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Gibberellins are produced by all vascular plants and several fungal and bacterial species that associate with plants as pathogens or symbionts. In the 60 years since the first experiments on the biosynthesis of gibberellic acid in the fungus *Fusarium fujikuroi*, research on gibberellin biosynthesis has advanced to provide detailed information on the pathways, biosynthetic enzymes and their genes in all three kingdoms, in which the production of the hormones evolved independently. Gibberellins function as hormones in plants, affecting growth and differentiation in organs in which their concentration is very tightly regulated. Current research in plants is focused particularly on the regulation of gibberellin biosynthesis and inactivation by developmental and environmental cues, and there is now considerable information on the molecular mechanisms involved in these processes. There have also been recent advances in understanding gibberellin transport and distribution and their relevance to plant development. This review describes our current understanding of gibberellin metabolism and its regulation, highlighting the more recent advances in this field.

**Keywords:** Gibberellin metabolism.

**Introduction**

The name gibberellin encompasses a large group of diterpenoid carboxylic acids that are classified as such according to their structure. They were first discovered as metabolites of the fungus *Gibberella fujikuroi*, reclassified as *Fusarium fujikuroi*, that promoted growth in higher plants, and their suspected presence in plants as natural hormones was confirmed in the late-1950s (MacMillan and Suter 1958). The fungal gibberellins were given the trivial names gibberellin A₁, A₂, etc. (Takahashi et al. 1955), a system that was adopted for gibberellins from all sources, with numbers being assigned in order of discovery and structural characterization (MacMillan and Takahashi 1968). Currently, 136 gibberellins have been assigned numbers, but the last to be characterized was over 15 years ago and it is unlikely that this system of nomenclature will be continued.

The most abundant gibberellin present in *F. fujikuroi* and the first to be structurally characterized is gibberellin A₃, which is also known as gibberellic acid (Curtis and Cross 1954). This gibberellin is produced on an industrial scale in fungal cultures for application in agriculture, the largest use being in the production of seedless grapes (Rademacher 2016). However, although it is present in some higher plant species as a minor gibberellin, there is little evidence that gibberellic acid plays an important role in plants.

It is now common practice to abbreviate gibberellin A₃ as GA₃, with the generic abbreviation GA used for gibberellin. This has resulted in some confusion, with many workers assuming that GA is an abbreviation for gibberellin acid, i.e. GA₃. The name gibberellin A was used in early gibberellin research on the fungal metabolites to distinguish it from a second biologically active fraction, which was named gibberellin B, although the identity of this material is still unclear (Yabuta and Sumiki 1938). An unfortunate consequence is that gibberellic acid has become synonymous with gibberellin and its concentration rather than that of the more relevant biologically active compounds GA₁ and GA₃ is frequently measured in plant tissues. GA₃ differs from GA₁ in possessing a double bond between C-atoms 1 and 2 (see Fig. 1), which protects it from 2β-hydroxylation, a major mechanism for inactivating GAs in higher plants (see below). An inability to regulate the concentration of GA₃ by this mechanism may explain its absence or low levels in plant organs. In contrast, its production by the phytopathogenic *F. fujikuroi* would benefit the fungus by compromising the plant host’s ability to protect itself from a high GA dosage.

Gibberellins are thought to be present in all vascular plants: in lower plants, such as lycophytes and ferns, GAs are involved in reproductive development (Tanaka et al. 2014, Miyazaki et al. 2018), whereas in higher plants GA function has expanded to the promotion of organ growth through enhanced cell elongation and/or cell division and, in many species, activation of developmental processes, such as seed germination, maturation and induction of flowering (Sponsel 2016). Gibberellins are also produced by some fungal and bacterial species that associate with plants, either as pathogens or symbionts. In these cases, GAs appear to have no developmental function in the producing organism but act on the plant host to aid infection by suppressing immunity (Navarro et al. 2008, Wiemann et al. 2013, Lu et al. 2015, Pieterse et al. 2014) or, in the case of nitrogen-fixing rhizobium bacteria, to regulate nodule formation (Tatsukami and Ueda 2016, McAdam et al. 2018). Remarkably, within the three kingdoms, plants, fungi and bacteria, the ability to synthesize these
complex molecules has been acquired independently by convergent evolution (Nett et al. 2017). Furthermore, the ability to inactivate GAs by 2β-hydroxylation is present only in higher plants: gymnosperms and angiosperms. Our knowledge of GA biosynthesis and signal transduction has progressed rapidly in recent years, enabled particularly by advances in molecular genetics and the utilization of mutants, either naturally occurring or created through forward or reverse genetic approaches. This review describes our current understanding, including the more recent developments. A historical account can be found in Hedden and Sponsel (2015). Fig. 1 provides an overview of GA biosynthesis in the three kingdoms, plants, fungi and bacteria, highlighting the distinct enzymes utilized for the same or similar reactions, while a more detailed biosynthetic pathway in plants is presented in Figs. 2, 3. Fig. 1 also includes the C-atom numbering system.

**Formation of ent-Kaurene**

As diterpenoids, GAs are formed from trans-geranylgeranyl diphosphate (GGPP), which is cyclized in two steps to the tetracyclic hydrocarbon precursor ent-kaurene via ent-copalyl diphosphate (Fig. 2). In plants, ent-kaurene is formed in plastids, predominantly via the methylerythritol 4-phosphate (MEP) pathway, although there is some contribution from the mevalonic acid (MVA) pathway, presumably dependent on the influx of isoprenoid intermediates of GGPP synthesis into the plastids from the cytosol (Kasahara et al. 2002, Flugge and Gao 2005). Aach et al. (1995) and Aach et al. (1997) have shown that ent-kaurene formation from GGPP occurs in the stroma of proplastids or developing chloroplasts, but not in mature chloroplasts. The proplastid inner membrane is more amenable to the import of small molecules than is the chloroplast membrane (Brautigam and Weber 2009), which could also enable crossover between the two terpene pathways. In immature plastids, there is likely to be less competition for GGPP from the major pathways of chlorophyll and carotenoid formation than in chloroplasts. The question of how GGPP is allocated to the different pathways and the potential role of channeling has attracted some attention (Beck et al. 2013, Ruiz-Sola et al. 2016, Zhou et al. 2017). Of the 10 functional GGPP synthase (GGPPS) genes in Arabidopsis thaliana (Arabidopsis), seven encode plastid-localized enzymes (Beck et al. 2013), of which GGPPS11 is most strongly and constitutively expressed and is suggested by Ruiz-Sola et al. (2016) to provide most of the
substrate for the biosynthesis of photosynthesis-related terpenoids in this species. However, on the basis of gene co-expression networks and mutant analysis, Ruiz-Sola et al. concluded that GGPPS11 was unlikely to contribute to GA biosynthesis, for which one or more of the minor enzymes may be responsible. Interestingly, expression of four of these associated more closely with genes of the MVA pathway than with those of the MEP pathway, suggesting that they may obtain their substrates from the cytosol. Ruiz-Sola et al. (2016) demonstrated that GGPPS11 interacted physically with enzymes involved in the biosynthesis of chlorophyll, carotenoids and plastoquinone and may form part of enzyme complexes, indicating the channeling of GGPP to these light harvesting components.

In contrast to Arabidopsis, rice is reported to contain one functional plastidic GGPPS, which must therefore be responsible for the biosynthesis of all diterpenoids in the plastid, including ent-kaurene (Zhou et al. 2017). The rice GGPPS resides as a homodimer in the plastid stroma, but by forming a heterodimer with OsGRP (GGPPS recruiting protein), it is recruited to the thylakoid membrane, where it forms part of a protein complex involved in the biosynthesis of chlorophyll and other components of the light harvesting machinery. Distribution of GGPPS between the thylakoid and stroma (e.g. for the biosynthesis of GAs) would therefore depend on the abundance of OsGRP, which is related to the small subunit of type-II geranyldiphosphate synthases (GPS). In contrast to OsGRP, which would divert GGPPS from GA biosynthesis, a functional GPS is necessary for GA biosynthesis in tomato and Arabidopsis (van Schie et al. 2007). The tomato GPS produced geranyl diphosphate and farnesyl diphosphate, but little GGPP from isopentenyl diphosphate and dimethylallyl diphosphate in vitro so must act in association with a GGPPS.

