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TITLE: The plant SDR superfamily: involvement in primary and secondary metabolism

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Running title: The plant SDR superfamily

ABSTRACT

Short-chain dehydrogenases/reductases (SDRs) comprise a large family of NAD(P)(H)-dependent oxidoreductases represented in plant kingdom by around 190 members in *Arabidopsis*. Here we will focus on the so-called 'classical' SDRs that account for about half of the SDRs and are characterized by a core structure of 250 amino acids and the conservations of specific motifs (cofactor binding site, catalytical residues). Although the 'classical' SDR family is highly divergent, an inventory of SDR from the model plant *Arabidopsis thaliana* genome reveals that it contains both large subfamilies with numerous members that share significant homologies (e.g. tropinone reductase, menthone/salutaridine reductase, NAD-dependent dehydrogenase) and small subfamilies with a limited number of members (e.g. protochlorophyllide reductase, β -ketoacyl-acyl carrier protein reductase and enoyl-reductase). The involvement of SDRs in plant metabolism illustrates the large functional diversity of the SDR superfamily's members. Functions of SDR encompass many aspects of primary (lipid synthesis, chlorophyll biosynthesis or degradation) and secondary (terpenoids, steroids, phenolics and alkaloids) metabolism. The present paper aims at reviewing the metabolic functions where the role of SDR has been documented and at discussing their phylogenetic diversification and structural features.

KEYWORDS

Short-Chain Dehydrogenase – Oxidoreductase - Multigenic Family – Primary and Secondary Metabolism – Plant – *Arabidopsis thaliana*

ABBREVIATIONS

ABA: Abscisic Acid; ACP: Acyl Carrier Protein; BKR: Beta-Keto (Acyl Carrier Protein) Reductase ; CCR: Cinnamoyl Coenzyme A Reductase; Chlb : Chlorophyll b; DFR: Dehydroflavonol-4-Reductase; DVR: 3,8- Divinyl Protochlorophyllide a 8-Vinyl Reductase; ENR: Enoyl Reductase; FAS: Fatty Acid Synthase; FEY: Forever Young; GA: Gibberellic Acid; HSD: Hydroxysteroid Dehydrogenase; 3 β -HSD/D: 3-Beta-Hydroxysteroid Dehydrogenase-Decarboxylase; IspIDH: Isopiperitenol Dehydrogenase; IspR: (-)-Isopiperitenone Reductase; JA: Jasmonic Acid; MMR: (-)-Menthone:(-)-(3R)-Menthol Reductase; MNR: (-)-Menthone:(-)-(3S)-Neomenthol Reductase; POR: Protochlorophyllide Reductase; PterAR: pterin aldehyde reductase; SalR: Salutaridine Reductase; SDH: Secoisolariciresinol Dehydrogenase; SDR: Short Chain Dehydrogenase-Reductase; TR: Tropinone Reductase; TS: Tasselseed.

INTRODUCTION

Short-chain dehydrogenases/reductases (SDRs) perform NAD(P)(H)-dependent

oxidoreduction reactions together with medium-chain alcohol dehydrogenases and aldo-keto reductases. It has been estimated that 25% of the identified dehydrogenases belong to the SDR superfamily with 47000 primary structures [1]. This ancient family, found in all domains of life (*Archea*, Eukaryotes, Prokaryotes) is characterized by a low sequence conservation, but several common properties [2,3]: (i) a conserved 3D structure with a common α/β folding pattern characterized by a 'Rossmann-fold' β -sheet with helices on either sides, (ii) an N-terminal dinucleotide cofactor binding motif, (iii) an active site with a catalytical residue motif YxxxK, and (iv) a variable substrate binding region at the C-terminal region of the protein. With the release of the genome sequences of a number of living organisms, the availability of around 300 crystal structures and the identification of many enzymatic functions, much attention was given to classify the members of the superfamily on the base of the primary sequences, structures and functions [1,3,4-8]. These efforts have led to the discrimination of five types of SDR: the 'classical' type, consisting of approximately 250 amino acids, the 'extended' type that has an additional 100-residue domain in the C-terminal region, the 'intermediary' and 'divergent' types, which harbour modifications on the cofactor and active sites motifs and the 'complex' SDR which are usually a part of large multi-domain enzymes, such as mammalian fatty acid synthases or bacterial polyketide synthases. It has become obvious that SDRs participate in many aspects of primary and secondary metabolism and are not only restricted to carbonyl-alcohol oxidoreduction, but comprise also isomerase, epimerase, dehydratase and decarboxylase activities. However, in all cases, the overall enzymatic reaction requires an oxidoreductive step. Among higher eukaryotes, the SDRs (either extended or classical) are highly represented in the plant kingdom. In *Arabidopsis thaliana*, Kavanagh *et al.* [3] listed 149 'non-redundant' SDR enzymes (all families included), in contrast with animals (46 in rat, 71 in human or 82 in fruit fly) or yeast (25 SDRs).

The present paper is aimed at providing an overview of the diversity of the SDR super-family with main focus on 'classical' SDRs and at reviewing the different metabolic pathways where they are involved. The question will be addressed of how SDR diversification has participated in the development of the large repertoire of chemical compounds encountered in plants.

1. OVERVIEW OF PLANT 'CLASSICAL' SDR

1.1 CLUSTERING *ARABIDOPSIS THALIANA* SDRS

The genome sequencing and the annotation data available for the model plant *Arabidopsis thaliana* [9,10] provide a reliable set of data useful for a global inventory of a multigenic family in a

plant species. Contrary to 'extended' or 'divergent' SDR, the classical SDR are defined by a single Pfam (PF00106) or INTERPRO (IPR002196). Hence, *Arabidopsis* genes coding for the classical SDRs were sorted and presented in a global clustering tree (Fig. 1). In order to give a better functional overview of plant SDRs, functionally-characterized SDRs of other plant species have been integrated together with the *Arabidopsis* genes. Since SDRs are a very ancient superfamily (probably polyphyletic) and the homologies between subfamilies are limited, the clustering tree does not necessarily reflect the overall phylogenetic relationships. However, within subfamilies, the tree clearly illustrates the existence of several clades: a large cluster of NAD-dependent dehydrogenase (Fig. 1B), which counts up to 11 genes in *Arabidopsis* genome, a cluster called HSD (Hydroxysteroid Dehydrogenase, Fig. 1C) with 8 different genes in *Arabidopsis*, the tropinone reductase cluster with 15 *Arabidopsis* genes (Fig. 1D), the Menthone/Salutaridine Reductase cluster, with 6 *Arabidopsis* genes (Fig. 1E), and the Protochlorophyllide Reductase (POR) cluster (Fig. 1F), with 3 *Arabidopsis* genes.

