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Multifunctional Enzymes in Oxylipin Metabolism

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The paper by Grechkin et al.[1] in this issue presents a new exciting example of how enzymes involved in oxylipin formation and metabolism carry out similar if not overlapping functions. Oxylipins are a diverse class of lipid metabolites that derive either from chemical or enzymatic oxidation of unsaturated fatty acids.[2] In plants a major portion of these molecules are polyunsaturated fatty acid-derived signals formed by different branches of the lipoxygenase (LOX) pathway.[3] Beside the initial reactions of oxygen insertion in positions C-9 or C-13 of fatty acids with 18 carbon atoms by different LOX isoforms, the largest diversity of oxylipins is provided by the family of CYP74 enzymes.[4] These proteins belong to an atypical cytochrome P450 subfamily and all of them catalyze the conversion of LOX-derived fatty acid hydroperoxides (Scheme 1, boxed area). According to their substrate specificity, they are divided into at least four different subfamilies: 1) the allene oxide synthases (AOS) that catalyze the first specific step in jasmonate biosynthesis are grouped as CYP74A; all of them are specific for (13*S*)-hydroperoxides; 2) hydroperoxide lyase (HPLs) enzymes catalyze formation of leaf aldehydes and alcohols;[5] HPLs that act specifically with (13*S*)-hydroperoxides form the CYP74B subfamily; 3) AOSs and HPLs that lack substrate specificity as well as AOSs that convert (9*S*)-hydroperoxides have been grouped into the CYP74C subfamily based on sequence similarities;[6] 4) enzymes grouped into the CYP74D subfamily convert (9*S*)- and/or (13*S*)-hydroperoxides to the corresponding divinyl ethers (DESs).[4]

Interestingly, although among the CYP74 enzymes a remarkable number of proteins with high sequence similarity exist, until now no example was described for which additional or at least bifunctional activities could be observed. In principle bifunctional enzymes have been described that cover catalysis within the group of oxylipins that might derive from CYP74 activity; but these functions have been in combination with completely different enzymes. Prominent examples include the following:

1) A fusion protein has been found in coral with LOX and AOS-like domains.[7] The AOS-like domain has only weak activity and shows sequence similarity to a catalase. As suggested by crystal-structure analysis, the LOX–AOS fusion protein might have evolved by mutations of interacting LOX and AOS proteins.[8] Such a dual function protein was recently also found in *Anabaena*.[9,10] In contrast to the enzymes from higher plants, both examples form and convert fatty acid hydroperoxides that are in the *R* configuration. It is tempting to speculate that like the coral AOS–LOX fusion protein other catalase-related AOS homologues corresponding to the plant CYP74 P450 family (e.g., HPL, DES and epoxyalcohol synthase (EAS)) might be part of a fusion protein.[8]
2) From the primitive land plant *Physcomitrella patens*, a *LOX* gene was cloned that as recombinant protein exhibited arachinoate 12-LOX activity, linoleate 13-LOX activity, hydroperoxidase activity as well as HPL activity.[11]
3) From mangrove plants, which are salt tolerant, an AOC protein was cloned with a 70 amino acid extension that was responsible for the salt-tolerant phenotype.[12] Although the product of this enzyme is not characterized yet, it might be another candidate for a bifunctional enzyme.

These examples are part of a scenario in which multifunctional proteins might have evolved in closely related subfamilies. Interestingly these multifunctional enzymes have yet not been described in higher plants. However, the CYP74 subfamilies are candidates due to their significant sequence similarities between different functionalities, and even in solanaceous species up to four isozymes have been described to occur in the same plant.[7]

The paper by Grechkin et al. is an exciting new example of versatile protein evolution in closely related proteins of this pathway. The work was initiated by unusual properties of a CYP74C family member, the 9-AOS of potato stolons and tomato roots.[13–15] This enzyme catalyzes formation of a substantial amount of cyclopentenone *cis*-10-oxo-11-phytoenoic acid from 9-hydroperoxy linoleic acid, whereas other AOSs do not cyclize the allene oxide due to the structural requirement with respect to the position of the double bound. Interestingly, a similar reaction was found in sunflower roots.[16]

Now, excellent proof by has been obtained by chemical and analytical approaches, which show that recombinant 9-AOS from tomato catalyzes not only the synthesis of the allene oxide, but also hydrolyzes the allene oxide to ketols and most importantly is able to cyclize it to the corresponding cyclopentenone compound. Such multifunctional properties of an enzyme of the oxylipin forming LOX pathways raise several new questions on lipid-derived signaling:

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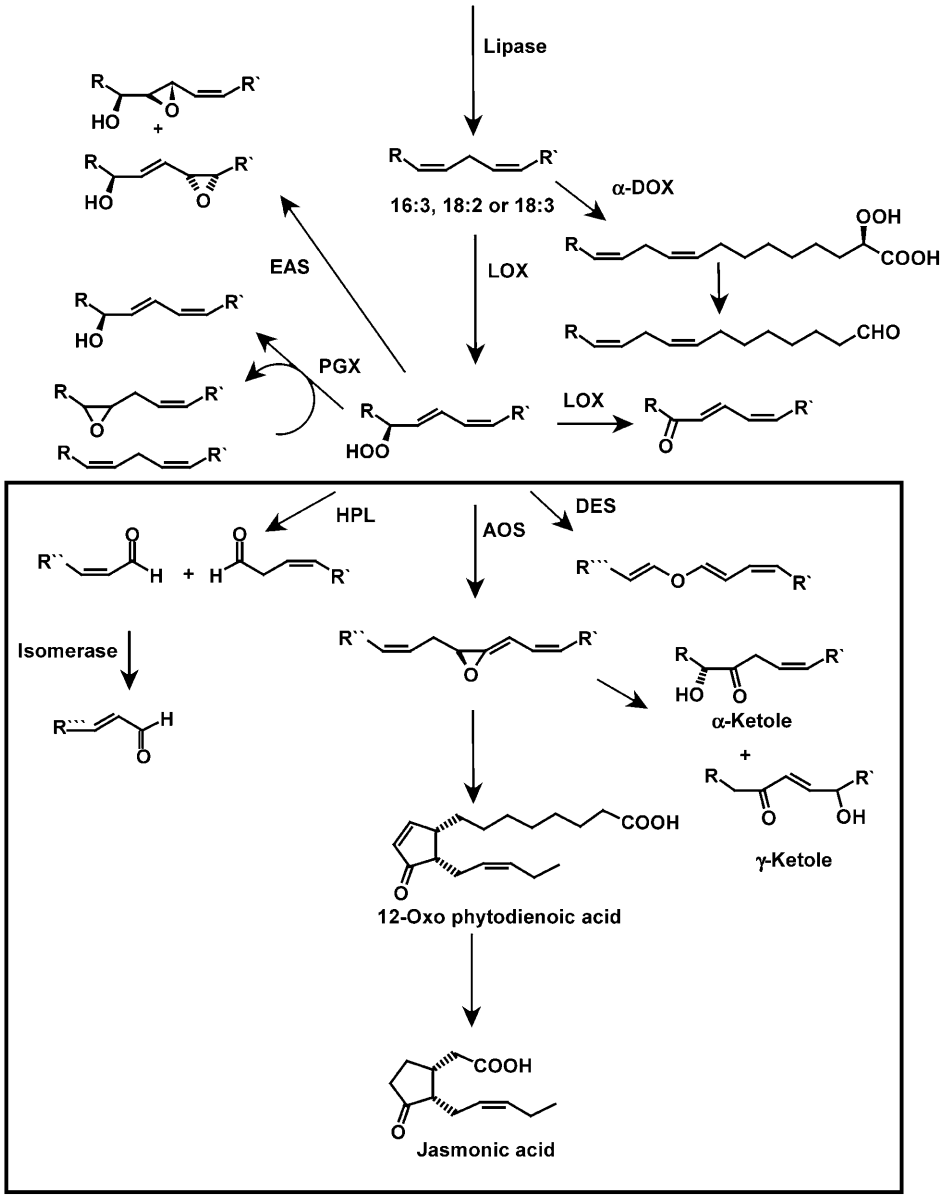

Scheme 1. The LOX pathway. The boxed area shows the reactions that derive from the activity of CYP74 enzymes. AOS: allene oxide synthase; DES: divinyl ether synthase; α-DOX: α-dioxygenase; EAS: epoxy alcohol synthase; HPL: hydroperoxide lyase; LOX: lipoxygenase; PXG: peroxygenase.

1) how did such enzyme properties evolve?
2) is the described multifunctional 9-AOS activity an example of the influence of environmental *and* developmental cues on the evolution of enzyme diversity in below-ground organs?

In the case of 9-AOS analyzed by Grechkin et al. important answers to these questions will be provided by crystallographic analyses and side-directed mutagenesis as have been obtained for coral AOS, AOC2 of *Arabidopsis*, and OPR1 and OPR3 of tomato.[8,17–19]

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