The two-step conversion of GGPP to ent-kaurene, proceeds by proton-initiated cyclization to the dicyclic ent-copalyl diphosphate (CPP) catalyzed by a type-II diterpene cyclase, ent-copalyl diphosphate synthase (CPS). Enzymes of this type contain a conserved DXDD motif, the middle aspartate donating a proton to initiate cyclization, while a water molecule coordinated to histidine and asparagine acts as the catalytic base to accept a proton and terminate the reaction (Koksal et al. 2014, Lemke et al. 2019). Using recombinant N-terminally truncated Arabidopsis CPS, Prisic and Peters (2007) demonstrated that enzyme activity was modified by Mg\(^{2+}\) and GGPP concentrations in a biphasic manner, with high concentrations of both inhibiting activity synergistically. As the concentration of Mg\(^{2+}\) and GGPP in plastids is promoted by light, Prisic and Peters (2007) suggested that this feedforward regulation of CPS activity reduced the flux into the GA pathway during deetiolation as part of the mechanism to decrease GA concentration. Mann et al. (2010) noted that a conserved histidine residue in CPS enzymes involved in GA biosynthesis was associated with this inhibition by Mg\(^{2+}\), whereas type-II diterpene cyclases involved in secondary metabolism, which are less sensitive to Mg\(^{2+}\) inhibition, contain arginine at the equivalent position. The authors proposed that these basic residues act as a counter ion to the DXDD motif and differentially influence the binding of Mg\(^{2+}\) to this motif, which, while necessary for enzyme activity, is inhibitory at higher concentrations. The second step by which ent-copalyl diphosphate is converted to ent-kaurene is catalyzed by a type-I cyclase, ent-kaurene synthase (KS). In this reaction, cyclization is initiated by metal-dependent heterolytic cleavage of the C–O bond to form a pimeren-8-yl carbocation, which undergoes rearrangement and loss of H\(^{+}\) to form the tetracyclic ent-kaurene (Zi et al. 2014). In common with other type-I terpene cyclases, KS contains DXXDD and RLX(N,D)DXX(S,T,G)XXX(E,D) motifs, which coordinate Mg\(^{2+}\) ions that associate with the diphosphate residue and participate in its ionization (Yamaguchi et al. 1996, Zhou and Peters 2009, Liu et al. 2014). Localization of CPS and KS in plastids was confirmed by Helliwell et al. (2001) using protein-GFP fusions. Furthermore, CPS contains an N-terminal plastid-targeting sequence, which is cleaved on entry to the plastid, the resulting protein being more catalytically active in vitro than the uncleaved precursor (Sun and Kamiya 1994, Sun and Kamiya 1997). KS also contains a putative plastid-targeting sequence (Yamaguchi et al. 1996, Yamaguchi et al. 1998), although import into plastids has not been demonstrated.

In the moss Physcomitrella patens, which synthesizes ent-kaurenoic acid derivatives, but not GAs (Miyazaki et al. 2018),
CPS and KS activities are present as a single bifunctional protein containing both the DxDD and DDXD motifs (Hayashi et al. 2006). The lycophyte Selaginella moellendorfii, thought to be one of the earliest plants to have evolved the capability of synthesizing GAs, has monofunctional CPS and KS enzymes (Shimane et al. 2014), which is a characteristic of vascular plants. While gymnosperms use bifunctional diterpene synthases to produce resin acids, ent-kaurene is formed by the two monofunctional enzymes (Keeling et al. 2010). The more primitive KS activities in P. patens and S. moellendorfii were found to have relatively low substrate specificity, converting different stereoismeric forms of CPP to a range of products, while angiosperm KS enzymes are specific for ent-CPP (Shimane et al. 2014). In angiosperms, CPS and KS have undergone considerable gene expansion and functional diversification to produce diterpenoids involved in plant defence, as has been particularly well documented in cereals (Peters 2006, Xu et al. 2007, Wu et al. 2012, Zhou et al. 2012, Fu et al. 2016, Murphy et al. 2018, Ding et al. 2019).

In GA-producing bacteria and fungi, the genes for GA biosynthesis are clustered in operons, which in most cases include a GGPPS gene dedicated to the GA pathway (Malonek et al. 2019). While the fungal operons contain a bifunctional CPS/KS, in bacteria these activities are separate, as in vascular plants (Moronne et al. 2009). On the basis of conservation of the catalytic amino acid dyad in CPS between bacteria and plants, but not fungi, Lemke et al. (2019) suggested a common origin of ent-kaurene synthesis for bacteria and plants, perhaps reflecting the endosymbiotic origin of chloroplasts. The bacterial and plant enzymes share some sequence and structural homology, but the bacterial enzymes are smaller than those in plants. As discussed by Moronne et al. (2009), fusion between CPS and KS may have occurred in early plant evolution, resulting in the bifunctional CPS/KS in bryophytes, such as P. patens, with gene duplication and respective loss of CPS or KS activities to produce separate enzyme activities occurring in lycophytes. This is supported by the considerable sequence homology between CPS and KS in vascular plants (Yamaguchi et al. 1996). As noted above, bifunctional diterpene synthases have been retained in conifers, but not for GA biosynthesis.

**Formation of C20-Gibberellins**

The conversion of ent-kaurene to GA12, the first C20-GA on the biosynthetic pathway, is catalyzed by two cytochrome P450 monooxygenases, ent-kaurene oxidase (KO) and ent-kaurenoic acid oxidase (KAO) (Helliwell et al. 1999, Hellwell et al. 2001) (see Fig. 2). Hellwell et al. (2001) showed that the Arabidopsis KO associates with the outer chloroplast membrane and possibly also the endoplasmic reticulum (ER), while the two Arabidopsis KAOs were located at the ER. This suggests that ent-kaurene is oxidized as it exits the plastid onto the ER, potentially through a membrane connection that allows the trafficking of nonpolar metabolites between the plastid and the ER (Meyers et al. 2013, Block and Jouhet 2015). KO, which in plants belongs to the subfamily CYP701A, catalyzes the three-step oxidation of ent-kaurene to ent-kaurenoic acid by repeated hydroxylation of C-19, with the intermediate diol apparently undergoing dehydration to form the aldehyde (ent-kaurenal) before further hydroxylation to ent-kaurenoic acid (Morrone et al. 2009, Nagel and Peters 2017). On the basis of kinetic analysis, it was concluded that the two intermediates in the reaction remain at the enzyme active site and that the first hydroxylation to ent-kaurenoic is the rate-limiting step (Moronne et al. 2009). In common with CPS and KS, the KO-like genes have proliferated in cereals with functional diversification to produce diterpenoids involved in plant defence. Rice has five KO-like genes arranged in tandem on chromosome 6, of which OsKO1, OsKO2 and OsKO5 (encoding CYP701A7, CYP701A6 and CYP701A9, respectively) are involved in GA biosynthesis (Itoh et al. 2004, Sakamoto et al. 2004, Chen et al. 2019, Zhang et al. 2020), while OsKO4 (CYP701A8) hydroxylates ent-kaurene and related diterpenes at the 3α position, which is in close proximity to C-19 (Wang et al. 2012). By coupling the N-terminal regions to GFP, Zhang et al. (2020) showed that OsKO2, similar to AtKO, is located in the plasmid outer membrane, whereas OsKO1 is present in the plastid and endomembranes. While the wheat KO family has not been functionally characterized, it is of interest to note that treatment of wheat seedlings with the KO-inhibitor paclobutrazol resulted in the accumulation inter alia of 3α-hydroxy-ent-kaurene (Croker et al. 1995), which is probably a product of a KO paralog. The fungal and bacterial KOs belong to the CYP503 (Tudzynski et al. 2001) and CYP117 (Nett et al. 2017) families, respectively, and are not closely related to the plant enzymes or to each other.

The reaction sequence catalyzed by KAO has been studied mainly in cell-free systems from developing seeds, particularly from endosperm of Cucurbitaceae, and from cultures and cell-free systems from the fungus F. fujikuroi (reviewed in Hedden and Sponsel 2015). In plants, the product of KAO (CYP88A subfamily) is GA12, which is formed from ent-kaurenoic acid in three steps via 7β-hydroxy-ent-kaurenoic acid and GA12-aldehyde. The first step involves stereospecific hydroxylation on C-7β, while the second reaction, in which ring B contracts from 6 to 5 carbon atoms through migration of the C-7–C-8 bond from C-7 to C-6 with the resulting extrusion of C-7 as the aldehyde, is initiated by stereospecific loss of the 6β-H (Graebe et al. 1975, Castellaro et al. 1990). In the final step, GA12-aldehyde is oxidized to GA12. The Cucurbitaceae Cucurbita maxima (pumpkin) and Cucumis sativa (cucumber) contain GA 7-oxidases (GA7ox), which are soluble 2-oxoglutarate-dependent dioxygenases (2-ODDs) that convert GA12-aldehyde to GA12, although they also have other activities. They are present particularly in developing seeds (Lange et al. 1994, Frisse et al. 2003, Lange et al. 2013) but are also expressed in vegetative tissues, including the roots (Lange et al. 2005, Sun et al. 2018). The species distribution of GA7ox appears to be restricted, with apart from the Cucurbitaceae, two reports of their presence in potato (Fixen et al. 2012, Katsarou et al. 2016).

