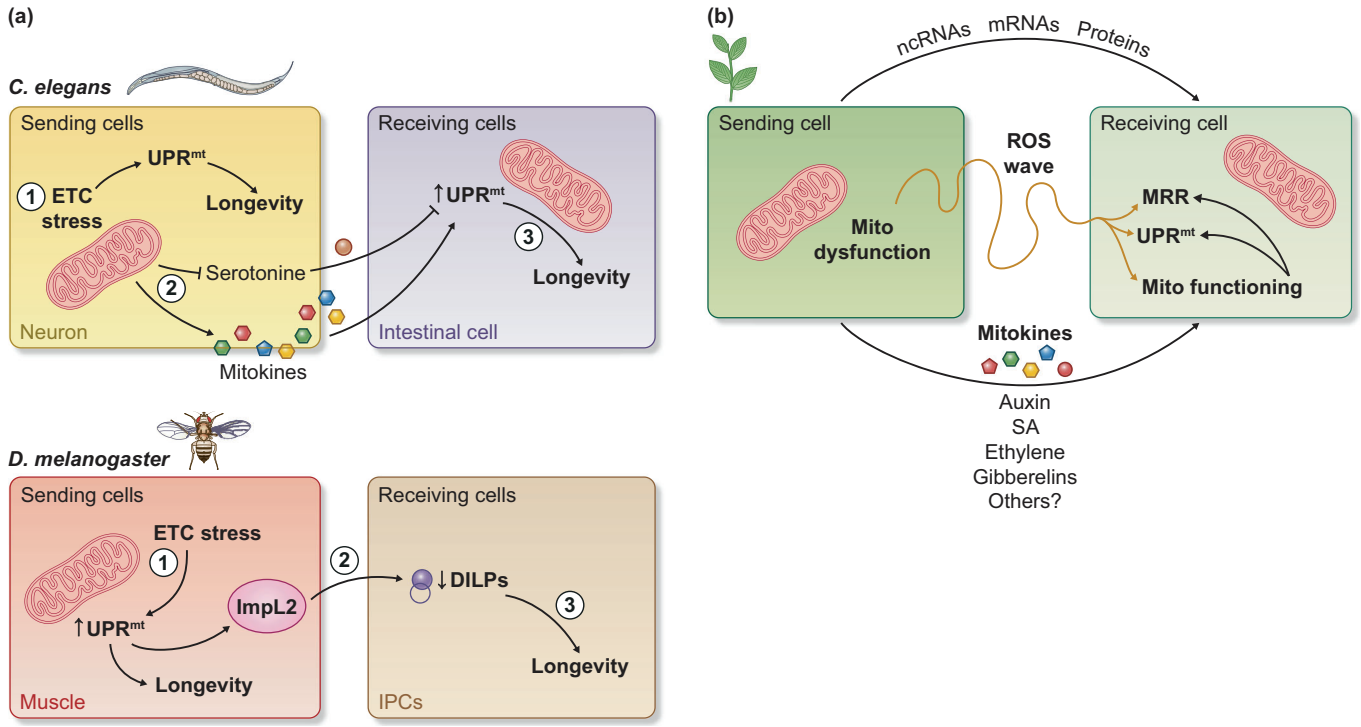

Fig. 2 Long-distance signalling: from sending to receiving cells. (a) Examples of cell non-autonomous mitochondrial signalling in *Caenorhabditis elegans* and *Drosophila melanogaster*. Mitochondrial stress (ETC stress) in 'sending cells' induces the unfolded protein response (UPR^{mt}) (1) that triggers the generation of mitokines or other signalling molecules (the neurotransmitter serotonin or the antagonist of insulin signaling ImpL2) (2), that travel to 'receiving cells' to produce a long-distance response (3). In the example of *C. elegans*, mitochondrial dysfunction in sending cells triggers a similar response (UPR^{mt}) in receiving cells. In the example of *D. melanogaster*, mitochondrial dysfunction in sending cells exerts changes in insulin (DILPs; *Drosophila* insulin-like peptides)-producing cells (IPCs), thus modifying hormonal homeostasis systemically. Modified from Miller *et al*. (2020). (b) By analogy, mitochondrial (Mito) dysfunction in 'sending cells' may be transmitted to 'receiving cells' in plants through hormones acting as plant mitokines, ROS waves (represented as a yellow wavy arrow) and/or mobile RNAs and proteins. Mitochondria can induce stress and retrograde signals by an unfolded protein response (UPR^{mt}) and mitochondrial retrograde responses (MRR) in receiving cells. This scheme is based on published literature showing the existence of multiple connections between mitochondria and different signalling pathways (see text for details). Apparent difference in width of the arrows is not significant.

