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Apoplastic calcium executes a shut-down function on plant peroxidases

A hypothesis

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Recently it was demonstrated that PO activity is switched by calcium within the typical range of apoplastic free calcium concentrations (Plieth and Vollbehr, Plant Signal Behav 2012;7: 650–660). The heat stability of POs is also dependent on calcium. Here, a scenario is put forward which assigns calcium a switch-off function under heat: Peroxidases are switched off by heat stress-triggered apoplastic calcium depletion. It is assumed that this initiates apoplastic accumulation of reactive oxygen species (ROS) and eventually triggers a self-amplifying cascade of cellular events involving plasma membrane ion transport. Calcium depletion-initiated ROS accumulation (CaDIRA) may also trigger signal percolation and the formation of systemic responses to many different stress factors in plants. This hypothesis can explain some as yet unexplained observations.

Keywords: calcium signaling, innate tolerance, peroxidase, positive feed-back signaling, self-amplifying signal

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Peroxidases Involved in Molecular Signaling

Class III Peroxidases (POs) of higher plants typically reside in the apoplast (http://peroxibase.toulouse.inra.fr/cellular_localisation.php). They are assumed to facilitate plants to withstand biotic stress situations and pathogen attack.[1] But, the whole diversity of their functions is not yet clear.[2] POs depend on peroxides and calcium. Both, hydrogen peroxide and calcium ions, are signals of plant abiotic stress response.[3-5] Interestingly, POs are the most heat stable enzymes of plants.[6-9] So, a typical questions at this point is: Are POs possibly nodes in signaling networks mediating heat stress response?

Previously we demonstrated that peroxidases (POs) are Ca^{2+} triggered molecular switches.[10] We also showed that heat stability of peroxidases from higher plants is dependent on Ca^{2+} too. However, POs are switched from heat sensitive to heat tolerant in the nM-range of $[Ca^{2+}]$ whereas their activity is switched in the μM range. The latter is the typical range of apoplastic free calcium $[Ca^{2+}]_{apo}$.[11] Since H_2O_2, is both, a signaling molecule and a PO substrate, it is assumed that POs play a role in plant innate heat tolerance, heat response, and possibly other stress response routes.

Heat Stress, and Acquired and Innate Heat Tolerance

Often plants are exposed to temperatures above their optimum for several hours a day. In their natural habitat, plants thrive because they employ many different mechanisms to cope with heat stress.[12,13] The current general model is that during heat stress, signaling cascades involving calcium[14-16] and H_2O_2[17,18] as signal molecules initiate the expression of heat shock proteins,[12,19] and other effectors[20] which then function as chaperones and in concert regenerate heat-damaged proteins and/or prevent further proteins misfolding.[21,22] This is acquisition of thermotolerance and is based on cellular events resulting from the exposure to high but sublethal temperatures.[13] Thermotolerance acquisition takes resources but enables the organism to cope better with subsequent periods of heat.

The innate (or basal) thermotolerance has to be distinguished from acquired thermotolerance.[23] It is the ability to withstand high temperatures without heat