The fungal KAO (belonging to the CYP68A subfamily) produces GA14, rather than GA12, by catalyzing an additional 3β-
hydroxylation (Rojas et al. 2001). The substrate for this reaction is thought to be GA12-aldehyde, to produce GA13α-aldehyde, and not GA12 as, in contrast to GA14-aldehyde and GA16, GA12 is not converted to 3β-hydroxyGAs by fungal cultures (Bearder et al. 1975). The seed and fungal KAOs possess remarkable multifunctionality, producing numerous by-products, of which the seco-ring B compounds fujieland fujenoic acid are major metabolites (Rojas et al. 2001). Fujienal results from oxidative ring cleavage of 6β,7β-dihydroxy-ent-kaurenoid acid, which is formed from 7β-hydroxy-ent-kaurenoid acid by stereospecific hydroxylation at C-6β (Castellaro et al. 1990). Ring contraction and hydroxylation are, thus, competing outcomes following the initial removal of the 6β-H to form a radical or carbocation (Graebe et al. 1975, Nett et al. 2016). A second group of by-products are the kaurenolides, which contain a C-19,6α lactone and a 7β-hydroxy group. They are formed from ent-kaurenoid acid via ent-kaura-6,16-dienoic acid, which is proposed to be converted to 7β-hydroxykaurenolide by nonenzymatic reaction of the C-4α carbonyl (C-19) with an intermediate 6β,7β-epoxide (Hedden and Graebe 1981, Beale et al. 1982). Dehydrogenation at C-6β, in the formation of ent-kaura-6,16-dienoic acid occurs with stereospecific removal of 7β-H, but non-stereospecific loss of H from C-6 (Castellaro et al. 1990). These ent-kaurenoid by-products are not converted to GAs, their formation detracts seriously from GA formation. However, it is notable that they are formed in plant organs and the fungus, which produce large quantities of GAs and related ent-kaurenoids with uncertain functions in the producing organs/organism. Only 7β-hydroxy-ent-kaurenoid acid and GA12 were formed when KAO from Arabidopsis and barley was incubated with ent-kaurenoid acid after expression in yeast (Hellwell et al. 2001). It is possible that KAOs in plant tissues that produce GAs in hormonal quantities exert much tighter catalytic control over the reaction, or that the reaction outcome is sensitive to substrate concentration.

In the bacterial GA operon, a CYP114 family P450 is responsible for the KAO activity (Nagel and Peters 2017, Nett et al. 2017). It converts ent-kaurenoid acid only to GA13α-aldehyde, which is oxidized to GA12 by a short-chain alcohol dehydrogenase, encoded from the operon. Full CYP114 activity is dependent on electrons from a dedicated ferredoxin within the operon, which is oxidized to GA12-aldehyde, to produce GA13α-aldehyde, and not GA12 as, in contrast to GA14-aldehyde and GA16, GA12 is not converted to 3β-hydroxyGAs by fungal cultures (Bearder et al. 1975). The seed and fungal KAos possess remarkable multifunctionality, producing numerous by-products, of which the seco-ring B compounds fujieland fujenoic acid are major metabolites (Rojas et al. 2001). Fujienal results from oxidative ring cleavage of 6β,7β-dihydroxy-ent-kaurenoid acid, which is formed from 7β-hydroxy-ent-kaurenoid acid by stereospecific hydroxylation at C-6β (Castellaro et al. 1990). Ring contraction and hydroxylation are, thus, competing outcomes following the initial removal of the 6β-H to form a radical or carbocation (Graebe et al. 1975, Nett et al. 2016). A second group of by-products are the kaurenolides, which contain a C-19,6α lactone and a 7β-hydroxy group. They are formed from ent-kaurenoid acid via ent-kaura-6,16-dienoic acid, which is proposed to be converted to 7β-hydroxykaurenolide by nonenzymatic reaction of the C-4α carbonyl (C-19) with an intermediate 6β,7β-epoxide (Hedden and Graebe 1981, Beale et al. 1982). Dehydrogenation at C-6β, in the formation of ent-kaura-6,16-dienoic acid occurs with stereospecific removal of 7β-H, but non-stereospecific loss of H from C-6 (Castellaro et al. 1990). These ent-kaurenoid by-products are not converted to GAs, their formation detracts seriously from GA formation. However, it is notable that they are formed in plant organs and the fungus, which produce large quantities of GAs and related ent-kaurenoids with uncertain functions in the producing organs/organism. Only 7β-hydroxy-ent-kaurenoid acid and GA12 were formed when KAO from Arabidopsis and barley was incubated with ent-kaurenoid acid after expression in yeast (Hellwell et al. 2001). It is possible that KAOs in plant tissues that produce GAs in hormonal quantities exert much tighter catalytic control over the reaction, or that the reaction outcome is sensitive to substrate concentration.

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**Gibberellin Biosynthesis from GA12**

The formation of the bioactive end products of the pathway from GA12 involving mainly 2-ODD enzymes in plants is illustrated in Fig. 3. The pathway branches from GA12, 13-hydroxylation to GA3, initiating the formation of 13-hydroxylated GAs, such as GA4, while a parallel non-13-hydroxylation pathway from GA12 results in GA3 formation.

**GA 13-hydroxylation and related activities**

13-Hydroxylation of GA12 in rice was shown by Magome et al. (2013) to be catalyzed by two cytochrome P450s, CYP714B1 and CYP714B2. Although vegetative tissues of rice contain predominantly 13-hydroxylated GAs (Kobayashi et al. 1988), the overexpression of either CYP714B gene in rice, which resulted in an increase in the concentration of 13-hydroxy GAs, including GA1, caused semidwarfism (Magome et al. 2013). This finding prompted Magome et al. to suggest that 13-hydroxylation was a mild inactivating reaction that adjusted the balance of bioactive GAs in favor of GA1 relative to the more active GA4. Higher biological activity of GA4 is in accord with the properties of the rice GA receptor, Gi1D, which has a greater affinity for GA4 than for the 13-hydroxy GAs GA1 and GA3 (Ueguchi-Tanaka et al. 2005), although GA1 and GA3 have similar activities in rice bioassays, while GA4 is considerably more active (Crozier et al. 1970, Nishijima and Katsura 1989). This anomaly may be related to differences in the efficiency of transport, or inactivation between the GAs when applied in bioassays.

Arabidopsis contains two members of the CYP714A subfamily, which are expressed in developing seeds (Zhang et al. 2011, Nomura et al. 2013). CYP714A1 converts GA12 to 16α-carboxy-17-norGA12 and caused severe dwarfism when overexpressed in Arabidopsis. CYP714A2, which caused mild dwarfism when overexpressed, converts GA12 to 12α-hydroxyGA12 (GA111) and, to a small extent, GA3 (by 13-hydroxylation) and also 13-hydroxylates ent-kaurenoid acid to form steviol (Nomura et al. 2013). Another CYP714 family member, CYP714D1, known also as ELONGATED UPPERMOST INTERNODE (EUI), present in rice, epoxidizes the 16,17-double bond of 13-deoxyGAs, including GA12, to form inactive products (see below) (Zhao et al. 2006). Thus, CYP714 family members have a generally GA-inactivating function by oxidizing GAs and/or ent-kaurenoids on the C and D rings (see below). It was reported recently that some members of the CYP72A subfamily have a similar function (He et al. 2019). Arabidopsis contains eight tandem CYP72A genes, one of which, CYP72A9, was shown by heterologous expression in yeast to 13-hydroxylate GA12 as well as GA1 and GA3. It also acted on ent-kaurenoid acid, but in this case 13-hydroxylation was a minor activity, the major product being the 16α,17-dihydroxy derivative, presumably formed via epoxidation of the 16,17-double bond. Most Arabidopsis organs contain much higher amounts of GA4 than of GA1, the exception being developing seeds, in which the CYP72A genes are most highly expressed. He et al. (2019) suggested that CYP72A9 and some paralogs may be the major source of 13-hydroxy GAs in the seed. They determined the function of CYP72A family members in Arabidopsis and other species and showed that some have related activities, although usually with a more restricted substrate range. Indeed, many of the enzymes for which activity could be shown acted only on ent-kaurenoid acid. Overexpression of CYP72A9, but not its paralogs, in Arabidopsis resulted in strong dwarfism, while seeds of cyp72a9 mutants germinated more rapidly than those of the wild type without stratification, suggesting that CYP72A9, in common with other GA-inactivating enzymes in seeds, may have a role in promoting dormancy (He et al. 2019). Low levels of 13-hydroxylase activity have also been noted for certain 2-ODDs for which the primary function is GA 3β-hydroxylase activity, such as the wheat enzyme TaGA3ox2 (Appleford...
et al. 2006) and MmGA3ox2 from *Marah macrocarpus* (Ward et al. 2010). As C-3 and C-13 are spatially widely separated on the GA molecule, 13-hydroxylation requires that the orientation of the substrate in the active site is rotated horizontally from that required for 3β-hydroxylation. Interestingly, a 2-ODD from *Tripterygium wilfordii* functions as a 13-hydroxylase, converting GA9 to GA20, but did not act on GA4, while other substrates were not tested (Zhang et al. 2019). Phylogenetic analysis showed that the *T. wilfordii* enzyme is related most closely to the GA 3-oxidases (GA3ox).

