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Cardiolipin at the heart of stress response across kingdoms

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Cardiolipin is a key phospholipid most specifically found in the membrane of mitochondria in yeasts, plants, and animals. Cardiolipins are essential for the maintenance, the integrity, and the dynamics of mitochondria. In most eukaryotes mitochondria play a central role in the response and adaptation to stress conditions especially through their importance in the control of programmed cell death. To assess the impact of the absence of cardiolipin, knock-down of the expression of cardiolipin synthase, the last enzyme of cardiolipin synthesis pathway in eukaryotes has been performed in yeasts, animals, and plants. These studies showed that cardiolipin is not only important for mitochondrial ultrastructure and for the stability of respiratory complexes, but it is also a key player in the response to stress, the formation of reactive oxygen species, and the execution of programmed cell death.

CLS mutants

Cardiolipins are dimeric anionic phospholipids composed of 4 acyl chains and are mostly found in the internal membranes of bacteria and mitochondria.[1,2] In eukaryotes mitochondria CLs are involved in membrane curvature and cristae formation.[2] CLs have been shown to bind several proteins of the internal mitochondrial membrane, including subunits of complexes of the respiratory chain, carrier family proteins (ADP-ATP-carrier, phosphate carrier, uncoupling protein), and other peripheral membrane proteins in yeasts and mammals.[2-6]

In eukaryotes, the ultimate step of CL synthesis is catalyzed from phosphatidyl-CMP and phosphatidylglycerol (PG) by cardiolipin synthase (CLS).[7,8] As the *CLS* gene is present in a single copy in yeasts,[9] *Arabidopsis*,[10,11] and mammals,[12] several *cls* mutants have been characterized. In yeast, *CLS* disrupted mutants (named *crd1*) are viable but show temperature-sensitive colony formation and multiple mitochondrial defects including impaired electron transport by the mitochondrial respiratory chain (MRC, **Figure 1**) and alterations of both the oxidative phosphorylation activities and the mitochondrial membrane potential.[6,13] In *Drosophila melanogaster* CLs are essential for physiological ATP synthesis as a *cls* mutant obtained by transposon insertion in the *CLS* gene displays impaired MRC functioning linked to the assembly and stability of respiratory supercomplexes. Indeed, this mutant exhibits dimerization of the ATP synthase, decreased respiratory activity and altered organization of the cristae membrane.[4] In cells from Barth syndrome patients, reduced CL content leads to mitochondrial dysfunction and pathological conditions.[14,15] Very recently, *cls* T-DNA insertion knockdown[16] and knockout[17] mutants have been characterized in *Arabidopsis*. At*cls* mutants harbor some of the features of yeast and mammal CL-deficient cells, such as low abundance of CI/CIII supercomplexes and abnormal mitochondria with few cristae.[16,17] Moreover giant mitochondria were observed suggesting deficiency of the plant mitochondrial fusion/fission machinery,[16,17] confirming previous results obtained in animals and yeast.[18-20] More specifically it was found that CL plays a dominant role in mitochondrial fission through the major mitochondrial fission factor, DYNAMIN-RELATED PROTEIN3 (DRP3).[16] These reports highlight the central role of CL in maintaining mitochondrial structure and function in yeast, plants, and animals. However, in contrast to yeast *crd1* mutant, At*cls* seedlings are severely affected, both in their growth and their development,[16,17] suggesting a broader role for CL in plant cell functioning.

Cardiolipin-mediated response to stress

In CL-deficient cells, several phenotypes involving mitochondria and the response to stress have been described. In animals it was found that CL externalization to the outer mitochondrial membrane promotes mitophagy (**Fig. 1**), indicating that redistribution of cardiolipin during stress response serves as a signal for the recycling of unwanted mitochondria in neuronal cells.[21] At elevated temperature, the yeast *crd1* mutant exhibits various vacuolar defects, demonstrating the existence of a mitochondria-vacuole signaling pathway dependent on CL synthesis.[22]