1.2 FUNCTIONAL DIVERSITY OF PLANT SDR

Traditionally, an opposition is made between primary and secondary metabolites. Primary metabolites are encountered in all plants and are involved in essential metabolic pathways such as lipid, sugar, nucleic acid and amino acid biosynthesis or degradation pathways, and also all the metabolic functions linked to photosynthesis. Secondary metabolites are perceived as compounds involved in environmental interactions such as defence against biotic agents (fungi, viruses, herbivores, insects), competition between plants (allelopathic agents), attraction of pollinators and seed-dispersing agents and adaptation to abiotic stresses [12].

Figure 1 shows that most of the enzymes involved in primary metabolism (β -ketoacyl-ACP-reductase or enoyl-ACP-reductase in lipid biosynthesis, chlorophyll degradation, folate salvage) exist as a single copy gene. On the contrary, the four subfamilies detached from the main phylogenetic tree (Fig. 1B, C, D and E) which are involved in the main routes of secondary metabolism (phenolics, isoprenoids and alkaloids) are largely diversified and the genes annotated have very precise functions (*Datura stramonium* or *Solanum tuberosum* tropinone reductase, *Papaver somniferum* salutaridine reductase, *Forsythia x intermedia* secoisolariciresinol dehydrogenase, *Mentha piperita* isopiperitenol dehydrogenase, menthone reductase, (-)-isopiperitenol/(-)-carveol dehydrogenase). Besides, several genes in *Arabidopsis* are located in adjacent positions, suggesting that these multigenic families may have evolved by duplication events. The significance of the multigenic families may be

analysed in two ways: either there are cases of functional redundancy, but the genes are differentially expressed; or the emergence of multigenic families served as a template for chemical diversification: random mutations and selection leading to the emergence of new metabolic pathways.

2. FUNCTIONS OF SDR IN PLANT METABOLISM

2.1. PRIMARY METABOLISM

2.1.1. Fatty acid biosynthesis.

2.1.1.1. Fatty acid synthase

The overall pathway of fatty acid biosynthesis is roughly identical in all living organisms. However, in plants, fatty acid synthase (named FAS II) is composed of separate soluble enzymes carrying single enzymatic reaction which are localized in the plastids, while in animal and yeast fatty acid synthase (FAS I) is composed of one or two multifunctional polypeptides. In the synthesis of lipids there are two reduction steps catalysed by two SDRs, a β ketoacyl-ACP reductase (BKR) with a function similar to bacterial FAB G (AT1G24360.1 in *Arabidopsis*) and an enoyl-ACP reductase (ENR) with a function similar to bacterial FAB I (AT2G05990.1 in *Arabidopsis*). BKR is NADPH-dependent while ENR is NADH-dependent [13, 14]. The fine crystal structure of the two reductases of *Brassica napus* has been determined. It reveals that the two enzymes are homotetramers with each subunit having a dinucleotide-binding fold. BKR harbours a triad of Ser154, Tyr167 and Lys171 residues in the active site characteristic of the SDR family [15] while ENR does not have the catalytic triad but is believed to utilize conserved tyrosine and lysine residues for catalysis [16]. Primary sequences analyses reveal that this function is probably conserved in all plant kingdom, a conserved orthologue being found in one or two copies in every sequenced genomes (Os08g23810.1 and Os09g10600.1 in *Oryza sativa*; Pp1s29252V2.1 and Pp1s9954V2.1 in *Physcomitrella patens*). In the case of BKR, the sequence displays also a high homology with the cyanobacteria *Synechocystis* (55% identity between At1g24360.1 and P73574), in agreement with the endosymbiotic origin of the plastids.

2.1.1.2. Fatty acid elongase

Very long chain fatty acids are biosynthesized by an endoplasmic reticulum-localized enzyme complex, named fatty acid elongase comprising a β -ketoacyl reduction as observed for *de novo* fatty acid synthesis [17]. Two β -ketoacyl reductase genes (*GL8a* and *GL8b*) belonging to the SDR family have been isolated and characterized in maize [18] and one in *Brassica napus* [19]. There are two potential *Arabidopsis* BKRs in the fatty acid elongase cluster, AtKCR1

(At1g67730) and AtKCR2, (At1g24470) but functional characterization indicated that only AtKCR1 was involved in microsomal fatty acid elongation [20]. Even if the reticulum β -ketoacyl reductase catalyses reactions similar to fatty acid synthase BKR, its sequences remain distant, suggesting that these two mechanisms emerged independently during evolution. This 'independent' evolution is even more obvious in the case of the enoyl-reductase reaction, catalysed in the reticulum by CER10 (At3g55360, [21]) which does not harbour any signature (PF00106, PF01073 or PF01370) of typical SDR. At last, the high similarity between yeast and plant fatty acid elongase complexes support the hypothesis of an ancestral eukaryotic elongation system, contrary to FAS-II which probably emerged as a result of plastid endosymbiosis.

2.1.2 Chlorophyll biosynthesis

SDR members are involved in the biosynthesis of chlorophyll by participating in the conversion of protochlorophyllide a into chlorophyllide a. This conversion is performed by a plastid-targeted NADPH:protochlorophyllide oxidoreductase A (POR). Several isoforms of the protein are present in the POR clade (Fig. 1F). There are three genes encoding POR proteins in *Arabidopsis*, PORA, B and C that share 84-89% identity and that exhibit functional redundancy [22]. In addition an enzyme belonging to the extended SDRs, 3,8-divinyl protochlorophyllide a 8-vinyl reductase (DVR) participates in the synthesis of monovinyl derivatives of chlorophyll by performing reduction of the 8-vinyl group to an ethyl group on the tetrapyrrole ring [23]. DVR is a single-copy gene having sequence similarity to isoflavone reductases. As observed with plant FAS-II (BKR), all POR proteins are also closely related to cyanobacterial POR (*Synechocystis* Q59987.2, 55% identity with *Arabidopsis* POR A). In the case of DVR, an orthologue copy is also found in cyanobacteria (*Synechococcus*), but only in certain classes which produce monovinyl chlorophyll [23].