A novel 2-ODD activity acting on GA12 in Arabidopsis was reported recently by two groups (Xiong et al. 2018, Liu et al. 2019). The gene, named GIM2 (Xiong et al. 2018) or GAS2 (Liu et al. 2019), both corresponding to At2g36690, encodes a 2-ODD and promotes seed germination when overexpressed. It was shown by both groups that the enzyme expressed in *Escherichia coli* acts on GA12, but in one case it produced an unidentified hydroxyGA12 (Xiong et al. 2018), while in the other it hydrated the 16,17-double bond (Liu et al. 2019), which is an unusual 2-ODD activity. Overexpression of GIM2 produced on overall increase in the GA content including that of GA4 in germinating seed, with reduced GA levels in a gim2 mutant, while GAS2 overexpression reduced the GA4 content in seeds while increasing the concentration of hydrated GA12. As both groups were working with the same gene, the discrepancy in their results is difficult to explain. There must still be some uncertainty about the function of this enzyme and whether GA12 is its only substrate.

**GA 20-oxidases**

The conversion of GA12 and GA53 to GA9 and GA20, respectively, is catalyzed in plants by a family of 2-ODDs, known as GA 20-oxidases (GA20ox), that cleave C-20 with the formation of the 19,10-γ-lactone characteristic of C19-GAs. In the reaction sequence, the C-20 methyl is oxidized to the alcohol and then to the aldehyde, from which C-20 is lost. The alcohol and aldehyde intermediates accumulate and are efficiently converted further, which is consistent with the mechanism of 2-ODD enzymes, in which the reaction products are released from the enzyme active site before the substrates are rebound for the next round of oxidation (Myllyla et al. 1977). The alcohol intermediates, i.e. GA15 and GA44 for the 13-H and 13-OH

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**Fig 3** Final stage of GA biosynthesis to the biologically active end products GA1 and GA4 in plants catalyzed by the 2-oxoglutarate-dependent dioxygenases GA20ox and GA3ox. Reactions producing minor by-products of GA20ox activity (GA25 and GA17) and of GA3ox activity (GA7 and GA3), present in some species, are shown in gray. Biologically active GAs are highlighted in a green box.
forms, respectively, are isolated as the 19,20-δ-lactones from plant tissues, probably due to spontaneous lactonization under the low pH conditions necessary to extract them. Most plant GA20ox enzymes preferentially accept the free C-20 alcohol as substrate, suggesting that in vivo the intermediates are present at least to some extent as the free alcohols as would be anticipated at the cytosolic pH of >7. An uncharacterized activity that oxidizes the δ-lactone to the aldehyde has been described, such as that found in spinach (Gilmour et al. 1986). While oxidation of the C-20 alcohol by GA20ox involves stereospecific loss of the 20-proR H, the spinach enzyme removed the 20-proS H from the lactone, in which the 20-proR H is fixed in a sterically hindered and poorly accessible position (Ward et al. 1997). The mechanism for the loss of C-20 from the aldehyde is not well understood. The 20-oic acid is normally a minor by-product of the reaction (Lange et al. 2013), except in the case of a GA20ox from C. maxima seeds, for which it is the main product (Lange et al. 1994). However, this carboxylic acid is not an intermediate in C19-GA biosynthesis (Kamiya and Graebe 1983). Nevertheless, C-20 is lost as CO2 rather than as formic acid, which would be the case if it was lost directly from the aldehyde (Kamiya et al. 1986). Ward et al. (2002) provided evidence consistent with the reaction proceeding via a C-10 radical, which it was suggested reacts with the 19-oic acid group to form the 19,10-lactone. The mechanism by which this radical is formed is consistent with the reaction proceeding via a C-10 radical, which it was suggested reacts with the 19-oic acid group to form the 19,10-lactone. The mechanism by which this radical is formed is unclear. In contrast to the alcohol and aldehyde intermediates, which accumulate often to relatively high concentrations, no intermediate between the aldehyde and C19-GA which accumulate often to relatively high concentrations, no intermediate may be enzyme-bound, although there is as yet no supporting evidence.

In fungi and bacteria, C-20 oxidation is catalyzed by the cytochrome P450s CYP68B and CYP112, respectively (Nagel and Peters 2018), which perform the same sequence of reactions as the plant GA20ox. It has not been possible to study the fungal enzyme in vitro, but work with cultures of the F. fujikuroi mutant B1-41a indicates that the intermediates are not released from the enzyme since they do not accumulate and are converted inefficiently to C19-GAs compared with earlier precursors when supplied to cultures (Bearder et al. 1975). Through the use of 18O-labeled substrates, it was also established that both O atoms in the 19,10-lactone originate from the 19-oic acid (Bearder et al. 1976). In contrast to the fungal enzyme, CYP112 from the bacterium Erwinia tracheiphila, prepared by expression in E. coli, converted GA12, GA15 (open and closed lactone) and GA24 to GA8 and accumulated the intermediates when incubated under NADPH-limiting conditions (Nagel and Peters 2018). On the basis of incubations in 18O2, Nagel and Peters (2018) confirmed that C-20 is lost as CO2 and proposed a mechanism in which the aldehyde, GA24, serves as the C-20 geminal diol or as the lactol, is oxidized to the C-19–20 anhydride with C-20 present as the geminal diol. Further oxidation of this intermediate would release C-20 as CO2 forming the 19,10-lactone by rearangement via the C-10 radical. While it is possible that hydrolysis of the anhydride to the dicarboxylic acid could account for the small amounts of 20-oic acid formed as a by-product of C19-GA formation in plants, it is also noteworthy that C-19,20 dioic acids readily form the anhydride in solution but are not substrates for GA20ox in plants, fungi or bacteria (Kamiya and Graebe 1983, Tudzynski et al. 2002, Nagel and Peters 2018).

Seed plants contain a family of GA20ox genes, with members differing in their developmental, environmental and tissue expression patterns. For example, Arabidopsis contains five GA20ox genes encoding functionally similar enzymes, except for AtGA200ox5, which produces the aldehyde without further conversion to the C19-GA (Plackett et al. 2012). AtGA20ox1 and AtGA20ox2 act partially redundantly in plant development, with AtGA20ox3 having a minor role, while the physiological function of the other two genes is unclear (Rieu et al. 2008, Plackett et al. 2012). Poaceae (grasses), including the cereals, typically contain four GA20ox genes, with the expression of one of them, GA20ox3, although relatively very high, restricted to the endosperm of developing grain, which produces large amounts of GAs of uncertain function (Pearce et al. 2015). It should be noted that gene annotation numbers, which usually relate to their order of discovery, do not denote orthology, except within plant families, as GA-oxidase gene multiplication and divergence seems to have occurred relatively late in evolution (Han and Zhu 2011, Huang et al. 2015). In many species, GA20ox activity limits the GA production (Fleet et al. 2003) and expression of GA20ox paralogs with major developmental roles is tightly regulated by developmental and environmental signals and by GA signaling to maintain GA homeostasis (see below).

**GA 3-oxidases**

In the final step in the biosynthesis of bioactive GAs, the C19-GAs GA8 and GA30 are 3β-hydroxylated to GA4 and GA9, respectively, by GA 3-oxidase (GA3ox) enzymes. GA3ox genes that are expressed in vegetative tissues of eudicots generally function only as 3β-hydroxylases with high regiospecificity, while those in monocots are less regiospecific, so that, e.g. GA3 is produced from GA20 as a minor by-product of GA3 production (see Fig. 3; Itoh et al. 2001). This side reaction occurs by oxidation at both the 2α and 3β positions to form the 2,3-unsaturated intermediate GA9, which is converted to GA3 by oxidation on C-1β by the same enzyme, followed by the migration of the double bond to the 1,2 position and hydroxylation on C-3β, (Albone et al. 1990, Fujioka et al. 1990). Thus, monocots contain low levels of GA9, usually <10% of the GA3 content, while it is usually undetectable in vegetative tissues from eudicots. GA3ox-like enzymes present in developing seeds of both eudicots and monocots may have quite diverse activities. For example, a GA3ox from C. maxima seed acts also on C20-GAs (Frisse et al. 2003) and two GA3ox-like enzymes act in sequence to produce GA3 in M. macrocarpa seeds via 2,3-didehydroGA9 (see Fig. 3) (Ward et al. 2010) and GA15 (1β-hydroxyGA3) via GA24, in wheat endosperm (Pearce et al. 2015). The wheat enzymes are closely related paralogs produced by a recent gene duplication with functional diversification such that, while one (TaGA3ox3) retains 3β-hydroxylase activity, the other acts as a 1β-hydroxylase and is annotated as TaGA1ox1 (Pearce et al. 2015). Barley contains an ortholog of these enzymes that hydroxylates at both the C-3β and C-18
positions to convert GA_20 to GA_30 (18-hydroxyGA_20) (Pearce et al. 2015). GA_54 and GA_131 are the major GAs present in developing seeds of wheat and barley, respectively (MacMillan 2001), although their function in seeds is unknown. GA biosynthesis is often very strong in developing seeds, which can produce a wide array of structures, reflecting the functional diversity of the enzymes involved, although in many cases the nature of these enzymes is still unknown.