be operative as plant mitokines to relay mitochondrial signals to distant tissues (Fig. 2b).

siRNAs, miRNAs, mRNAs and proteins

In addition to chemical messengers, other forms of long-distance communication exist in plants to coordinate growth and development. Mobile transcription factors and proteins regulating growth in response to external stimuli have been described (Miyashima *et al*., 2019; Jiang *et al*., 2020), but no examples of mitochondrial proteins moving between cells are available. In addition, the ability of siRNAs, miRNAs and mRNAs to move over long distances through PD and the phloem stream is well known (Maizel *et al*., 2020). Thieme *et al*. (2015) identified *c*. 2000 protein-coding transcripts that are translocated to distant tissues both from shoot to root and *vice versa* and proposed that mRNA mobility may constitute a specialised signal influenced by plant nutrients. Evidence for translation of the moving mRNAs at distant sites was also obtained, indicating their functionality. Gene ontology analysis identified 'mitochondrion' as an enriched term in the population of mobile transcripts (Thieme *et al*., 2015). Mobile mRNAs encoding mitochondrial proteins involved in RNA metabolism, ribosomal proteins, mETC and protein import machinery components, protein chaperones, and enzymes involved in mitochondrial carbon and redox metabolisms have been identified. Of special interest are those encoding ALTERNATIVE OXIDASE 1a (AOX1a), the alternative oxidase isoform involved in MRR and stress responses, OUTER MITOCHONDRIAL MEMBRANE PROTEIN of 66 kDa (OM66), an outer membrane protein that participates in cell death, pathogen responses and amplification of SA signalling (Zhang *et al*., 2014), MICU, a regulator of a mitochondrial transporter involved in calcium signalling (Wagner *et al*., 2015), and INCREASED SIZE EXCLUSION LIMIT 1 (ISE1), a DEAD-box helicase involved in regulating transport through PD (Stonebloom *et al*., 2009). Moreover, mRNAs for the Arabidopsis NAC domain transcription factor ANAC017, the ER-tethered transcription factor involved in triggering the MRR and other stress responses mentioned before (Ng *et al*., 2013), and WRKY DNA-BINDING PROTEIN40 (WRKY40), involved in the coordination of the expression of stress responsive genes encoding chloroplast and mitochondrial proteins (Van Aken *et al*., 2013), were also found among the mobile mRNAs. The biological significance of the long-distance transport of these mRNAs remains unexplored.

ROS waves and spread signals

ROS signals can also be transmitted systemically through the so-called 'ROS wave' (Fichman & Mittler, 2020) that is accompanied by a change in cytosolic redox conditions in distant cells (Fichman & Mittler, 2021). This implies the participation of electrical signals, ion movements, hormones, and also mobile proteins and RNAs. ROS long-distance signalling is dependent on ROS generated in the apoplast by the plasma membrane enzyme RESPIRATORY BURST OXIDASE HOMOLOG D (RBOHD) (Miller *et al.*, 2009). It has been proposed that a communication between RBOHD and ROS-producing organelles, like chloroplasts, peroxisomes and mitochondria, may be established in plant cells, as observed in animals for mitochondria and plasma membrane NADPH oxidases (Zandalinas & Mittler, 2018). Changes in mitochondrial PROLINE DEHYDROGENASE, an enzyme linked to the mETC, affect ROS production by RBOHD and signalling during biotic stress (Fabro *et al.*, 2016). In addition, changes in mitochondrial redox state have been detected after elicitor-induced ROS production by RBHOD (Nietzel *et al.*, 2019). These results indicated that mitochondrial function may indeed be connected to ROS signalling through RBOHD. In addition, recent evidence has suggested that these rapid systemic signals can spread not only through plant vascular bundles but also through mesophyll cells, triggering systemic acquired acclimation (SAA) and safeguarding growth and defence responses (Zandalinas & Mittler, 2021). ROS also increase intercellular transport though PD and this is required for the propagation of the systemic signal (Fichman *et al.*, 2021). As mentioned above, mitochondria are also connected to PD function and may then also influence this process. It was also suggested that different types of stresses trigger different types of signals, therefore conferring specificity to the response (Zandalinas & Mittler, 2021). ROS carry information by themselves, but amplify their power by promoting post-translational modifications of key proteins that regulate plant growth and developmental and stress responses (Considine & Foyer, 2020; Møller *et al.*, 2020; Nietzel *et al.*, 2020; Bailly & Merendino, 2021). The connection of mitochondrial ROS to the propagation of the ROS wave deserves further investigation. It has been well established that mitochondrial changes influence plant stress responses (Box 1) (Welchen *et al.*, 2021), but if this is a local or a systemic effect has not been evaluated.