2.1.3 Chlorophyll degradation

Chlorophyll b is the major pigment of Light-Harvesting Complexes LHCII. The conversion of chlorophyll b to 7-hydroxymethylchlorophyll a is the first step of chlorophyll degradation and is catalysed by the chlorophyll b reductases Non Yellow Coloring (NYC1) and NYC1-like (NOL), two SDR-type enzymes [24-25]. Both proteins exhibit Chlorophyll b reductase activity in rice (Q5N800.1 and Q84ST4.1) and in *Arabidopsis* (At4g13250.1 and At5g04900.1). The two proteins interact *in vitro*, which suggests that they may form a complex that acts as Chl b reductase.

2.1.4 Folate synthesis

Folates are a group of essential dietary compounds referring to all derivatives of tetrahydrofolic acid, a water-soluble vitamin B (B9). They are acting as co-factors in one-carbon metabolism. Folates undergo oxidative cleavage, releasing a pterin aldehyde moiety which is re-used in folate biosynthesis after reduction of the aldehyde group. The reduction can be performed by several NADPH-dependent aldehyde reductases having broad-specificity towards diverse aromatic and aliphatic aldehydes. A gene of *Arabidopsis* (At1g10310) has been identified whose corresponding recombinant protein exhibited non specific pterin aldehyde reductase (PterAR) activity [26]. Interestingly, the recombinant protein encoded by a gene located in a neighbour cluster (At4g20760) proved to be inactive towards pterin aldehydes.

2.1.5 Protein associated with plastid import translocon (Tic32)

A member of the SDR family, Tic32 is a component of the protein import translocon of the inner envelope of chloroplast (Tic). The protein interacts with other members of the translocon and could function as a redox sensor, but its molecular substrate is unknown [27]. Originally described in pea (*Pisum sativum*), Tic32 is very similar to a cluster of 5 *Arabidopsis* SDRs (At4g23420, At4g23430, At4g11410, At5g02540, At2g37540, Fig. 1). Only At4g23430 encodes a protein with a predicted chloroplastic peptide signal at the N-terminal extremity, but none of these genes have been characterised yet.

2.2 SECONDARY METABOLISM

2.2.1 Steroids

In animals, the diversity of SDR partly relies on hydroxysteroid metabolism. On the SDR classification proposed by Kallberg *et al.* [1], enzymes involved in hydroxysteroids metabolism are found in 15 distinct subfamilies (SDR9C, SDR11E, SDR12C, SDR13C, SDR16C, SDR26C, SDR28C, SDR30C, SDR31E, SDR37C, SDR47C, SDR60E, SDR75U, SDR119C, SDR133E). In plants, only three (SDR31E, SDR75E, SDR119C) comprise genes encoding SDRs putatively involved steroids metabolism.

2.2.1.1 3- β -hydroxysteroid dehydrogenase-decarboxylase (3 β -HSD/D)

Sterols are commonly found in all eukaryotic cell membranes. Sterol biosynthesis implies the removal of two methyl groups at the C-4 position. In plants, this removal is carried out by the successive action of a sterol C-4 methyl oxidase, a 4- α -carboxysterol-3 β -hydroxysteroid dehydrogenase/C-4 decarboxylase (3 β -HSD/D) and a NADPH-dependent 3-oxosteroid reductase. Rahier

et al. [28,29] recently characterized two NAD-dependent *Arabidopsis* 3 β -HSD/D (At1g47290 and At2g26260) and demonstrated, by yeast functional complementation and by VIGS-mediated silencing, their involvement in sterol biosynthesis. Both isoforms are classified as extended SDR (SDR31E family, in the Kallberg *et al.* [1] nomenclature).

2.2.1.2 11 β - and 17 β - hydroxysteroid dehydrogenase

The level of active glucocorticoids in animals is regulated by an 11 β -hydroxysteroid dehydrogenase (HSD). Although these compounds have not been identified in plants, 8 genes coding for 11 β -HSD-like proteins were found in the *Arabidopsis* genome (SDR119C family, Fig. 1C). These proteins, also named steroleosin, belong to the SDR family and possess 11 β - and 17 β -HDS activity, but the actual substrates *in vivo* are unknown [30]. AtHSD1 is thought to regulate plant growth and development by promoting or mediating the effects of brassinosteroids [31]. The protein, located at the surface of oil bodies, is highly similar to Sop2 from sesame [32].

2.2.1.3 Progesterone 5 β -Reductase

Digitalis species comprise plants of medical interest for their capacity to accumulate cardenolides, a class of steroids with cardiotonic properties that are efficient for treating cardiac insufficiencies. The reduction of progesterone by progesterone 5 β -reductase constitutes a central step in cardenolides biosynthesis. This reaction is catalyzed by a stereospecific NADPH-dependent 'extended'-SDR (belonging to the SDR75E family defined by Kallberg *et al.* [1]) which has been also crystallized [33]. Herl *et al.* [34] recently characterized an *Arabidopsis* orthologue, VEP1 (At4g24220) of *Digitalis* progesterone 5 β -reductase and confirmed, by heterologous expression, its $\Delta^{4,5}$ – steroid 5 β -reductase activity.

2.2.2 Alkaloids biosynthesis

Plant alkaloids are pharmacologically active nitrogen-containing compounds originating from amino acids, polyamines or purines. SDRs were found to be involved in the biosynthesis of at least two major classes of alkaloids: benzylisquinoline alkaloids (morphine, codeine) and tropane-derived alkaloids (scopolamine, atropine, cocaine).

2.2.2.1 Benzylisquinoline pathway

By comparative transcripts and metabolites profiling in different *Papaver* (Poppy) species, Ziegler *et al.* [35] identified a stereospecific NADPH-dependent salutaridine reductase (SalR) belonging to the same cluster as menthone reductase or neomenthol reductase (Fig. 1E). Contrary to most SDRs, SalR-SDR is active as a monomer and contains an additional 40 amino acids sequence

upstream of the catalytic YxxxK motif. Several molecular modelling studies supported by site-directed mutagenesis enabled the characterisation of the substrate binding pocket. Amino acids of the binding sites are located on three areas which are poorly conserved in other proteins belonging to the Salutaridine/menthone reductase clade (Fig. 1E). Interestingly, several mutations of these amino acids led to the formation of enzymes with lower affinity but higher turnover than the native enzyme [36,37].