GA3ox genes are present as small families, with Arabidopsis containing four members and rice and barley only two. Two of the Arabidopsis enzymes, AtGA3ox1 and AtGA3ox2, and only one in cereals, GA3ox2, have major roles in the development of vegetative organs. A second rice enzyme OsGA3ox1, which contributes particularly toward reproductive development, does not have close orthologs in barley and wheat, in which, apart from GA3ox2, the other GA3ox-like genes are mainly expressed in developing seeds.

In F. fujikuroi and other related GA-producing Fusarium species, 3β-hydroxylation occurs early in the pathway catalyzed by the highly multifunctional P450-1 (CYP68A) (see above). A close ortholog has the same function in the Cassava pathogen Sphaceloma manihoticola, which produces GA_15 (Bömke et al. 2008), while the GA_13-producing Phaeosphaeria spp. utilizes an unrelated CYP to convert GA_9 to GA_4 in a pathway resembling that in plants (Kawaide 2006). In F. fujikuroi, the last two steps in GA biosynthesis are the desaturation of GA_9 to GA_15, catalyzed by a GA20ox, DES (Bhattacharya et al. 2012), and 13-hydroxylation, catalyzed by P450-3 (Tudzynski et al. 2003). The genes encoding DES and P450-3 lie on opposite ends of the GA operon in F. fujikuroi and are missing in the S. manihoticola operon. In the GA-biosynthetic operons of GA_13-producing plant pathogenic bacteria, such as Xanthomonas oryzae, CYP115 encodes a 3β-hydroxylase that converts GA_20 to GA_15 (Nagel et al. 2017). However, the operons of most GA-producing symbiotic rhizobia lack CYP115 so that these bacteria produce GA_15, which must be converted to GA_13 by the host plant (Nagel and Peters 2017), although some rhizobia contain CYP115 and are able to produce GA_15 (Nett et al. 2017). Production of bioactive GAs by fungal and bacterial plant pathogens is proposed to facilitate infection by suppressing jasmonic acid signaling that promotes immunity, whereas in the symbiotic N2-fixing rhizobia, in which GA has a role in nodule formation, the ability of the plant to regulate GA production must be advantageous (Nagel and Peters 2017).

### Inactivation

**GA 2-oxidases**

Inactivation, i.e. introducing structural modifications that decrease affinity for the receptor, is an essential activity to regulate the concentration of biologically active GAs in plant tissues. A number of inactivating reactions have been described (illustrated in Fig. 4), the most universal being 2β-hydroxylation, which can occur on the bioactive end products of the pathway or on the C_{19} or C_{20} GA precursors, so preventing formation of the active species. Gibberellin 2-oxidases (GA2ox) form two major families of 2-ODDs, consisting of enzymes that act primarily on C_{19} GA substrates and those that act mainly on C_{20} GAs. While these families are phylogenetically not closely related, there is functional overlap, with some enzymes belonging to the C_{19} GA2ox family acting on C_{20} GAs, usually as a minor activity, and vice versa (Lange et al. 2013, Pearce et al. 2015). The C_{19} GA2ox family is the largest of the GA 2-ODD families and, on the basis of sequence, falls into two sub-families, this division preceding the divergence of the monocots and eudicots (Kawai et al. 2014, Huang et al. 2015). While GA2ox genes are present in seed plants (Niu et al. 2014, Huang et al. 2015), 2β-hydroxyGAs are apparently not produced in lycophytes and ferns, suggesting the absence of functional GA2ox genes, which therefore evolved later than the other GA 2-ODDs. 2β-HydroxyGAs are also not produced by fungi and bacteria (MacMillan 2001), in which GAs do not have a developmental role so that precise regulation of their concentration may not be critical. The exceptions are symbiotic GA-producing bacteria for which GA production may need to be more tightly regulated, but as noted above, they produce the precursor, GA_9, allowing the plant host to regulate the synthesis of bioactive GA. Some C_{19} GA2ox enzymes have been shown to oxidize the 2β-hydroxyGA product further to the 2-ketone (Thomas et al. 1999). These products are isolated as the so-called ‘GA catabolites’, in which the 19,10-lactone has opened with formation of a double bond between C-10 and C-1 (Gaskin et al. 1981). It is likely that the catabolites are artifacts of isolation and that the product in planta is the unrearranged ketone.

The recent determination of the X-ray crystal structure of the rice C_{19} GA2ox OsGA2ox3 revealed that it formed a tetramer in the presence of its substrate GA_15, with the monomers linked via two GA_4 molecules and two disulfide bridges (Takehara et al. 2020). The tetramer was shown to be more active than the monomer, exhibiting a lower Km for GA_4 by an energetically more favorable pathway to the active site. Thus, increasing GA_4 concentration promotes multimer formation and enhances enzyme activity, providing an allosteric feedforward mechanism to maintain GA homeostasis. GA_4 also promoted dimerization of the C_{20} GA2ox OsGA2ox6, for which it is not the preferred substrate. Remarkably, Takehara et al. 2020 found a similar mechanism, involving dimerization of the auxin catabolic enzyme indoleacetic acid oxidase, also a 2-ODD, in the presence of its substrate.

GA2ox enzymes have an essential function in regulating GA concentration during normal plant development, and also in response to changes in environmental conditions. The expansion of the GA2ox families has enabled some specificity in gene expression at the tissue/organ level (Li et al. 2019) and in response to stress (Colebrook et al. 2014), although there is considerable redundancy (Ross and Reid 2010). In their assessment of GA-metabolic gene specialization in rice through CRISPR/Cas9 gene knock-out, Chen et al. (2019) found that two of the three C_{20} GA2ox genes have specific roles in fertility and grain development, whereas loss of individual genes had no effect on stem extension, potentially due to redundancy. The wheat reduced height alleles Rht18 and Rht14 cause increased expression of the C_{20} GA2ox gene TaGA2oxA9 in stems, while some
loss-of-function *taga2ox9* mutants generated from *Rht18* exhibited overgrowth phenotypes compared with the tall parent of *Rht18*, which suggests a potential role for this gene in the control of stem height (Ford et al. 2018). Increased internode length in tomato was also reported for a mutant of the tomato C20-GA2ox gene *SlGA2ox7* (Schrager-Lavelle et al. 2019). The reduction in the bioactive GA content in leaves as they mature was shown in pea to be due to high rates of 2-oxidation rather than reduced biosynthesis (Ross et al. 2003). In developing seeds, GA2ox activity may increase to high levels as the seed approaches maturity (Albone et al. 1984), ensuring that bioactive GAs do not accumulate in mature seed and thereby induce precocious germination and/or abnormal seedling growth. This is illustrated by the *slender* (*sln*) mutant of pea, which has an overgrowth phenotype due to a mutation in the *PsGA2ox1* gene that allows GA20 to accumulate in the mature seed (Lester et al. 1999). Conversion of GA20 to GA1 following seed imbibition promotes the excessive seedling growth. It has been reported for several species that GA2ox expressed at the base of the shoot apical meristem limits the influx of bioactive GA to the meristem to control meristem function (Sakamoto et al. 2001, Jasinski et al. 2005, King et al. 2008, Bolduc and Hake 2009). Induction of GA2ox expression by stress is a common mechanism for growth control and enhanced stress tolerance with different GA2ox genes being targeted according to the stress (reviewed in Colebrook et al. 2014). In a recent example, touch-induced growth reduction in Arabidopsis was associated with increased expression of *AtGA2ox7*, a C20-GA2ox gene (Lange and Lange 2015).