IV. Conclusion

For the establishment and growth of complex sessile organisms such as plants, an exquisite coordination of mitochondrial function with other cell compartments, mainly the chloroplast and the nucleus, must exist. The existence of multiple interconnections that allow this coordination has been firmly established. As plants are multicellular organisms, it makes sense to also postulate the existence of signalling mechanisms that operate beyond cell boundaries, either over relatively short or long distances. From the transmission of entire mitochondria to the modification of hormonal pathways, which *per se* participate in cell-to-cell signalling mechanisms, available evidence has indicated the existence of cell nonautonomous effects of changes in mitochondrial function. Grafting techniques, as well as the effects of altering mitochondrial components in specific cell types or tissues, may be useful to ascertain the extent and physiological roles of such effects and their participation in systemic acclimation responses (Box 2). Questions also remain open concerning the nature of the systemic signals that would be triggered in every situation. Are ROS, the redox state, the energy state and chemical or electrical signals, post-translational modifications of proteins, mobile RNAs or proteins, and/or hormonal signals involved? It will be challenging to determine how motile organelles such as mitochondria regulate growth, development and stress responses in sessile organisms such as plants.

Acknowledgements

Work from our laboratory has been supported by grants from Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina (PICT2017-2340 to EW and PICT2018-01439 to DHG) and Universidad Nacional del Litoral (CAI+D 50620190100067LI to EW). EW and DHG are members of CONICET. We apologise to our colleagues whose relevant work was not cited due to space limitations.

ORCID

Daniel H. Gonzalez https://orcid.org/0000-0002-3137-8095
Elina Welchen https://orcid.org/0000-0003-4025-573X

References

Azim MF, Burch-Smith TM. 2020. Organelles-nucleus-plasmodesmata signaling (ONPS): an update on its roles in plant physiology, metabolism and stress responses. *Current Opinion in Plant Biology* **58**: 48–59.

Bailly C, Merendino L. 2021. Oxidative signalling in seed germination and early seedling growth: an emerging role for ROS trafficking and inter-organelle communication. *Biochemical Journal* **478**: 1977–1984.

Belt K, Huang S, Thatcher LF, Casarotto H, Singh KB, Van Aken O, Millar AH. 2017. Salicylic acid-dependent plant stress signaling via mitochondrial succinate dehydrogenase. *Plant Physiology* **173**: 2029–2040.

Berendzen KM, Durieux J, Shao LW, Tian Y, Kim HE, Wolff S, Liu Y, Dillin A. 2016. Neuroendocrine coordination of mitochondrial stress signaling and proteostasis. *Cell* **166**: 1553–1563.

Berkowitz O, De Clercq I, Van Breusegem F, Whelan J. 2016. Interaction between hormonal and mitochondrial signalling during growth, development and in plant defence responses. *Plant, Cell & Environment* **39**: 1127–1139.

Blanco NE, Liebsch D, Díaz MG, Strand Å, Whelan J. 2019. Dual and dynamic intracellular localization of *Arabidopsis thaliana* SnRK1.1. *Journal of Experimental Botany* **70**: 2325–2338.

Brioudes F, Jay F, Sarazin A, Grentzinger T, Devers EA, Voinnet O. 2021. HASTY, the Arabidopsis EXPORTIN5 ortholog, regulates cell-to-cell and vascular microRNA movement. *EMBO Journal* **6**: e107455.

Brunkard JO. 2020. Exaptive evolution of target of rapamycin signaling in multicellular eukaryotes. *Developmental Cell* **54**: 142–155.