In the *Arabidopsis cls* mutant, the absence of CL leads to an important perturbation of mitochondrial metabolism, that results in an increase of the levels of tricarboxylic acid (TCA) derived compounds and amino acids, especially glycine and serine, indicating an alteration of the photorespiratory pathway[17] (**Fig. 1**). A deficient photorespiration would impair the chloroplast Calvin-Benson cycle leading to a decreased stability of

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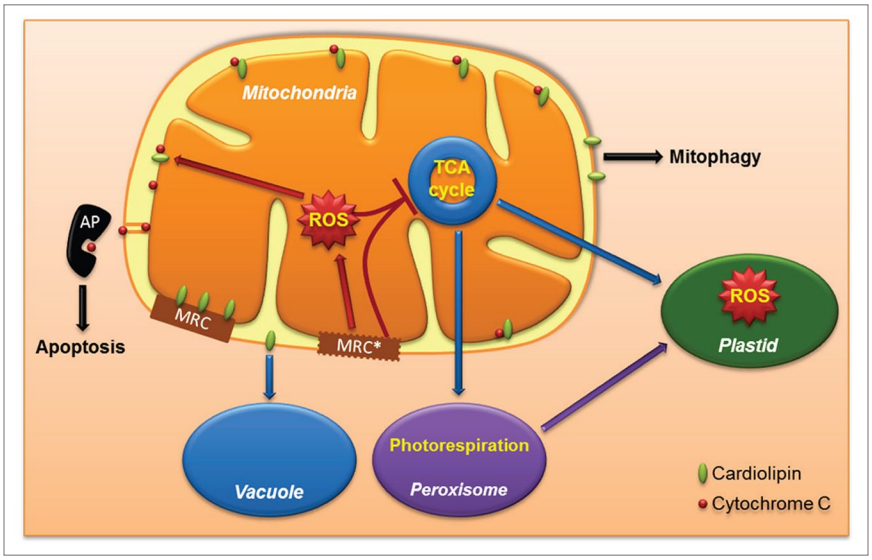

Figure 1. Schematic representation of cellular signaling pathways found to involve cardiolipins in yeast, plant, and animals. In the cls mutant of Arabidopsis devoid of cardiolipin (CL), the mitochondrial respiratory chain (MRC) is perturbed, and as a result reactive oxygen species (ROS), are generated in mitochondria. Also in animals, mitochondrial ROS are produced during apoptosis and trigger CL peroxidation. Cytochrome c is detached from peroxidised CL and is more efficiently released into the cytosol to contribute to the formation of the apoptosome (AP), which leads to caspase activation and cell death. In Arabidopsis the lack of CL results in a perturbation of the tricarboxylic acid (TCA) cycle, putatively through MRC destabilization and ROS production. In Arabidopsis, photorespiration involves plastid, peroxisome, and mitochondria, in cls alteration of the TCA cycle in mitochondria leads to metabolic changes that affect photorespiration, which results in the inhibition of the Calvin-Benson cycle and the production of ROS in plastids. In addition altered mitochondria are probably less efficient to dissipate redox equivalents from the chloroplast, which may induce photoinhibition and ROS production in plastid. In animals it was found that CL externalization to the outer mitochondrial membrane during stress response is used as a signal for mitophagy. In yeast, at elevated temperature, the lack of CL leads to several vacuolar defects, showing the existence of a mitochondria-vacuole signaling pathway mediated by CL. AP, apoptosome; MRC, Mitochondrial respiratory chain MRC; MRC*, MRC without cardiolipin, MRC; ROS, reactive oxygen species; TCA, tricarboxylic acid.

photosystem II and to photooxidative stress.[23] In *Arabidopsis cls* seedlings, the expression of a singlet oxygen-responsive gene was clearly enhanced,[17] consistent with a photooxidative stress induced by photosystem II lack of stability.[24,25] Additionally, as mitochondria also participate to the protection from photoinhibition by dissipating chloroplast redox equivalents,[26] alteration in the ultrastructure and respiratory function of mitochondria in the *Arabidopsis cls* mutant, might directly contribute to the production of singlet oxygen in plastids (**Fig. 1**). Probably as a result of that propensity to photoinhibition, *Arabidopsis* seedlings of the *cls* mutant are highly sensitive to light.[17]

Therefore the absence of CL in *Arabidopsis* mitochondria may lead to perturbation of the function of other organelles such as peroxisomes and plastids (**Fig. 1**). The crucial role of CL in yeasts, plants, and animals is therefore, not only restricted to the functioning of mitochondria, but is also involved at a broader level in the response to stress by interacting with other cellular compartments.