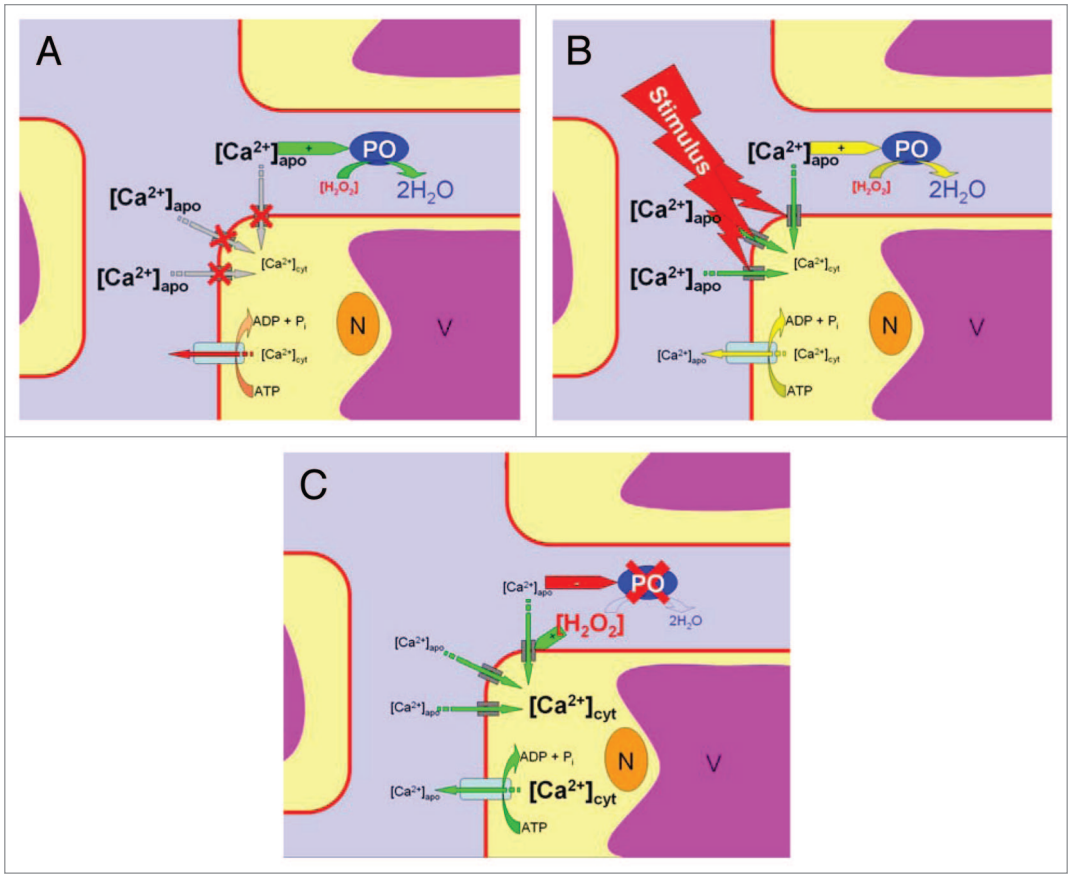

Figure 1. The calcium-depletion-initiated-ROS-accumulation (CaDIRA) scenario. Calcium 'chairs' stimulus-response coupling and signal amplification in the apoplast through a positive feed-back loop. Color coding: pale blue, apoplast; yellow, cytoplasm; magenta, vacuole; brown, nucleus; red, plasma membrane. (A) In a resting system cytoplasmic calcium $[Ca^{2+}]_{cyt}$ is kept low by active transport. Apoplastic $[Ca^{2+}]_{apo}$ in contrast is high. This promotes peroxidase (PO) activities which keep H_2O_2 concentration low in the apoplast. (B) Upon heat stress ('stimulus') cation channels are activated and Ca^{2+} ions flow downstream, producing a $[Ca^{2+}]_{cyt}$ increase at the expense of extracellular Ca^{2+}. The apoplastic Ca^{2+} pool starts to deplete. (C) At low $[Ca^{2+}]_{apo}$, PO activities are reduced. This, among other processes, causes H_2O_2 accumulation. H_2O_2 in turn promotes Ca^{2+} flux from the apoplast into the cell which causes persisting $[Ca^{2+}]_{apo}$ depletion, reduced PO activity, increased H_2O_2, and finally a burst of extracellular ROS and intracellular $[Ca^{2+}]_{cyt}$.

preconditioning. The primary thermostability of unadapted cellular modules like proteins and membranes thus constitutes innate thermotolerance. Consequently, species from habitats of different maximum day temperature have different innate thermotolerance because evolution maintained molecular modules of appropriate thermostability.