2.2.2.2 Tropane pathway

Tropane alkaloids (scopolamine, hyoscamine, atropine, cocaine) have received much attention given their interest as drugs. Tropinone reduction, either into tropine or into pseudotropine is considered as a branching point of alkaloids metabolism. In Solanaceous species, where both tropine stereoisomers-derived compounds are found, two stereospecific NADPH dependent-SDRs were identified: tropinone reductase I (TR-I), yielding tropine and tropinone reductase II (TR-II), yielding pseudotropine [38]. The crystal structures of both TR-I and TR-II were described [39,40]; interestingly, the stereospecificity appeared to be controlled by few charged amino acids which control the binding orientation of tropinone within the substrate binding site. In TR-II, a glutamate (E156), absent in TR-I directly interacts with the positively-charged tropinone nitrogen while the orientation of tropinone in TR-I is controlled by electrostatic repulsion between the substrate and a histidine (H112).

Yet, in all sequenced plant species, several 'tropinone reductases' are predicted that show high similarity to either TR-I or TR-II (Fig. 1D). In rice or *Arabidopsis*, the adjacent positions of various TR on different chromosome segments suggest that, in higher plants, the tropinone reductase family was diversified by several independent duplication events. To understand the significance of this diversification, Brock *et al.* [41] characterized tropinone reductase from the Brassicaceae *Cochlearia officinalis* and *Arabidopsis* and after mutagenesis, evaluated the importance of certain amino acids in substrate specificity and stereospecificity. Both enzymes show broad substrate specificity but only *Cochlearia* TR-I can reduce tropinone in tropine and pseudotropine. As observed with *Solanaceous* TR, the enzyme specificity can be altered by few amino acids mutations. Thus, the authors suggested a scenario for the emergence of TR among the SDR superfamily. From a TR prototype (probably with relaxed specificity and low turnover), the family first diversified into two major families (TR-I and TR-II) and subsequent gene duplication has generated a wide spectrum of non-specific isoforms. Some of these isoforms (as in Solanaceae) were selected for specialization and acquired higher specificities. The

high number of duplications in the alkaloid pathway highlights the importance of co-evolution of the secondary metabolism in response to various biotic stresses or interactions (detoxification of xenobiotics, defence against pests and herbivores).

SDRs involved in alkaloids biosynthesis may not be restricted to the tropinone reductase or salutaridine reductase clusters. Indeed, FgaDH, an NAD-chanoclavine-I dehydrogenase involved ergot alkaloid biosynthesis was recently characterized in *Aspergillus fumigatus* and *Claviceps purpurea* [42]. Interestingly, this protein harbours motifs similar to the conserved motifs of plant NAD-dependent dehydrogenase cluster (Fig. 1B), which is involved in several metabolic pathways (phenolics, isoprenoids, hormones).

2.2.3 Phenolics

Two SDR families play a central role in phenylpropanoid metabolism: the dehydroflavonol-4-reductase (DFR) family (classified SDR108E by Kallberg *et al.* [1]) and the 'isoflavone-reductase' family (reviewed by Ferrer *et al.* [43]). However, none of them are classified as 'classical' SDRs.

Besides DFR, its representative member, which converts flavanones into flavanols [44], the DFR family includes cinnamoyl-CoA reductases, the first reaction of the monolignol biosynthesis pathway [45] and anthocyanidin reductase encoded by the *BANYULS* genes [46]. Like tropinone reductase, this subfamily shows a high diversification pattern. In *Arabidopsis*, 24 proteins belong to this SDR-subfamily, most of them still awaiting functional characterization. Yet, the characterization and further annotation of some CCR-like proteins may be puzzling. A CCR-like NADPH-reductase (which is present as two close isoforms in *Arabidopsis*, tobacco and tomato) was investigated in *Eucalyptus gunnii* [47], Mungbean (*Vigna radiata*, [48]), grapevine [49], tobacco [50] and tomato [51]. Depending on the publications, the authors focused on the reduction of either cinnamaldehyde [47,50], phenylacetaldehyde [51] or the fungal toxin eutypine [48,49]. Although the enzymatic constants (K_m , V_{max}) are variable between the different substrates, these CCR-like proteins can reduce a broad range of aldehydes. However, it can be hypothesized that these NADPH-reductases play specific roles in plant-micro-organisms interaction. In grapevine, this hypothesis is supported by the high affinity of NADPH-reductase towards eutypine, a xenobiotic toxin emitted by the pathogen *Eutypa lata*, and by the fact that over-expression of mungbean eutypine reductase in grapevine increases the detoxification capacity against eutypine [52]. In other species, the level of NADPH-reductase activity may control the release of pollinator-attractive aroma volatiles. The selection and evolution of enzymes may then be controlled by very specific ecological niche interactions.

The second family (enzymes related to isoflavone-reductase) comprises only 9 members in *Arabidopsis thaliana*. Several functions have already been described in various plant species: pinoresinol reductase in *Forsythia x intermedia* [53], eugenol synthase in sweet basil (*Ocimum basilicum*) [54], vestitone reductase in alfalfa (*Medicago sativa*), phenylcoumaran benzylic ether reductase in *Pinus taeda* and *Populus trichocarpa* [55], leucoanthocyanidin reductase in *Arabidopsis* [56]. Many of these enzymes have been crystallized recently [43].

In addition to these two 'extended' SDR subfamilies, a secoisolariciresinol dehydrogenase (SDH) was purified and identified in *Forsythia x intermedia* and *Podophyllum peltatum* [57], the latter being crystallized [58]. This 'classical' NAD-dependent homotetrameric SDR catalyzes the two step lactonization (oxidation) of secoisolariciresinol into matairesinol. Interestingly, this enzyme is closely related to xanthoxine reductase ABA2 (52% identity, Fig. 1B), involved in ABA biosynthesis and also performing a two-steps oxidation as mentioned below.