Colombatti F, Gonzalez DH, Welchen E. 2014. Plant mitochondria under pathogen attack: a sigh of relief or a last breath? *Mitochondrion* **19**: 238–244.

Considine MJ, Foyer CH. 2020. Oxygen and reactive oxygen species-dependent regulation of plant growth and development. *Plant Physiology* **186**: 79–92.

De Clercq I, Vermeirssen V, Van Aken O, Vandepoele K, Murcha MW, Law SR, Inzé A, Ng S, Ivanova A, Rombaut D *et al.* 2013. The membrane-bound NAC transcription factor ANAC013 functions in mitochondrial retrograde regulation of the oxidative stress response in *Arabidopsis*. *Plant Cell* **25**: 3472–3490.

Decaestecker W, Buono RA, Pfeiffer ML, Vangheluwe N, Jourquin J, Karimi M, Van Isterdael G, Beeckman T, Nowack MK, Jacobs TB. 2019. CRISPR-TSKO: a technique for efficient mutagenesis in specific cell types, tissues, or organs in Arabidopsis. *Plant Cell* **31**: 2868–2887.

Dmitrieva VA, Domashkina VV, Ivanova AN, Sukhov VS, Tyutereva EV, Voitsekhovskaja OV. 2021. Regulation of plasmodesmata in Arabidopsis leaves: ATP, NADPH and chlorophyll b levels matter. *Journal of Experimental Botany.* **11**: erab205.

Dong L-F, Kovarova J, Bajzikova M, Bezawork-Geleta A, Svec D, Endaya B, Sachaphibulkij K, Coelho AR, Sebkova N, Ruzickova A *et al.* 2017. Horizontal transfer of whole mitochondria restores tumorigenic potential in mitochondrial DNA-deficient cancer cells. *eLife* **6**: e22187.

Durieux J, Wolff S, Dillin A. 2011. The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* **144**: 79–91.

Elena-Real CA, González-Arzola K, Pérez-Mejías G, Díaz-Quintana A, Velázquez-Campoy A, Desvoyes B, Gutiérrez C, De la Rosa MA, Díaz-Moreno I. 2020. Proposed mechanism for regulation of H_2O_2-induced programmed cell death in plants by binding of cytochrome c to 14-3-3 proteins. *The Plant Journal* **106**: 74–85.

Fabro G, Rizzi YS, Alvarez ME. 2016. Arabidopsis proline dehydrogenase contributes to Flagellin-Mediated PAMP-Triggered immunity by Affecting RBOHD. *Molecular Plant–Microbe Interactions* **29**: 620–628.

Fichman Y, Mittler R. 2020. Rapid systemic signaling during abiotic and biotic stresses: is the ROS wave master of all trades? *The Plant Journal* **102**: 887–896.

Fichman Y, Mittler R. 2021. A systemic whole-plant change in redox levels accompanies the rapid systemic response to wounding. *Plant Physiology* **186**: 4–8.

Fichman Y, Myers RJ, Grant DAG, Mittler R. 2021. Plasmodesmata-localized proteins and ROS orchestrate light-induced rapid systemic signaling in Arabidopsis. *Science Signaling* **14**: 322.

Frank MH, Chitwood DH. 2016. Plant chimeras: the good, the bad, and the 'Bizzaria'. *Developmental Biology* **419**: 41–53.

Ganusova EE, Reagan BC, Fernandez JC, Azim MF, Sankoh AF, Freeman KM, McCray TN, Patterson K, Kim C, Burch-Smith TM. 2020. Chloroplast-to-nucleus retrograde signalling controls intercellular trafficking via plasmodesmata formation. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**: 20190408.

García L, Welchen E, Gey U, Arce AL, Steinebrunner I, Gonzalez DHD, Garcia L, Welchen E, Gey U, Arce AL *et al.* 2016. The cytochrome c oxidase biogenesis factor AtCOX17 modulates stress responses in Arabidopsis. *Plant, Cell & Environment* **39**: 628–644.

Gras DE, Mansilla N, Rodríguez C, Welchen E, Gonzalez DH. 2020. Arabidopsis thaliana SURFEIT1-like genes link mitochondrial function to early plant development and hormonal growth responses. *The Plant Journal* **103**: 690–704.

Gurdon C, Svab Z, Feng Y, Kumar D, Maliga P. 2016. Cell-to-cell movement of mitochondria in plants. *Proceedings of the National Academy of Sciences, USA* **113**: 3395–3400.

Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH. 2016. Transfer of mitochondria from astrocytes to neurons after stroke. *Nature* **535**: 551–555.

Hertle AP, Haberl B, Bock R. 2021. Horizontal genome transfer by cell-to-cell travel of whole organelles. *Science Advances* **7**: eabd8215.

Ivanova A, Law SR, Narsai R, Duncan O, Lee JH, Zhang B, Van Aken O, Radomiljac JD, van der Merwe M, Yi KK *et al.* 2014. A functional antagonistic relationship between auxin and mitochondrial retrograde signaling regulates alternative oxidase1a expression in arabidopsis. *Plant Physiology* **165**: 1233–1254.

Jaipargas EA, Barton KA, Mathur N, Mathur J. 2015. Mitochondrial pleomorphy in plant cells is driven by contiguous ER dynamics. *Frontiers in Plant Science* **6**: 783.

Jiang X, Xu J, Lin R, Song J, Shao S, Yu J, Zhou Y. 2020. Light-induced HY5 functions as a systemic signal to coordinate the photoprotective response to light fluctuation. *Plant Physiology* **184**: 1181–1193.

Jurdak R, Launay-Avon A, Paysant-Le Roux C, Bailly C. 2021. Retrograde signalling from the mitochondria to the nucleus translates the positive effect of ethylene on dormancy breaking of *Arabidopsis thaliana* seeds. *New Phytologist* **229**: 2192–2205.

Kacprzak SM, Dahlqvist A, Van Aken O. 2020. The transcription factor ANAC017 is a key regulator of mitochondrial proteotoxic stress responses in plants. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**: 20190411.

Lan J, Rollins JA, Zang X, Wu Di, Zou L, Wang Zi, Ye C, Wu Z, Kapahi P, Rogers AN *et al.* 2019. Translational regulation of non-autonomous mitochondrial stress response promotes longevity. *Cell Reports* **28**: 1050–1062.e6.

Lane N. 2017. Serial endosymbiosis or singular event at the origin of eukaryotes? *Journal of Theoretical Biology* **434**: 58–67.

Liu D, Dong Z, Wang J, Tao Y, Sun X, Yao X. 2020. The existence and function of mitochondrial component in extracellular vesicles. *Mitochondrion* **54**: 122–127.

Liu Q, Galli M, Liu X, Federici S, Buck A, Cody J, Labra M, Gallavotti A. 2019. NEEDLE1 encodes a mitochondria localized ATP-dependent metalloprotease required for thermotolerant maize growth. *Proceedings of the National Academy of Sciences, USA* **116**: 19736–19742.

Maizel A, Markmann K, Timmermans M, Wachter A. 2020. To move or not to move: roles and specificity of plant RNA mobility. *Current Opinion in Plant Biology* **57**: 52–60.

Mansilla N, Welchen E, Gonzalez DH. 2019. Arabidopsis SCO proteins oppositely influence cytochrome c oxidase levels and gene expression during salinity stress. *Plant & Cell Physiology* **60**: 2769–2784.

Mathur J. 2021. Organelle extensions in plant cells. *Plant Physiology* **185**: 593–607.

Matilla AJ. 2021. Cellular oxidative stress in programmed cell death: focusing on chloroplastic $^1O_2$ and mitochondrial cytochrome-c release. *Journal of Plant Research* **134**: 179–194.

Mencia R, Céccoli G, Fabro G, Torti P, Colombatti F, Ludwig-Müller J, Alvarez ME, Welchen E. 2020. OXR2 increases plant defense against a hemibiotrophic pathogen via the salicylic acid pathway. *Plant Physiology* **184**: 1112–1127.

Merendino L, Courtois F, Grübler B, Bastien O, Straetmanns V, Chevalier F, Lerbs-Mache S, Lurin C, Pfannschmidt T. 2020. Retrograde signals from mitochondria reprogramme skoto-morphogenesis in *Arabidopsis thaliana* via alternative oxidase 1a. *Philosophical Transactions of the Royal Society B: Biological Sciences* **375**: 20190567.

Middleton AM, Dal Bosco C, Chlap P, Bensch R, Harz H, Ren F, Bergmann S, Wend S, Weber W, Hayashi K-I *et al.* 2018. Data-driven modeling of intracellular auxin fluxes indicates a dominant role of the ER in controlling nuclear auxin uptake. *Cell Reports* **22**: 3044–3057.