Timing is Decisive: Heat Shock-Induced Calcium Flux may Cause a Deactivation of Peroxidases in the Apoplast and Trigger a Self-Amplifying Positive Feed-Back Loop

A movement of calcium ions between apoplast and cytoplasm during periods of heat is controversially reported. Some studies report an increase in cytoplasmic free calcium $[Ca^{2+}]_{cyt}$ during heating[14,16] whereas others do not.[24] This is due to experimental timing as has been also shown for cold treatment.[25,26] With rapid temperature changes a $[Ca^{2+}]_{cyt}$ transient can be detected, however with moderate changes, $[Ca^{2+}]_{cyt}$ transients are small or even below detection level. Thus, not the temperature alone but the rapidity of its change is the principal factor eliciting $[Ca^{2+}]_{cyt}$ transients. Ca^{2+} involved in heat signaling is of extracellular (i.e., apoplastic) origin.[12] Due to the high cytoplasmic calcium buffer capacity,[27] a detectable increase of free $[Ca^{2+}]_{cyt}$ in the cytoplasm reports a bulk Ca^{2+} ion flux from the apoplast into the cell. Thus, in a heat shock situation the cytoplasmic $[Ca^{2+}]_{cyt}$ increases at the expense of apoplastic $[Ca^{2+}]_{apo}$. This causes massive Ca^{2+} depletion in the apoplast due to the much lower calcium buffer capacity in this compartment.[28] In consequence, a shutdown of apoplastic PO activity occurs as has been demonstrated recently in reference 10. This, in turn, causes a H_2O_2 accumulation in the apoplast because H_2O_2 consumption by POs is halted (**Fig. 1**). It is known that H_2O_2 activates cation transporters and favors further cellular calcium ion influx.[29-32] This accelerates apoplastic calcium depletion. Thereby, PO inhibition and ROS accumulation is potentiated. ROS, accumulating in the apoplast, may then spread by diffusion and trigger other cells and serve as messengers for signal cascades. Hence, it is a calcium-depletion-initiated-ROS-accumulation (CaDIRA) in the apoplast (**Fig. 1**) providing a positive feed

back amplification which probably makes an effect and initiates pathways of stress response in general and of thermotolerance acquisition in particular. In other words, calcium 'chairs' stimulus-response coupling and signal amplification in the apoplast through CaDIRA. Other scenarios have been proposed earlier. Among these are also models involving self-amplifying feed-back loops[33] and depletion-refilling of Ca^{2+} stores.[34]

The CaDIRA Scenario can Answer Some Yet Open Questions

This self amplifying mechanism may further deplete calcium from the apoplast, so that finally nM levels of $[Ca^{2+}]_{apo}$ are reached. This in turn switches POs from heat stable to heat sensitive.[10] This way, under continuing heat, POs are irreversibly heat inactivated. Thus, CaDIRA (**Fig. 1**) can explain the finding that even moderate heat (42°C) is sufficient to reduce PO activity by almost 50%,[35] when temperature was increased quickly (i.e., 'heat shock' was applied). A slower increase in temperature probably causes a reduced calcium depletion in the apoplast, since calcium pumps, employed for cytosolic calcium clearance and apoplast refilling, can keep up with heat-induced Ca^{2+} influx. Consequently, with moderate temperature changes, PO activity is reduced, but the heat stability of the enzymes is less affected since ROS fail to accumulate above precarious levels.

Other abiotic and biotic stimuli are known to elicit $[Ca^{2+}]_{cyt}$ transients.[36,37] Some $[Ca^{2+}]_{cyt}$ transients originate from Ca^{2+} release from intracellular stores[38] and others from the extracellular space. The latter can influence apoplastic enzymes and plasma membrane ion transport. This is known for chloride transporters for instance, when the plant is under salt stress. Chloride transport into the cells during salt stress is accelerated when Ca^{2+} is depleted outside the cells and conversely, Ca^{2+} addition outside the cell reduces chloride transport and alleviates salt stress.[39] A direct interdependence between $[Ca^{2+}]_{cyt}$ and $[Ca^{2+}]_{apo}$ has been demonstrated.[40] When salt is withdrawn from salt-adapted root cells then $[Ca^{2+}]_{apo}$ decreases in parallel to a $[Ca^{2+}]_{cyt}$ increase. This suggests that such an effect can also be seen with heat stress. Evidently, more detailed investigations are needed.