2.2.4 Terpenoids metabolism

2.2.4.1 Menthol biosynthetic pathway

Monoterpenes represent the major components of essential oils. The monoterpene (-)-menthol and its derivatives are particularly abundant in peppermint (*Mentha x piperita*) and spearmint (*Mentha spicata*). The biosynthetic pathways of menthol and menthol isomers comprise several reduction steps that are catalysed by monomeric reductases of the SDR family (Fig. 1E). Two double-bond NADPH-dependent reductases, (-)-isopiperitenone reductase and (+)-pulegone reductase represent important steps in the biosynthesis of (-)-menthone. The corresponding genes have been isolated and characterized in peppermint [59]. Thereafter, (-)-menthone is reduced into (-)-menthol and (+)-neomenthol by two types of stereospecific and NADPH-dependent menthone reductases: (-)-menthone:(-)-(3R)-menthol reductase (MMR) and (-)-menthone:(-)-(3S)-neomenthol reductase (MNR). The *MMR* and *MNR* genes of peppermint have been isolated and functionally characterized [60]. Both belong to the SDR superfamily. *MNR* genes have later been characterized in pepper (*Capsicum annuum*, *CaMNR1*) and *Arabidopsis* (*AtSDR1*, At3g61220). They share 59% and 54% sequence identity with the peppermint *MNR* gene and increased plant resistance against a broad spectrum of pathogens [61]. Interestingly, *MNR* are closely related to *Papaver* salutaridine reductase (*SalR*), involved in benzylisquinoline alkaloids biosynthesis (Fig. 1E). Ziegler *et al.* [37] formulated a scenario where *MNR* and *SalR* evolved from a common ancestor

potentially involved in pathogen defence. Speciation (after duplication events) of this gene in poppy and peppermint would have led to specific salutaridine or (-)-menthol producing reductases. Spearmint and peppermint monoterpene pathway involves also, in the early step, the NAD-dependent oxidation of isopiperitenone (in peppermint) or carveol (in spearmint). Contrary to the reductions described above, this enzymatic steps involves an (-)-isopiperitenol/(-)-carveol dehydrogenase belonging to the NAD-dependent dehydrogenase cluster (Fig. 1B, [62]). This example illustrates that metabolic diversification may arise from independent enzymatic families.

2.2.4.2 Phytoalexins

When plants are attacked by pathogens, they produce secondary metabolites called phytoalexins which are generally diterpenoids. A gene cluster for the synthesis of momilactones, one type of diterpenoid phytoalexins, has been characterized in rice. The cluster harbours a *SDR* gene encoding a reductase capable of converting 3 β -hydroxy-9 β H-primara-7,15-dien-19,6 β -olide into momilactone A [63].

2.2.5 Plant aromas

Given its chemical diversity and its importance in ecological interactions between plants and animals, volatile compounds must be considered as an important class of secondary metabolites. Several independent pathways are involved in aroma formation: terpenoid and phenolic metabolism, lipids and amino acids degradation [64,65]. However, the diversity of aroma compounds is also linked to the presence of large multigenic families such as acyl-transferases, *O*-methyl-transferases, or Cytochrome P450 mono-oxygenases [66]. In that view, the involvement and diversification of SDR in the monoterpene-derived menthol pathway gives credit to the idea that SDR evolution played a major role in metabolic diversification. Similarly, extended SDR have been described either in the formation of eugenol [54] or in the interconversion of phenylacetaldehyde into 2-phenylethanol [51].

At last, expression analyses in various fruits (melon, strawberries, banana, apricot, [67,68]) report the expression of a well conserved SDR during ripening, its expression being enhanced along other genes involved in aroma biosynthesis (acyltransferases, medium-chain alcohol dehydrogenase). Conversely, in *Arabidopsis*, GENEVESTIGATOR microarray database reveals that the orthologue (At3g01980) is highly expressed in flowers [69]. This SDR, original by its weakly conserved cofactor binding sites or by its 'divergent' catalytic site displays the other features of classical SDR, yet, it still awaits full characterization.

2.2.6 Plant hormones

Plants hormones are synthesized through independent metabolic pathways (steroids, terpenoids, phenolics, lipids catabolism, decarboxylation of tryptophan).

In analogy with animal SDRs largely involved in steroids or prostaglandins synthesis, modifications, or catabolism [3], it may be rational to assume that several plant SDRs play a major role regarding hormone metabolism.

2.2.6.1 ABA biosynthesis

Abscisic Acid (ABA) is a sesquiterpenoid plant hormone involved in the control of many physiological processes such as seed development and germination and in the response to various stresses. The biosynthesis pathway comprises an NAD-dependent conversion of xanthoxin into ABA aldehyde. The enzyme responsible for this conversion is short-chain alcohol dehydrogenase (At1g52340) named ABA2 in *Arabidopsis* [70,71]. Although ABA2 belongs to the 'diversified' NAD-dehydrogenase cluster (Fig. 1B), the ABA2 sequence is well conserved among higher plants (55 % identity between rice and *Arabidopsis*) but is absent in the *Physcomitrella patens* moss. This suggests that ABA biosynthesis emerged as a consequence of higher plant metabolism diversification and was probably selected and conserved when it acquired a major physiological function.

2.2.6.2 Auxin

Indole-3-butyric acid (IBA) and 2,4-dichlorophenoxybutyric acid (2,4-DB) are synthetic auxins that mimic auxin effects in plants. In fact their activity is due to the metabolisation by β -oxidation into indole-3-acetic acid (IAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) respectively [72]. *Arabidopsis* mutants have been identified that are resistant to IBA and 2,4-DB. One of these mutants has been characterized and the encoding protein, identified simultaneously by two separate groups of researchers, was named IBR1 by Zolman *et al.* [73] and SDRa by Wiszniewski *et al.* [72]. IBR1/SDRa (At4g055630.1) protein is a NADP(H)-dependent classical SDR member very distant from other classical SDRs (Fig. 1) but close (49% identity) to the human DHRS4 peroxysomal protein, an SDR thought to be involved either in 3 β -hydroxysteroid reduction or in xenobiotic detoxification [74]. The *IBR1/SDRa* mutant phenotype does not show any alteration of fatty acid metabolism, indicating that the primary role of the encoded protein is in hormone metabolism. The protein possesses a C-terminal peroxisomal-targeting signal and has been localized in the peroxisome by the GFP fusion method [72] or proteomics [75].