Miller G, Schlauch K, Tam R, Cortes D, Torres MA, Shulaev V, Dangl JL, Mittler R. 2009. The plant NADPH oxidase RBOHD mediates rapid systemic signaling in response to diverse stimuli. *Science Signaling* **2**: ra45.

Miller HA, Dean ES, Pletcher SD, Leiser SF. 2020. Cell non-autonomous regulation of health and longevity. *eLife* **9**: 1–28.

Miyashima S, Roszak P, Sevilem I, Toyokura K, Blob B, Heo J-o, Mellor N, Help-Rinta-Rahko H, Otero S, Smet W *et al.* 2019. Mobile PEAR transcription factors integrate positional cues to prime cambial growth. *Nature* **565**: 490–494.

Møller IM, Igamberdiev AU, Bykova NV, Finkemeier I, Rasmusson AG, Schwarzländer M. 2020. Matrix redox physiology governs the regulation of plant mitochondrial metabolism through posttranslational protein modifications. *Plant Cell* **32**: 573–594.

Ng S, Ivanova A, Duncan O, Law SR, Van Aken O, De Clercq I, Wang Y, Carrie C, Xu L, Kmiec B *et al.* 2013. A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in Arabidopsis. *Plant Cell* **25**: 3450–3471.

Nietzel T, Elsässer M, Ruberti C, Steinbeck J, Ugalde JM, Fuchs P, Wagner S, Ostermann L, Moseler A, Lemke P *et al.* 2019. The fluorescent protein sensor roGFP2-Orp1 monitors *in vivo* H_2O_2 and thiol redox integration and elucidates intracellular H_2O_2 dynamics during elicitor-induced oxidative burst in Arabidopsis. *New Phytologist* **221**: 1649–1664.

Nietzel T, Mostertz J, Ruberti C, Née G, Fuchs P, Wagner S, Moseler A, Müller-Schüssele SJ, Benamar A, Poschet G *et al.* 2020. Redox-mediated kick-start of mitochondrial energy metabolism drives resource-efficient seed germination. *Proceedings of the National Academy of Sciences, USA* **117**: 741–751.

Ohbayashi I, Huang S, Fukaki H, Song X, Sun S, Morita MT, Tasaka M, Millar AH, Furutani M. 2019. Mitochondrial pyruvate dehydrogenase contributes to auxin-regulated organ development. *Plant Physiology* **180**: 896–909.

Oikawa K, Imai T, Thagun C, Toyooka K, Yoshizumi T, Ishikawa K, Kodama Y, Numata K. 2021. Mitochondrial movement during its association with chloroplasts in *Arabidopsis thaliana*. *Communications Biology* **4**: 1–13.

Owusu-Ansah E, Song W, Perrimon N. 2013. Muscle mitohormesis promotes longevity via systemic repression of insulin signaling. *Cell* **155**: 699–712.

Racca S, Welchen E, Gras DE, Tarkowská D, Turečková V, Maurino VG, Gonzalez DH. 2018. Interplay between cytochrome *c* and gibberellins during Arabidopsis vegetative development. *The Plant Journal* **94**: 105–121.

Riley JS, Tait SW. 2020. Mitochondrial DNA in inflammation and immunity. *EMBO Reports* **21**: e49799.

Simon NML, Kusakina J, Fernández-López Á, Chembath A, Belbin FE, Dodd AN. 2018. The energy-signaling Hub SnRK1 is important for sucrose-induced hypocotyl elongation. *Plant Physiology* **176**: 1299–1310.

Sperschneider J, Catanzariti AM, Deboer K, Petre B, Gardiner DM, Singh KB, Dodds PN, Taylor JM. 2017. LOCALIZER: subcellular localization prediction of both plant and effector proteins in the plant cell. *Scientific Reports* **7**: 1–14.

Stonebloom S, Burch-Smith T, Kim I, Meinke D, Mindrinos M, Zambryski P. 2009. Loss of the plant DEAD-box protein ISE1 leads to defective mitochondria and increased cell-to-cell transport via plasmodesmata. *Proceedings of the National Academy of Sciences, USA* **106**: 17229–17234.