**Other inactivation mechanisms**

As described above, cytochrome P450s belonging to the CYP714 family have a generally inactivating activity by oxidizing GAs on the C and D rings (see Fig. 4B). Of particular significance, EU11 (CYP714D1) has an important developmental function in rice by restricting culm height, acting particularly on the upper internodes (Zhu et al. 2006). Introduction of *eui1* mutant alleles into male sterile rice to allow adequate panicle exsertion was an important development for hybrid rice production.
Sites of GA Biosynthesis and GA Mobility

The sites of GA synthesis and their relationship to the sites of action are of major relevance to any consideration of function. There is renewed interest in GA distribution stemming from the identification of GA transporters and the development of in vivo methods to determine GA distribution and movement at the cellular level (Rizza et al. 2017, Wexler et al. 2019). The topic has been reviewed recently (Lacombe and Achard 2016, Binenbaum et al. 2018, Rizza and Jones 2019) and will be discussed only briefly here. The sites of GA synthesis are usually inferred from the expression of biosynthesis genes on the basis of reporter activity, in situ hybridization or, in the case of Arabidopsis roots, transcript analysis in combination with cell isolation and sorting (Birnbaum et al. 2003, Dugardeyn et al. 2008). However, this does not allow for differences in translational efficiency or enzyme stability. Treatment of spinach with GA biosynthesis inhibitors resulted in elevated levels of SoGA20ox1 protein, measured by Western blotting, in the petioles and shoot tip, but no change in the transcript level (Lee and Zeenervaat 2007), emphasizing the need to consider posttranscriptional regulation. The location of GA biosynthesis has been investigated more directly from the application of radioactively labeled GAs, e.g. in pea (Smith 1992, O’Neill and Ross 2002), but such studies provide limited spatial resolution. Normal development under non-stressful conditions depends on appropriate coordination of GA biosynthesis and inactivation. Reinecke et al. (2013) were able to complement the dwarf phenotype of the pea ga3ox1 (le) mutant more effectively by introgressing the native PgGA3ox1 gene than by constitutive expression of its cDNA from the 35S promoter. Ectopic expression of PgGA3ox1, which is rate limiting for GA biosynthesis in pea, resulted in strong upregulation of the GA-catabolic gene PgSA2ox1, which the authors suggested would be normally segregated from cells responsible for GA biosynthesis.

In vegetative organs, GAs are synthesized mainly in growing regions, such as elongating stems and leaves, and root tips. Very high rates of synthesis occur in certain tissues, including anthers (Hirano et al. 2008) and the cereal scutellar epithelium (Kaneko et al. 2003), which act as sources for other tissues (see below), and in developing seeds, in which the function of GA is unclear. While there is evidence based on transcript localization for GA biosynthesis occurring at or close to the site of action, e.g. in cereal stems (Kaneko et al. 2003, Pearce et al. 2011) or Arabidopsis roots (Dugardeyn et al. 2008), there are also examples of mobility between organs, where tissues act as a source of GA for neighbouring GA-nonautonomous organs. Examples are the cereal embryo scutellum as a source of GA for the aleurone (Appleford and Lenton 1997), the suspensor as a GA source for the embryo in several species (reviewed in Jacob and Brian 2020) and GA or precursors from the anther tapetum being required for filament elongation and petal growth (Weiss and Halevy 1989, Silverstone et al. 1997, Hu et al. 2008). In female cucumber flowers, GAs produced in ovaries moves to the petals and sepals where it is converted to GA4, which promotes the expansion of these organs (Lange and Lange 2016). In these cases, the hormone acts to coordinate the growth and development of neighboring, physiologically related organs. As suggested above, GA from the anthers in cereals may also stimulate peduncle elongation to ensure adequate emergence of the spike. There are also examples of long distance GA transport, such as from leaves to induce the transition to flowering at the shoot apex in Arabidopsis (Eriksson et al. 2006) and Lolium (King et al. 2001). In some cases, long distance movement of precursors rather than the active hormone has been noted (Probsting et al. 1992, Regnault et al. 2015). It is unclear what specifies the structure of the mobile molecules, but it may be determined by the properties of transmembrane transporters. So far only influx transporters have been identified and these lack specificity, transporting other hormones as well as unrelated molecules (reviewed in Binenbaum et al. 2018). According to the ion-trap hypothesis, the high pH environment of the cytosol would deter efflux of GAs by passive diffusion through the cell membrane, while influx from the more acidic apoplast would be more favored (Kramer 2006). However, passive diffusion across biological membranes, which are rich in proteins and other molecules that can interact with mobile signals, may be limited (Kell 2015), such that both influx
Regulation of GA Metabolism

The concentration of biologically active GAs in GA-responsive tissues is tightly regulated through biosynthesis, inactivation and transport. The mechanisms involved in regulating the expression of GA biosynthesis and inactivation genes in higher plants in response to developmental and environmental signals are active areas of research. The literature on this topic has been reviewed in detail (Hedden and Thomas 2012, Hedden 2016, Magome and Kamiya 2016) and will be summarized here, as well as highlighting some recent findings (see Fig. 5). CPS catalyzes the first committed step and is suggested to be the gateway to the GA-biosynthetic pathway with a role in developmental regulation (Silverstone et al. 1997), but bioactive GA production is limited by later enzymes in the pathway, particularly GA20ox (Fleet et al. 2003). Members of the 2-ODD gene families, which differ in their spatial and temporal expression patterns, are major sites of regulation. Transcription factors that specify spatial and temporal expression patterns of GA-biosynthetic and inactivation genes have been identified, including KNOX, MADS-box and bHLH proteins. A recent example is the MADS-box protein OsMAD557, which directly promotes expression of the inactivation genes OsGA20ox3 and OsEUI1 to limit internode elongation and panicle exsertion in rice (Chu et al. 2019). Expression of OsEUI1 was shown also to be promoted directly by the homeodomain-leucine zipper transcription factor HOX12 to regulate panicle exsertion (Gao et al. 2016). In Arabidopsis, expression of an EU1-like gene EU1-LIKE P450 A1 (ELA1) is upregulated directly by LEAFY in floral primordia to suppress GA accumulation and promote flower formation (Yamaguchi et al. 2014). In rice, mutation of the leucine zipper (HD-ZIP II) transcription factor SMALL GRAIN AND DWARF 2 caused dwarfism, which was associated with reduced expression of OsGA20ox1 and OsGA20ox2 and increased expression of several GA2ox genes, although it is not known if the regulation is direct (Chen et al. 2019).

Transcriptional regulation of GA metabolism genes via the GA signaling pathway provides a mechanism for GA homeostasis (Fig. 5): some GA20ox and GA3ox gene family members are downregulated by GA signaling whereas there is upregulation of GA20ox genes (see, e.g. Thomas et al. 1999). Details of the GA signaling pathway are well established (Sun 2011, Nelson and Steber 2016). Briefly, binding of GA to its receptor GID1 results in a conformational change in the receptor that promotes its interaction with DELLA proteins, which then through association with the F-box component of an E3 ubiquitin ligase are targeted for degradation via the ubiquitin-proteasome pathway. DELLAs belong to the GRAS family of transcriptional regulators and uniquely contain an N-terminal sequence with conserved DELLA, LExLE and VHYNP domains that binds to the GA-GID1 complex to allow GA-induced degradation. DELLA function includes growth suppression, which is thus relieved by GA action. A major activity of DELLAs is to modify transcription in association with transcription factors (Davière and Achard 2016, Thomas et al. 2016): this can be inhibitory through the sequestration of transcription factors so preventing their binding to gene promoters, or they can promote transcription by the sequestration of inhibitors or by acting...
as co-activators in partnership with transcription factors. This last mechanism is involved in the promotion of GA20ox and GA3ox expression by DELLAs to enable GA homeostasis through negative feedback regulation. In Arabidopsis, the DELLA protein GAI promotes transcription of AtGA20ox2, AtGA3ox1 and the GA-receptor gene AtGID1b in association with the C2H2 zinc finger protein GAI-ASSOCIATED FACTOR1 (GAF1), also known as INDETERMINATE DOMAIN 2 (IDD2) (Fukazawa et al. 2014). Activation of AtGA20ox2 expression by GAF1 is suppressed by interaction with the WD-repeats protein TOPLESS-RELATED (TPR) such that expression is regulated by the balance between GAI and TPR. Fukazawa et al. (2017) identified cis elements in the promoter of AtGA20ox2 that are necessary for the binding of GAF1 and for feedback regulation indicating that, while other feedback mechanisms have been reported for some genes (reviewed in Hedden and Thomas 2012), GA regulation of AtGA20ox2 expression is predominantly via GAF1. This transcription factor was also shown to specify the expression of AtGA20ox2 in the shoot apex and root tip (Fukazawa et al. 2017). A notable target of gene activation by IDD-DELLA encodes the non-DELLA GRAS protein SCARECROW-LIKE3 (SCL3) (Yoshida et al. 2014). As SCL3 also interacts with IDDs, it attenuates its own expression by competing with DELLAs, as well as suppressing the expression of feedback regulated genes. The involvement of DELLAs in GA-induced upregulation of GA20ox genes is less clear and this process may involve other mechanisms (Livne et al. 2015). While the 2-ODD genes are mainly implicated in homeostasis through GA metabolism, GA signaling has been reported to modify the expression of genes involved in earlier biosynthetic reactions, including downregulation of KAO expression in rice by the GA-responsive WUSCHEL-related homeobox factor OsWOX3A (Cho et al. 2016). As discussed above, feedback regulation may also occur at the protein level (Lee and Zeevaart 2007) and, although it is more difficult to study, it warrants further investigation. In addition, allosteric regulation has been described for CPS, the activity of which is suppressed synergistically by Mg$^{2+}$ and GGPP (Prisic and Peters 2007), and for OsGA2ox3x3, which is activated by its substrate GA$_4$ (Takehara et al. 2020), both mechanisms contributing to GA homeostasis.