2.2.6.3 Role of SDRs in developmental processes via the putative interference in the synthesis or action of hormones

Contrary to ABA2, where a metabolic role has clearly been assigned to a SDR in ABA biosynthesis, several papers describe spectacular developmental perturbations induced by alteration of SDR-encoding genes expression [76-78]. These papers suggest that SDRs may interfere with the biosynthesis, catabolism or action of some hormones. The *TASSELSEED2* gene (*TS2*), originally described in maize as involved in feminization of the flower [78] is representative of this situation. *TS2* belongs to the NAD-dependent dehydrogenase clade (Fig. 1B). Phylogenetic studies in grass lineages [79] have shown that the *TS2*-like family has undergone a large diversification in Monocots. However, the identity of the reaction catalyzed by *TS2* remains enigmatic. Wu *et al.* [80] showed that *TS2* could bind a broad spectrum of substrates, with a probable preference towards steroids. However, the recent positional cloning of *ts1* gene suggests alternative role for *TS2* [81]. Indeed, *TS1* encodes a 13-lipoxygenase involved in jasmonic acid synthesis and application of exogenous jasmonic acid can rescue feminization either in *ts1* or *ts2* mutant rice. The authors proposed that *TS2* could be involved in jasmonic acid (JA) biosynthesis (in the peroxysomal β -oxidation steps), but the activity has not been demonstrated. The elucidation of the accurate function of *TS2* will be of great interest, especially if it highlights the cross-talk between different hormones (GA, JA or steroids for example).

A second example is the *TDF511* potato gene involved in several aspects of plant development including tuber morphology, early sprouting and stolon elongation. The gene shows homology to steroid dehydrogenases but the activity of the encoded protein has not been determined. Nevertheless, it was shown that *TDF511*-inhibited transgenic potatoes had elevated levels of biologically active GAs and their respective precursors [77]. However the direct involvement in GA catabolism has not been demonstrated. Rather, *TDF511* could play an indirect role via the synthesis or breakdown of other hormones.

CONCLUDING REMARKS

The present review shows the diversity of the plant short chain dehydrogenase superfamily. This diversity is first illustrated by the size of the family: in *Arabidopsis*, 92 genes code for 'classical'-SDRs and 98 genes code for 'intermediary', 'extended', 'divergent', 'complex' or 'unknown' SDR. Plants contain SDRs with both prokaryote and eukaryote origins. Some plastid SDRs (POR, FASII) clearly emerged from cyanobacteria as a consequence of the

plastidial endosymbiosis, while other SDRs (hydroxysteroid dehydrogenase, progesterone reductase, fatty acid elongase) are common to all eukaryotes. However, the most striking diversity concerns the functions of the enzymes. While some SDRs are involved in primary metabolism pathways (lipid biosynthesis, chlorophyll synthesis and degradation, folate salvage), a large number of SDRs intervene in all the secondary metabolism routes (steroids, terpenoids, alkaloids, phenolics). In primary metabolism, the enzymes show a clear relationship with other eukaryotes or prokaryotes (for plastid enzymes) homologues, suggesting that these essential functions were conserved during evolution. By contrast, SDRs involved in secondary metabolism often belong to large subfamilies that probably have arisen by several duplication events. Moreover, the observation that stereo- or substrate specificity relies on a small number of amino acids and the coexistence of 'broad' or 'specialised' SDRs among the different subfamilies suggests that during evolution, SDR diversification enabled the development of an enzymatic 'repertoire', some of its members being selected and specialised as they acquired major functions (hormone synthesis, synthesis of abundant metabolite). In that view, the emergence of complex family such as SDR, but also acyltransferase, glucosyltransferase or cytochrome P450-dependent monooxygenases can be considered as a prerequisite to plant chemical diversification.

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REFERENCES

- [1] Kallberg, Y., Oppermann, U., and Persson, B. 2010, *FEBS J.*, 277, 2375.
- [2] Filling, C., Berndt, K. D., Benach, J., Knapp, S., Prozorovski, T., Nordling, E., Ladenstein, R., Jornvall, H., and Oppermann, U. 2002, *J. Biol. Chem.*, 277, 25677.
- [3] Kavanagh, K. L., Jornvall, H., Persson, B., and Oppermann, U., 2008, *Cell Mol Life Sci.* 65, 3895.
- [4] Persson, B., Krook, M., and Jornvall, H. 1991, *Eur. J. Biochem.*, 200, 537.
- [5] Oppermann, U., Filling, C., Hult, M., Shafqat, N., Wu, X., Lindh, M., Shafqat, J., Nordling, E., Kallberg, Y., Persson, B., and Jornvall, H. 2003, *Chem. Biol. Interact.*, 143-144, 247.
- [6] Kallberg, Y., Oppermann, U., Jornvall, H., and Persson, B. 2002, *Eur. J. Biochem.*, 269, 4409.

- [7] Persson, B., Kallberg, Y., Bray, J. E., Bruford, E., Dellaporta, S. L., Favia, A. D., Duarte, R. G., Jornvall, H., Kavanagh, K. L., Kedishvili, N., Kisiela, M., Maser, E., Mindnich, R., Orchard, S., Penning, T. M., Thornton, J. M., Adamski, J., and Oppermann, U. 2009, *Chem. Biol. Interact.*, 178, 94.
- [8] Bray, J. E., Marsden, B. D., and Oppermann, U. 2009, *Chem. Biol. Interact.*, 178, 99.
- [9] Arabidopsis Genome Initiative 2000, *Nature*, 408,796.
- [10] Swarbreck, D., Wilks, C., Lamesch, P., Berardini, T. Z., Garcia-Hernandez, M., Foerster, H., Li, D., Meyer, T., Muller, R., Ploetz, L., Radenbaugh, A., Singh, S., Swing, V., Tissier, C., Zhang, P., and Huala, E., 2008, *Nucleic Acids Res.*, 36, D1009.
- [11] Tamura K, Dudley J, Nei M. and Kumar S. 2007, *Mol. Biol. Evol.*, 24, 1596.
- [12] Croteau, R., Kutchan, T. M., and Lewis, N. G., 2000, *Biochemistry & Molecular Biology of Plants*, Buchanan, B.B., Gruissem, W., Jones, R. (Eds.), American Society of Plant Physiologists, Rockville, 1250.
- [13] Ohlrogge, J.B. and Jaworski, J.G. 1997, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 48, 109.
- [14] O'Hara, P., Slabas, A. R., and Fawcett, T. 2002, *Plant Physiol.*, 129, 310.
- [15] Fisher, M., Kroon, J. T., Martindale, W., Stuitje, A. R., Slabas, A. R., and Rafferty, J. B., 2000, *Structure* 8, 339.
- [16] Rafferty, J. B., Simon, J. W., Baldock, C., Artymiuk, P. J., Baker, P. J., Stuitje, A. R., Slabas, A. R., and Rice, D. W. 1995, *Structure* 3, 927.
- [17] Kunst, L. and Samuels, L. 2009, *Curr. Opin. Plant Biol.*, 12, 721.
- [18] Dietrich, C. R., Perera, M. A., D. Yandean-Nelson M, Meeley, R. B., Nikolau, B. J., and Schnable, P. S. 2005. *Plant J.*, 42, 844.
- [19] Puyaubert, J., Dieryck, W., Costaglioli, P., Chevalier, S., Breton, A., Lessire, R., 2005 *Biochim. Biophys. Acta.* 1687, 152.
- [20] Beaudoin, F., Wu, X., Li, F., Haslam, R. P., Markham, J. E., Zheng, H., Napier, J. A., Kunst, L. 2009, *Plant Physiol.*, 150, 1174.
- [21] Zheng, H., Rowland, O., and Kunst, L. 2005, *Plant Cell* 17, 1467.
- [22] Paddock, T. N., Mason, M. E., Lima, D. F., and Armstrong, G. A. 2010. *Plant Mol. Biol.*, 72, 445.
- [23] Nagata, N., Tanaka, R., Satoh, S., and Tanaka, A. 2005, *Plant Cell*, 17, 233.
- [24] Horie, Y., Ito, H., Kusaba, M., Tanaka, R., Tanaka, A. 2009, *J. Biol. Chem.*, 284, 17449.
- [25] Sato, Y., Morita, R., Katsuma, S., Nishimura, M., Tanaka, A., and Kusaba, M. 2009, *Plant J.*, 57, 120.
- [26] Noiriél, A., Naponelli, V., Bozzo, G. G., Gregory, J. F., and Hanson, A. D. 2007, *Plant J.*, 51, 378.