Thieme CJ, Rojas-Triana M, Stecyk E, Schudoma C, Zhang W, Yang L, Miñambres M, Walther D, Schulze WX, Paz-Ares J *et al.* 2015. Endogenous Arabidopsis messenger RNAs transported to distant tissues. *Nature Plants* **1**: 1–9.

Todkar K, Chikhi L, Desjardins V, El-Mortada F, Pépin G, Germain M. 2021. Selective packaging of mitochondrial proteins into extracellular vesicles prevents the release of mitochondrial DAMPs. *Nature Communications* **12**: 1971.

Tran HC, Van Aken O. 2020. Mitochondrial unfolded protein-related responses across kingdoms: similar problems, different regulators. *Mitochondrion* **53**: 166–177.

Van Aken O. 2021. Mitochondrial redox systems as central hubs in plant metabolism and signaling. *Plant Physiology* **186**: 36–52.

Van Aken O, Pečenková T, Van De Cotte B, De Rycke R, Eeckhout D, Fromm H, De Jaeger G, Witters E, Beemster GTS, Inzé D *et al.* 2007. Mitochondrial type-I prohibitins of Arabidopsis thaliana are required for supporting proficient meristem development. *The Plant Journal* **52**: 850–864.

Van Aken O, Zhang B, Law S, Narsai R, Whelan J. 2013. AtWRKY40 and AtWRKY63 modulate the expression of stress-responsive nuclear genes encoding mitochondrial and chloroplast proteins. *Plant Physiology* **162**: 254–271.

Wagner S, Behera S, De Bortoli S, Logan DC, Fuchs P, Carraretto L, Teardo E, Cendron L, Nietzel T, Füßl M *et al.* 2015. The EF-Hand Ca2+ binding protein MICU choreographs mitochondrial Ca^{2+} dynamics in Arabidopsis. *Plant Cell* **27**: 3190–3212.

Wang W, Zhang X, Niittylä T. 2019. OPENER is a nuclear envelope and mitochondria localized protein required for cell cycle progression in arabidopsis. *Plant Cell* **31**: 1446–1465.

Wang X, Auwerx J. 2017. Systems phytohormone responses to mitochondrial proteotoxic stress. *Molecular Cell* **68**: 540–551.e5.

Welchen E, Canal MV, Gras DE, Gonzalez DH. 2021. Cross-talk between mitochondrial function, growth, and stress signalling pathways in plants. *Journal of Experimental Botany* **72**: 4102–4118.

Welchen E, García L, Mansilla N, Gonzalez DH. 2014. Coordination of plant mitochondrial biogenesis: keeping pace with cellular requirements. *Frontiers in Plant Science* **4**: 551.

Welchen E, Schmitz J, Fuchs P, García L, Wagner S, Wienstroer J, Schertl P, Braun HP, Schwarzländer M, Gonzalez DH *et al.* 2016. D-lactate dehydrogenase links methylglyoxal degradation and electron transport through cytochrome. *Plant Physiology* **172**: 901–912.

White RR, Lin C, Leaves I, Castro IG, Metz J, Bateman BC, Botchway SW, Ward AD, Ashwin P, Sparkes I. 2020. Miro2 tethers the ER to mitochondria to promote mitochondrial fusion in tobacco leaf epidermal cells. *Communications Biology* **3**: 1–8.

Yamashita A, Fujimoto M, Katayama K, Yamaoka S, Tsutsumi N, Arimura S. 2016. Formation of mitochondrial outer membrane derived protrusions and vesicles in *Arabidopsis thaliana*. *PLoS ONE* **11**: e0146717.

Zandalinas SI, Mittler R. 2018. ROS-induced ROS release in plant and animal cells. *Free Radical Biology and Medicine* **122**: 21–27.

Zandalinas SI, Mittler R. 2021. Vascular and non-vascular transmission of systemic reactive oxygen signals during wounding and heat stress. *Plant Physiology* **186**: 1721–1733.

Zhang B, Van Aken O, Thatcher L, De Clercq I, Duncan O, Law SR, Murcha MW, van der Merwe M, Seifi HS, Carrie C *et al.* 2014. The mitochondrial outer membrane AAA ATPase AtOM66 affects cell death and pathogen resistance in *Arabidopsis thaliana*. *The Plant Journal* **80**: 709–727.

Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, Hauser CJ. 2010. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* **464**: 104–107.