Crosstalk between hormone signaling pathways is well established with DELLA proteins acting as a major hub. The evidence for other hormone signaling pathways targeting GA metabolism is conflicting (Ross et al. 2011, Ross and Quittenden 2016), but there are examples for most hormones, which target primarily the GA 2-ODD genes (reviewed in Ross et al. 2016). Notably, GA mediates growth stimulation by auxin, which promotes GA biosynthesis in a number of physiological contexts, including stem extension in response to auxin from the shoot apex (O’Neill and Ross 2002) and fruit growth induction by seed-derived auxin (Ozga and Reinecke 2003). It has been shown for several GA-biosynthetic genes that regulation by auxin occurs via the IAA/AUX/ARF signaling pathway and is independent of DELLA (Frigerio et al. 2006, O’Neill et al. 2010).

A major function of GA is to mediate growth and developmental responses to environmental changes, which can cause rapid modification in GA concentration through altered metabolism. Environmental factors, including temperature, mechanical stimulation, abiotic and biotic stress and the duration, intensity and quality of light, have all been shown to affect GA biosynthesis and inactivation, acting primarily on the expression of the 2-ODD genes. In many cases, the transcription factors mediating these responses have been identified (reviewed in Hedden and Thomas 2012, Hedden 2016, Magome and Kamiya 2016). For example, stimulation of Arabidopsis seed germination by red light is associated with phytochrome-mediated degradation of the bHLH transcription factor PHYTOCHROME INTERACTING FACTOR-LIKE5 (PIF5), which restricts GA production by suppressing the expression of AtGA3ox1 and AtGA3ox2 and promoting AtGA2ox2 expression in the dark via the intermediary of SOMNUS, a C3H-type zinc finger protein (Kim et al. 2008). In the shade avoidance response, growth promotion under a low red/far-red light ratio was associated with enhanced expression of GA20ox genes in Arabidopsis petioles (Hisamatsu et al. 2005), whereas in seedlings of the gymnosperm Pinus tabuliformis, under these conditions, KAO expression was strongly induced (Li et al. 2020). Promotion of Arabidopsis seedling growth by transfer to higher temperature is associated with increased expression of AtGA20ox1 and AtGA3ox1 and decreased expression of AtGA2ox1 in the hypocotyl (Stavang et al. 2009). It was shown recently that in response to increased temperature, AtGA20ox1 expression was directly upregulated by the class I TESINTE BRANCHED 1, CYCOIDEA, PCF (TCP) transcription factors TCP14 and TCP15, which are induced by the temperature master regulator PHYTOCHROME INTERACTING FACTOR4 (PIF4) (Ferrero et al. 2019). As the function of both TCP (Davière et al. 2014) and PIF4 (de Lucas et al. 2008) is attenuated by interaction with DELLA proteins, the PIF4-TCP-GA signaling pathway is subject to complex feedback loops (Ferrero et al. 2019). Growth suppression by abiotic stress through upregulation of GA20ox genes has been shown to be mediated by the stress-related APETALA2/EThYLENE RESPONSE FACTOR (AP2/ERF)-type transcription factors (reviewed in Colebrook et al. 2014). Recently, it was shown that microRNA regulation of an AP2 protein promoted stem elongation in barley, but the authors propose that AP2 acts through the jasmonate, rather than GA pathway to restrict internode elongation in this case (Patil et al. 2019).

In common with other secondary metabolites, GA production by the fungus F. fujikuroi requires depletion of nitrogen sources, such as ammonium or glutamine (Tudzynski 2014). Through a complex interaction of the GATA transcription factors AreA and AreB, low nitrogen promotes the expression of six of the seven genes in the GA-biosynthetic cluster (Michielse et al. 2014). Factors affecting the expression of the GA-biosynthetic operon in bacteria are less well understood, although in symbiotic rhizobia GA production is highly dependent on the developmental stage of the host plant (Mendez et al. 2014).

### Concluding Remarks

Gibberellin biosynthesis is a mature field, which began in the late-1950s with work on GA$_3$ biosynthesis in the fungus...
F. *fujikuroi*. Progress in the field was initially slow but accelerated with the development of sensitive methods for compound identification, the increasing availability of mutants and, more recently, of full genome sequences. These advances led first to the establishment of the metabolic pathways and then to the identification of the enzymes and finally of the relevant genes, in plants, fungi and recently bacteria. While genome sequences have proved extremely valuable for gene identification, it is important that gene function is not assumed from the sequence and is confirmed by biochemical means and/or through the use of mutants. The availability of genome sequences has prompted interest in the evolution of GA metabolism. Current information indicates that in plants GA biosynthesis evolved with vascularization, emphasizing the importance of GAs as a mobile signal. GA biosynthesis evolved also in fungi and bacteria that associate with plants enabling these organisms to function as pathogens or symbionts by modifying their hosts' development and immunity. It is remarkable that the ability to produce these complex molecules has evolved three times in different kingdoms: plants, fungi and bacteria. There are reports of GAs occurring in algae, but as nonvascular land plants had not evolved the capability to synthesize GAs, it is unclear where algae fit in the evolutionary scheme. The reports need to be confirmed and the relevant enzymes identified.

Current work with flowering plants is focused on the mechanisms involved in regulating GA concentrations in response to developmental and environmental cues. While there is particular emphasis on the expression of GA biosynthesis and catabolism genes, it is also necessary to determine the sites of GA biosynthesis and action, ideally at the cellular level, and the mechanisms involved in linking them. Indeed, GA localization and movement is currently attracting considerable interest, e.g. associated with the identification of transporters. Locating the sites of GA accumulation at the cellular levels is an important goal that is being addressed through in vivo methods. Currently, for practical reasons, this has been restricted to locating the biologically active compounds, but determining the location of precursors can provide important information on how GA production is regulated. This will need to be addressed, and although the quantification of GAs and their precursors and catabolites at the cellular level by physicochemical methods is challenging, it is becoming more realistic with the increasing sensitivity of methods, such as UPLC–MS. The field has come a very long way in the last 60 years, but, through the implementation of technological advances, many more exciting discoveries can be anticipated.

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### References

Aach, H., Bode, H., Robinson, D.G. and Graebe, J.E. (1997) *ent*-Kaurene synthase is located in proplastids of meristematic shoot tissues. *Planta* 202: 211–219.

Aach, H., Böse, G. and Graebe, J.E. (1995) *ent*-Kaurene biosynthesis in a cell-free system from wheat (*Triticum aestivum* L) seedlings and the localization of *ent*-kaurene synthetase in plastids of three species. *Planta* 197: 333–342.

Albone, K.S., Gaskin, P., Macmillan, J., Phinney, B.O. and Willis, C.L. (1990) Biosynthetic origin of gibberellin A<sub>1</sub> and gibberellin A<sub>2</sub> in cell-free preparations from seeds of *Marah macropus* and *Malus domestica*. *Plant Physiol.* 94: 132–142.

Albone, K.S., Gaskin, P., Macmillan, J. and Sponsel, V.M. (1984) Identification and localization of gibberellins in maturing seeds of the cucurbit *Sechium edule*, and a comparison between this cucurbit and the legume *Phaseolus cocineus*. *Planta* 162: 560–565.

Appleford, N.E.J, Evans, D.J, Lenton, J.R, Gaskin, P., Croker, S.J, Devos, K.M, et al. (2006) Function and transcript analysis of gibberellin-biosynthetic enzymes in wheat. *Planta* 223: 568–582.

Appleford, N.E.J. and Lenton, J.R. (1997) Hormonal regulation of α-amylase gene expression in germinating wheat (*Triticum aestivum*) grains. *Physiol. Plant.* 100: 534–542.

Beale, M.H., Bearder, J.R., Down, G.H., Hutchison, M., Macmillan, J. and Phinney, B.O. (1982) The biosynthesis of kaurenolide diterpenoids by *Gibberella fujikuroi*. *Phytochemistry* 21: 1279–1287.

Bearder, J.R., MacMillan, J. and Phinney, B.O. (1975) Fungal products. Part XIV. Metabolic pathways from *ent*-kaurenolic acid to fungal gibberellins in mutant B4-1A of *Gibberella fujikuroi*. *J. Chem. Soc. Perkin Trans. 1* 1: 721–726.