Cardiolipin and programmed cell death

In animals, mitochondria are key organelles for the control of programmed cell death (PCD) execution. In mitochondrial membranes, the balance between proteins from the B-cell/lymphoma 2 (Bcl2) family, that are either pro-apoptotic (e.g., Bcl-xS, Bax, Bid, Bad) or antiapototic (e.g., Bcl-2, Bcl-xL, Mcl-1) will determine whether the cell will live or self-destruct.[27] The irreversible decision to undergo PCD is taken when, together with other factors, cytochrome c is released from the mitochondria into the cytosol.[28] This crucial step is followed by formation of the apoptosome (**Fig. 1**) and activation of a family of proteases called caspases, leading to the dismantling of the cell.[29]

As cardiolipins anchor cytochrome c in mitochondrial internal membrane,[30] a particular effort has been made in the last decade to determine if CLs could have a role in the control of PCD. In particular, ROS production and peroxidation of cardiolipins were shown to be involved in the control of cytochrome c release[31,32] (**Fig. 1**). Nevertheless, the role of CL on the onset of PCD is still not clear in animals, as in human cells experiments aiming at knocking down *CLS* expression showed conflicting results. Indeed, a more pronounced apoptosis has been described after treatment with anti-Fas antibody or staurosporine,[33] but an increased resistance to apoptosis induced by actinomycin D (ActD) has also been reported.[34] However, in both cases CL levels were manipulated using RNA interference, as experiments using *cls* mutants fully devoid of CL remain to be done.

In plants, where the release of cytochrome c from mitochondria has also been described during PCD,[35,36] the impact of *CLS* downregulation on stress susceptibility has been tested on both whole plants of RNAi lines[16] and protoplasts from *cls* mutants.[17] In both cases *cls* defective mutants displayed a much higher susceptibility to stress, as in animals where cardiolipin deficiency

facilitates the release of cytochrome c into the cytosol, thereby intensifying the execution of PCD.[31,33] Alternatively, ROS accumulation (**Fig. 1**) suggested by increased transcript levels for both alternative oxidase (AOX) and external NAD(P)H dehydrogenase (NDB2), 2 markers of oxidative stress in plant mitochondria,[37] might also have an impact on the susceptibility to PCD, as mitochondrial ROS have been reported to be involved in the execution of PCD in both animals and plants.[38,39]

Future challenges

Taken together, the results obtained with *cls* mutants show that cardiolipins are essential for *Arabidopsis* growth and development, and the lack of CL leads to more pronounced perturbations than in yeast. The multiplicity of CL-linked phenotypes (mitochondrial ultrastructure, assembly of CI-CIII supercomplexes, metabolism perturbation, ROS production), might explain the striking consequences of CL deficiency on plant growth and morphology.[16,17] The impact of the lack of CL on PCD being demonstrated after several treatments (UV-C, heat shock, dark) in *Arabidopsis*,[16,17] a more detailed analysis would help us to understand further the mechanism(s) involved. As the lack of CL leads to a higher susceptibility to stress, it would be interesting to try to correlate that with a stronger release of cytochrome c from mitochondria to the cytosol. Also it would be essential to test the peroxidation of CL during PCD execution in *Arabidopsis* as it has been detected in animals. As CLs have been shown to be involved in mitophagy control in animals, and as there is a clear link between autophagy and PCD, it would be interesting to evaluate how these aspects participate in the response to stress in *Arabidopsis*. Also the vacuole playing an important role in plant PCD,[40] it might be important to investigate if the mitochondria-vacuole signaling pathway identified in yeast at elevated temperature, also exists in *Arabidopsis*. The *Arabidopsis cls* mutant will certainly be a tool of great help to unravel the different roles of CL in the control of PCD in *Arabidopsis*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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