Often, physiological responses take place away from the primary site of stimulation. This requires cell-to-cell communication and a systemic spread of signals.[41] Here, the CaDIRA scenario depicted above (**Fig. 1**) can put ideas across and provide explanations: With a local stimulation (e.g., attack of a phytophagous insect or local heat treatment) few cells around the site of stimulation undergo CaDIRA and switch to an 'excited state'. Since membrane depolarization and calcium flux are involved, apoplastic calcium depletion quickly occurs at the other end of the excited cell with almost no time lag and initiates CaDIRA at neighboring cells. This way, the signal could percolate through the plant tissue with velocities not limited to diffusion rates in the apoplast.

The notion of apoplastic calcium signaling is in line with other studies showing that $[Ca^{2+}]_{apo}$ also controls other extracellular enzymes like cell wall phosphatases.[42] In addition, apoplastic calmodulin plays an important role in signal transduction.[43-45]

References

1. Penel C, Dunand C. Signaling via Plant Peroxidases. In: Baluška F, Vivanco J, Eds. Signaling in Plants: Springer Berlin Heidelberg 2009; 155-71.
2. Bakalovic N, Passardi F, Ioannidis V, Cosio C, Penel C, Falquet L, et al. PeroxiBase: a class III plant peroxidase database. Phytochemistry 2006; 67:534-9; PMID:16442574; http://dx.doi.org/10.1016/j.phytochem.2005.12.020.
3. Knight H, Knight MR. Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci 2001; 6:262-7; PMID:11378468; http://dx.doi.org/10.1016/S1360-1385(01)01946-X.
4. Neill S, Desikan R, Hancock J. Hydrogen peroxide signalling. Curr Opin Plant Biol 2002; 5:388-95; PMID:12183176; http://dx.doi.org/10.1016/S1369-5266(02)00282-0.
5. Laloi C, Apel K, Danon A. Reactive oxygen signalling: the latest news. Curr Opin Plant Biol 2004; 7:323-8; PMID:15134754; http://dx.doi.org/10.1016/j.pbi.2004.03.005.
6. Lee CY, Pennesi AP. Isolation and further characterization of a heat resistant peroxidase isoenzyme from Cauliflower. J Food Sci 1984; 49:1616-7; http://dx.doi.org/10.1111/j.1365-2621.1984.tb12859.x.
7. Burnette FS. Peroxidase and its relationship to food flavor and quality: a review. J Food Sci 1977; 42:1-6; http://dx.doi.org/10.1111/j.1365-2621.1977.tb01204.x.
8. Hendrickx M, Saraiva J, Lyssens J, Oliveira J, Tobback P. The influence of water activity on thermal stability of horseradish peroxidase. Int J Food Sci Technol 1992; 27:33-40; http://dx.doi.org/10.1111/j.1365-2621.1992.tb01175.x.
9. Morales-Blancas EF, Chandia VE, Cisneros-Zevallos L. Thermal inactivation kinetics of peroxidase and lipoxygenase from broccoli, green asparagus and carrots. J Food Sci 2002; 67:146-54; http://dx.doi.org/10.1111/j.1365-2621.2002.tb11375.x.