- [27] Hormann, F., Kuchler, M., Sveshnikov, D., Oppermann, U., Li, Y., Soll, J. 2004, *J. Biol. Chem.*, 279, 34756.
- [28] Rahier, A., Bergdoll, M., Genot, G., Bouvier, F., and Camara, B. 2009, *Plant Physiol.*, 149, 1872.
- [29] Rahier, A., Darnet, S., Bouvier, F., Camara, B., and Bard, M. 2006, *J. Biol. Chem.*, 281, 27264.
- [30] D'Andrea, S., Canonge, M., Beopoulos, A., Jolivet, P., Hartmann, M. A., Miquel, M., Lepiniec, L., Chardot, T. 2007, *Biochimie*, 89, 222.
- [31] Li, F., Asami, T., Wu, X., Tsang, E. W., Cutler, A. J. 2007, *Plant Physiol.*, 145, 87.
- [32] Baud, S., Dichow, N. R., Kelemen, Z., d'Andrea, S., To, A., Berger, N., Canonge, M., Kronenberger, J., Viterbo, D., Dubreucq, B., Lepiniec, L., Chardot, T., and Miquel, M. 2009, *Plant Cell Physiol.*, 50, 1463.
- [33] Thorn, A., Egerer-Sieber, C., Jager, C. M., Herl, V., Muller-Uri, F., Kreis, W., Muller, Y. 2008, *J. Biol. Chem.*, 283, 17260.
- [34] Herl, V., Fischer, G., Reva, V. A., Stiebritz, M., Muller, Y. A., Muller-Uri, F., Kreis, W. 2009, *Biochimie*, 91, 517.
- [35] Ziegler, J., Voigtlander, S., Schmidt, J., Kramell, R., Miersch, O., Ammer, C., Gesell, A., and Kutchan, T. M. 2006, *Plant J.*, 48, 177.
- [36] Geissler, R., Brandt, W., Ziegler, J. 2007, *Plant Physiol.*, 143, 1493.
- [37] Ziegler, J., Facchini, P.J., Geissler, R., Schmidt, J., Ammer, C., Kramell, R., Voigtlander, S., Gesell, A., Pienkny, S., and Brandt, W. 2009, *Phytochemistry*, 70, 1696.
- [38] Nakajima, K., Hashimoto, T., and Yamada, Y. 1993, *Proc. Natl. Acad. Sci. USA*, 90, 9591.
- [39] Nakajima, K., Yamashita, A., Akama, H., Nakatsu, T., Kato, H., Hashimoto, T., Oda, J., and Yamada, Y. 1998, *Proc. Natl. Acad. Sci. USA*, 95, 4876.
- [40] Yamashita, A., Kato, H., Wakatsuki, S., Tomizaki, T., Nakatsu, T., Nakajima, K., Hashimoto, T., Yamada, Y., and Oda, J. 1999, *Biochemistry*, 38, 7630.
- [41] Brock, A., Brandt, W., and Drager, B. 2008, *Plant J.*, 54, 388.
- [42] Wallwey, C., Matuschek, M., and Li, S. M. 2010, *Arch. Microbiol.*, 192, 127.
- [43] Ferrer, J. L., Austin, M. B., Stewart, C., Jr., and Noël, J. P. 2008, *Plant Physiol. Biochem.*, 46, 356.
- [44] Petit, P., Granier, T., d'Estaintot, B. L., Manigand, C., Bathany, K., Schmitter, J. M., Lauvergeat, V., Hamdi, S., Gallois, B. 2007, *J. Mol. Biol.*, 368, 1345.
- [45] Lacombe, E., Hawkins, S., Van Doorselaere, J., Piquemal, J., Goffner, D., Poeydomenge, O., Boudet, A. M., Grima-Pettenati, J. 1997, *Plant J.*, 11, 429.