10. Plieth C, Vollbehr S. Calcium promotes activity and confers heat stability on plant peroxidases. Plant Signal Behav 2012; 7:650-60; http://dx.doi.org/10.4161/psb.20065
11. Felle HH, Hanstein S, Sattelmacher B, Horst WJ. Probing apoplastic ion relations in *Vicia faba* as influenced by nutrition and gas exchange. In: Sattelmacher B, Horst W, Eds. The Apoplast of higher plants: Compartment of storage, transport and reactions. Dordrecht: Springer Netherlands 2007; 295-306.
12. Saidi Y, Finka A, Goloubinoff P. Heat perception and signalling in plants: a tortuous path to thermotolerance. New Phytol 2011; 190:556-65; PMID:21138439; http://dx.doi.org/10.1111/j.1469-8137.2010.03571.x.
13. Wahid A, Gelani S, Ashraf M, Foolad MR. Heat tolerance in plants: An overview. Environ Exp Bot 2007; 61:199-223; http://dx.doi.org/10.1016/j.envexpbot.2007.05.011.
14. Gong M, van der Luit AH, Knight MR, Trewavas AJ. Heat-shock-induced changes in intracellular Ca^{2+} level in tobacco seedlings in relation to thermotolerance. Plant Physiol 1998; 116:429-37; http://dx.doi.org/10.1104/pp.116.1.429.
15. Liu HT, Gao F, Cui SJ, Han JL, Sun DY, Zhou RG. Primary evidence for involvement of IP_3 in heat-shock signal transduction in Arabidopsis. Cell Res 2006; 16:394-400; PMID:16617335; http://dx.doi.org/10.1038/sj.cr.7310051.
16. Liu HT, Li B, Shang ZL, Li XZ, Mu RL, Sun DY, et al. Calmodulin is involved in heat shock signal transduction in wheat. Plant Physiol 2003; 132:1186-95; PMID:12857801; http://dx.doi.org/10.1104/pp.102.018564.
17. Dat JF, Lopez-Delgado H, Foyer CH, Scott IM. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol 1998; 116:1351-7; PMID:9536052; http://dx.doi.org/10.1104/pp.116.4.1351.
18. Gong M, Chen Bo, Li ZG, Guo LH. Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2. J Plant Physiol 2001; 158:1125-30; http://dx.doi.org/10.1078/0176-1617-00327.
19. Voellmy R, Boellmann F. Chaperone regulation of the heat shock protein response. In: Csermely P, Vígh L, Eds. Molecular Aspects of the Stress Response: Chaperones, Membranes and Networks. New York: Springer 2007; 89-99.
20. Sung DY, Kaplan F, Lee KJ, Guy CL. Acquired tolerance to temperature extremes. Trends Plant Sci 2003; 8:179-87; PMID:12711230; http://dx.doi.org/10.1016/S1360-1385(03)00047-5.
21. Wang W, Vinocur B, Shoseyov O, Altman A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 2004; 9:244-52; PMID:15130550; http://dx.doi.org/10.1016/j.tplants.2004.03.006.
22. Waters ER, Lee GJ, Vierling E. Evolution, structure and function of the small heat shock proteins in plants. J Exp Bot 1996; 47:325-38; http://dx.doi.org/10.1093/jxb/47.3.325.
23. 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; 138:882-97; PMID:15923322; http://dx.doi.org/10.1104/pp.105.062257.