- [46] Xie, D. Y., Sharma, S. B., Paiva, N. L., Ferreira, D., and Dixon, R. A. 2003, *Science*, 299, 396.
- [47] Goffner, D., Van Doorselaere, J., Yahiaoui, N., Samaj, J., Grima-Pettenati, J., and Boudet, A. M. 1998, *Plant Mol. Biol.*, 36, 755.
- [48] Guillen, P., Guis, M., Martinez-Reina, G., Colrat, S., Dalmayrac, S., Deswarte, C., Bouzayen, M., Roustan, J. P., Fallot, J., Pech, J. C., and Latché, A. 1998, *Plant J.*, 16, 335.
- [49] Colrat, S., Latché, A., Guis, M., Pech, J. C., Bouzayen, M., Fallot, J., and Roustan, J. P., 1999. *Plant Physiol.*, 119, 621.
- [50] Damiani, I., Morreel, K., Danoun, S., Goeminne, G., Yahiaoui, N., Marque, C., Kopka, J., Messens, E., Goffner, D., Boerjan, W., Boudet, A. M., Rochange, S., 2005. *Plant Mol. Biol.*, 59, 753.
- [51] Tieman, D. M., Loucas, H. M., Kim, J. Y., Clark, D. G., and Klee, H. J. 2007, *Phytochemistry*, 68, 2660.
- [52] Afifi, M., El-Kereamy, A., Legrand, V., Chervin, C., Monje, M. C., Nepveu, F., and Roustan, J. P. 2003, *J. Plant Physiol.*, 160, 971.
- [53] Dinkova-Kostova, A. T., Gang, D. R., Davin, L. B., Bedgar, D. L., Chu, A., and Lewis, N. G. 1996, *J. Biol. Chem.*, 271, 29473.
- [54] Koeduka, T., Fridman, E., Gang, D. R., Vassao, D. G., Jackson, B. L., Kish, C. M., Orlova, I., Spassova, S. M., Lewis, N. G., Noel, J. P., Baiga, T. J., Dudareva, N., and Pichersky, E. 2006, *Proc. Natl. Acad. Sci. USA*, 103, 10128.
- [55] Min, T., Kasahara H., Bedgar, D.L., Youn, B., Lawrence, P.K., Gang, D.R., Halls, S.C., Park, H., Hilsenbeck, J.L., Davin, L.B., and Lewis NG, Kang C. 2003, *J. Biol. Chem.*, 278, 50714.
- [56] Abrahams, S., Lee, E., Walker, A. R., Tanner, G. J., Larkin, P. J., Ashton, A. R. 2003, *Plant J.* 35, 624.
- [57] Xia, Z. Q., Costa, M. A., Pelissier, H. C., Davin, L. B., Lewis, N. G. 2001, *J. Biol. Chem.*, 276, 12614.
- [58] Youn, B., Moinuddin, S. G., Davin, L. B., Lewis, N. G., and Kang, C. 2005, *J. Biol. Chem.*, 280, 12917.
- [59] Ringer, K. L., McConkey, M. E., Davis, E. M., Rushing, G. W., and Croteau, R. 2003, *Arch. Biochem. Biophys.*, 418, 80.
- [60] Davis, E. M., Ringer, K. L., McConkey, M. E., Croteau, R. 2005, *Plant Physiol.*, 137, 873.
- [61] Choi, H. W., Lee, B. G., Kim, N. H., Park, Y., Lim, C. W., Song, H. K., and Hwang, B. K. 2008, *Plant Physiol.*, 148, 383.
- [62] Ringer, K. L., Davis, E. M., Croteau, R. 2005, *Plant Physiol.*, 137, 863.
- [63] Shimura, K., Okada, A., Okada, K., Jikumaru, Y., Ko, K. W., Toyomasu, T., Sassa, T., Hasegawa, M., Kodama, O., Shibuya, N., Koga, J., Nojiri, H., and Yamane, H. 2007, *J. Biol. Chem.*, 282, 34013.

- [64] Pichersky, E., Noel, J. P., Dudareva, N. 2006, *Science*, 311, 808.
- [65] Pech, J. C., Latché, A., van der Rest, B. 2008, *Fruit and Vegetable Flavour*, Brückner, B. and Grant Wyllie, S. (Eds.), Woodhead Publishing Limited, Cambridge, 254.
- [66] Gang, D. R. 2005, *Annu. Rev. Plant Biol.*, 56, 301.
- [67] Manriquez, D., El-Sharkawy, I., Flores, F. B., El-Yahyaoui, F., Regad, F., Bouzayen, M., Latché, A., and Pech, J. C. 2006, *Plant Mol. Biol.*, 61, 675.
- [68] Gonzalez-Aguero, M., Troncoso, S., Gudenschwager, O., Campos-Vargas, R., Moya-Leon, M. A., and Defilippi, B. G. 2009, *Plant Physiol. Biochem.*, 47, 435.
- [69] Zimmermann P, Hirsch-Hoffmann M, Hennig L, and W Gruissem. 2004, *GENEVESTIGATOR: Plant Physiology*, 136, 2621.
- [70] Cheng, W. H., Endo, A., Zhou, L., Penney, J., Chen, H. C., Arroyo, A., Leon, P., Nambara, E., Asami, T., Seo, M., Koshiba, T., Sheen, J. 2002, *Plant Cell*, 14, 2723.
- [71] Gonzalez-Guzman, M., Apostolova, N., Belles, J. M., Barrero, J. M., Piqueras, P., Ponce, M. R., Micol, J. L., Serrano, R., Rodriguez, P. L. 2002, *Plant Cell*, 14, 1833.
- [72] Wiszniewski, A. A., Zhou, W., Smith, S. M., Bussell, J. D. 2009, *Plant Mol. Biol.*, 69, 503.
- [73] Zolman, B. K., Martinez, N., Millius, A., Adham, A. R., and Bartel, B. 2008, *Genetics*, 180, 237.
- [74] Matsunaga, T., Endo, S., Maeda, S., Ishikura, S., Tajima, K., Tanaka, N., Nakamura, K. T., Imamura, Y., Hara, A. 2008, *Arch. Biochem. Biophys.*, 477, 339.
- [75] Reumann, S., Babujee, L., Ma, C., Wienkoop, S., Siemsen, T., Antonicelli, G.E., Rasche, N., Luder, F., Weckwerth, W., and Jahn, O. 2007, *Plant Cell*, 19, 3170.
- [76] Callos J.D., DiRado M, Xu B, Behringer F.J., Link BM, Medford J.I. 1994, *Plant J.*, 6, 835.
- [77] Bachem, C. W., Horvath, B., Trindade, L., Claassens, M., Davelaar, E., Jordi, W., Visser, R. G. 2001, *Plant J.*, 25, 595.
- [78] DeLong, A., Calderon-Urrea, A., and Dellaporta, S. L. 1993, *Cell* 74, 757.
- [79] Malcomber, S.T. and Kellogg, E.A. 2006, *New Phytol.*, 170, 885.
- [80] Wu, X., Knapp, S., Stamp, A., Stammers, D. K., Jornvall, H., Dellaporta, S. L., and Oppermann, U. 2007, *Mol. Cell. Endocrinol.*, 265, 71.
- [81] Acosta, I. F., Laparra, H., Romero, S. P., Schmelz, E., Hamberg, M., Mottinger, J. P., Moreno, M. A., and Dellaporta, S. L. 2009, *Science*, 323, 262.