24. Larkindale J, Knight MR. Protection against heat stress-induced oxidative damage in Arabidopsis involves calcium, abscisic acid, ethylene and salicylic acid. Plant Physiol 2002; 128:682-95; PMID:11842171; http://dx.doi.org/10.1104/pp.010320.
25. Plieth C, Hansen UP, Knight H, Knight MR. Temperature sensing by plants: the primary characteristics of signal perception and calcium response. Plant J 1999; 18:491-7; PMID:10417699; http://dx.doi.org/10.1046/j.1365-313X.1999.00471.x.
26. Nagel-Volkmann J, Plieth C, Becker D, Lüthen H, Dörffling K. Cold-induced cytosolic free calcium ion concentration changes in wheat. J Plant Physiol 2009; 166:1955-60; PMID:19520454; http://dx.doi.org/10.1016/j.jplph.2009.05.002.
27. Plieth C, Sattelmacher B, Hansen UP. Cytoplasmic Ca^{2+}-H^{+}-exchange buffers in green algae. Protoplasma 1997; 198:107-24; http://dx.doi.org/10.1007/BF01282136.
28. Oja V, Savchenko G, Jakob B, Heber U. pH and buffer capacities of apoplastic and cytoplasmic cell compartments in leaves. Planta 1999; 209:239-49; PMID:10436227; http://dx.doi.org/10.1007/s004250050628.
29. Rentel MC, Knight MR. Oxidative stress-induced calcium signaling in Arabidopsis. Plant Physiol 2004; 135:1471-9; PMID:15247375; http://dx.doi.org/10.1104/pp.104.042663.
30. Price AH, Taylor A, Ripley SJ, Griffiths A, Trewavas AJ, Knight MR. Oxidative signals in tobacco increase cytosolic calcium. Plant Cell 1994; 6:1301-10; PMID:12244272; http://dx.doi.org/10.1105/tpc.6.9.1301.
31. Pei ZM, Murata Y, Benning G, Thomine S, Klüsener B, Allen GJ, et al. Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature 2000; 406:731-4; PMID:10963598; http://dx.doi.org/10.1038/35021067.
32. Mori IC, Schroeder JI. Reactive oxygen species activation of plant Ca^{2+} channels. A signaling mechanism in polar growth, hormone transduction, stress signaling and hypothetically mechanotransduction. Plant Physiol 2004; 135:702-8; PMID:15208417; http://dx.doi.org/10.1104/pp.104.042069.
33. Sagi M, Fluhr R. Production of reactive oxygen species by plant NADPH oxidases. Plant Physiol 2006; 141:336-40; PMID:16760484; http://dx.doi.org/10.1104/pp.106.078089.
34. Bowler C, Fluhr R. The role of calcium and activated oxygens as signals for controlling cross-tolerance. Trends Plant Sci 2000; 5:241-6; PMID:10838614; http://dx.doi.org/10.1016/S1360-1385(00)01628-9.
35. Saleh L, Plieth C. Fingerprinting antioxidative activities in plants. Plant Methods 2009; 5:2; PMID:19171044; http://dx.doi.org/10.1186/1746-4811-5-2.
36. Plieth C. Calcium: just another regulator in the machinery of life? Ann Bot 2005; 96:1-8; PMID:15845557; http://dx.doi.org/10.1093/aob/mci144.
37. Plieth C. Plant calcium signaling and monitoring: pros and cons and recent experimental approaches. Protoplasma 2001; 218:1-23; PMID:11732314; http://dx.doi.org/10.1007/BF01288356.
38. Plieth C, Sattelmacher B, Hansen UP, Thiel G. The action potential in Chara: Ca^{2+} release from internal stores visualized by Mn^{2+}-induced quenching of fura-dextran. Plant J 1998; 13:167-75; http://dx.doi.org/10.1046/j.1365-313X.1998.00019.x.
39. Lorenzen I, Aberle T, Plieth C. Salt stress-induced chloride flux: a study using transgenic Arabidopsis expressing a fluorescent anion probe. Plant J 2004; 38:539-44; PMID:15086798; http://dx.doi.org/10.1111/j.0960-7412.2004.02053.x.
40. Gao D, Knight MR, Trewavas AJ, Sattelmacher B, Plieth C. Self-reporting Arabidopsis expressing pH and $[Ca^{2+}]$ indicators unveil ion dynamics in the cytoplasm and in the apoplast under abiotic stress. Plant Physiol 2004; 134:898-908; PMID:15020753; http://dx.doi.org/10.1104/pp.103.032508.
41. Plieth C. Signal percolation through plants and the shape of the calcium signature. Plant Signal Behav 2010; 5:379-85; PMID:20139732; http://dx.doi.org/10.4161/psb.5.4.10717.
42. DeMarty M, Morvan C, Thellier M. Calcium and the Cell Wall. Plant Cell Environ 1984; 7:441-8; http://dx.doi.org/10.1111/j.1365-3040.1984.tb01434.x.
43. Ma L, Sun DY. The effects of extracellular calmodulin on initiation of *Hippeastrum rutilum* pollen germination and tube growth. Planta 1997; 202:336-40; http://dx.doi.org/10.1007/s004250050135.
44. Ma L, Xu X, Cui S, Sun D. The presence of a heterotrimeric G protein and its role in signal transduction of extracellular calmodulin in pollen germination and tube growth. Plant Cell 1999; 11:1351-64; PMID:10402434; http://dx.doi.org/10.1105/tpc.11.7.1351.
45. Tang J, Wu S, Bai J, Sun DY. Extracellular calmodulin-binding proteins in plants: purification of a 21-kDa calmodulin-binding protein. Planta 1996; 198:510-6.