Bearder, J.R, MacMillan, J and Phinney, B.O. (1976) Origin of oxygen atoms in lactone bridge of C<sub>19</sub>-gibberellins. *J. Chem. Soc. Chem. Commun.* 834–835.

Beck, G., Coman, D., Herren, E., Ruiz-Sola, M., Rodriguez-Concepcion, M., Gruissem, W., et al. (2013) Characterization of the GGPP synthase gene family in *Arabidopsis thaliana*. *Plant Mol. Biol. 82*: 393–416.

Bhattacharya, A., Kourmpetli, S., Ward, D.A., Thomas, S.G., Gong, F., Powers, S.J., et al. (2012) Characterization of the fungal gibberellin desaturase as a 2-oxoglutarate-dependent dioxygenase and its utilization for enhancing plant growth. *Plant Physiol.* 160: 837–845.

Binenbaum, J., Wein斯坦, R. and Shani, E. (2018) Gibberellin localization and transport in plants. *Trends Plant. Sci.* 23: 410–421.

Birnbaum, K., Shasha, D.E., Wang, J.Y., Jung, J.W., Lambert, G.M., Galbraith, D. W., et al. (2006) A gene expression map of the *Arabidopsis* root. *Science* 302: 1596–1600.

Block, M.A. and Jouhet, J. (2015) Lipid trafficking at endoplasmic reticulum-chloroplast membrane contact sites. *Curr. Opin. Cell Biol.* 35: 21–29.

Bolduc, N. and Hake, S. (2009) The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga2ox1. *Plant Cell* 21: 1647–1658.

Bömke, C., Rojas, M.C., Gong, F., Hedden, P., and Tuzelynski, B. (2008) Isolation and characterization of the gibberellin biosynthetic gene cluster in *Sphaceloma manihoticola*. *Appl. Environ. Microbiol.* 74: 5325–5339.

Brautigam, A. and Weber, A.P.M. (2009) Proteomic analysis of the proplastid envelope membrane provides novel insights into small molecule and protein transport across proplastid membranes. *Mol. Plant* 2: 1247–1261.

Castellaro, S.J., Dolan, S.C., Hedden, P., Gaskin, P. and Macmillan, J. (1990) Stereocchemistry of the metabolic steps from kaurenolic acids to kaurenolides and gibberellins. *Phytochemistry* 29: 1833–1839.

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1844
Chen, W.W., Cheng, Z.L., Liu, L.L., Wang, M., You, X.M. and Wang, J. (2019) Small Grain and Dwarf 2, encoding an HD-Zip II family transcription factor, regulates plant development by modulating gibberellin biosynthesis in rice. Plant Sci. 288: 110208.

Chen, X., Tian, X.J., Xue, L., Zhang, X.H., Yang, S.H., Traw, M.B., et al. (2019) CRISPR-based assessment of gene specialization in the gibberellin metabolic pathway in rice. Plant Physiol. 180: 2091–2105.

Cho, S.H., Kang, K., Lee, S.H., Lee, U.J. and Paek, N.C. (2016) OsWOX3A is involved in negative feedback regulation of the gibberellin acid biosynthetic pathway in rice (Oryza sativa). J. Exp. Bot. 67: 1677–1687.

Chu, Y.L., Xu, N., Wu, Q., Yu, B., Li, X.X., Chen, R.R., et al. (2019) Rice transcription factor OsMADS57 regulates plant height by modulating gibberellin catabolism. Rice 12: 38.

Colebrook, E.H., Thomas, S.G., Phillips, A.L. and Hedden, P. (2014) The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. 217: 67–75.

Crozier, A., Kuo, C.C., Durley, R.C. and Pharis, R.P. (1970) Biological activities of 26 gibberellins in nine plant bioassays. Can. J. Bot. 48: 867–877.

Croker, S.J., Gaskin, P., Beale, M.H. and Lenton, J.R. (1995) bet-Kaurenolide biosynthesis in a cell-free system from spinach shoot apex determines plant height. Curr. Biol. 24: 1923–1928.

de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pinto, M., Iglesias-Pedraza, J.M., Lorrain, S., et al. (2008) A molecular framework for light and gibberellin control of cell elongation. Nature 454: 480–484.

Ding, Y., Murphy, K.M., Poresky, E., Mafu, S., Yang, B., Char, S.N., et al. (2019) Multiple genes recruited from hormone pathways partition maize distylopodinoid defences. Nat. Plants 5: 1043–1056.

Dugardeyn, J., Vandenbergbusche, F. and Van Der Straeten, D. (2008) To grow or not to grow: what can we learn on ethylene-gibberellin cross-talk by in silico gene expression analysis? J. Exp. Bot. 59: 3–16.

Eriksson, S., Bohlenius, H., Moritz, T. and Nilsson, O. (2006) GA4 is the active gibberellin in the regulation of gibberellin biosynthesis in Arabidopsis. Plant Physiol. 142: 1053–1067.

Ferrero, L.V., Viola, L.L., Ariel, F.D. and Gonzalez, D.H. (2019) Class I TCP Fixen, K.R., Thomas, S.C. and Tong, C.B.S. (2012) Blue light inhibition of ceterstate of gibberellin in shoots of Zea mays L. Plant Physiol. 94: 127–131.

Fujikawa, J., Mori, M., Watanabe, S., Miyamoto, C., Ito, T. and Takahashi, Y. (2017) DELLA-GAF1 complex is a main component in gibberellin feedback regulation of GA 20-oxidase2. Plant Physiol. 175: 1395–1406.

Gao, S.P., Fang, J., Xu, F., Wang, W. and Chu, C.C. (2016) Rice BOX12 regulates panicle exertion by directly modulating the expression of elongated uppermost internode1. Plant Cell 28: 680–695.

Gao, S.P., Kirkwood, P.S. and Macmillan, J. (1981) Partial synthesis of ent-13-hydroxy-2-oxo-20-norgibberella-1(10),16-diene-7,19-dioic acid, a catabolite of gibberellin A20, and of related compounds. J. Chem. Soc. Perkin Trans. 1: 1083–1091.

Gilmour, S.J., Zeevaart, J.A.D., Schwenen, L. and Graebe, J.E. (1986) Gibberellin metabolism in cell-free extracts from spinach leaves in relation to photoperiod. Plant Physiol. 82: 190–195.

Graebe, J.E., Hedden, P. and Macmillan, J. (1975) Ring contraction step in gibberellin biosynthesis. J. Chem. Soc. Chem. Commun. 1975: 161–162.

Han, F.M. and Zhu, B.G. (2011) Evolutionary analysis of three gibberellin oxidases in rice, Arabidopsis, and soybean. Gene 473: 23–35.

Hayashi, K., Kawaiha, N., Noromi, M., Sakagi, Y., Matsu, A. and Nozaki, H. (2006) Identification and functional analysis of bifunctional ent-kaurene synthase from the moss Physcomitrella patens. FEBS Lett. 580: 6175–6181.

He, J., Chen, Q., Xin, P., Yuan, J., Ma, Y., Wang, X., et al. (2019) CYP72A enzymes catalyse 13-hydroxylation of gibberellins. Nat. Plants 5: 1057–1065.

Hedden, P. (2016) Gibberellin biosynthesis in higher plants. Annu. Plant Rev. 49: 37–72.

Hedden, P. and Graebe, J.E. (1981) Kaurenolide biosynthesis in a cell-free system from Cucurbita maxima seeds. Phytochemistry 20: 1011–1015.

Hedden, P. and Sporsel, V. (2015) A century of gibberellin research. J. Plant Growth Regul. 34: 740–760.

Hedden, P. and Thomas, S.G. (2012) Gibberellin biosynthesis and its regulation. Biochem. J. 444: 11–25.

Hellwell, C.A., Chandler, P.M., Poole, A., Dennis, E.S. and Peacock, W.J. (2001) The CYP88A2 cytochrome P450, ent-kaurenoic acid oxidase, catalyzes three steps of the gibberellin biosynthesis pathway. Proc. Natl. Acad. Sci. USA 98: 2065–2070.

Hellwell, C.A., Poole, A., Peacock, W.J. and Dennis, E.S. (1999) Arabidopsis ent-kaurene oxidase catalyzes three steps of gibberellin biosynthesis. Plant Physiol. 119: 507–510.

Hellwell, C.A., Sullivan, J.A., Mould, R.M., Gray, J.C., Peacock, W.J. and Dennis, E.S. (2001) A plastid envelope location of Arabidopsis ent-kaurene oxidase links the plastid and endoplasmic reticulum steps of the gibberellin biosynthesis pathway. Plant J. 28: 201–208.

Hirano, K., Aya, K., Hoso, T., Sakakibara, H., Kojima, M., Shim, R.A., et al. (2008) Comprehensive transcriptome analysis of phytohormone biosynthesis and signaling genes in microspore/pollen and tapetum of rice. Plant Cell Physiol. 49: 1429–1450.

Hisamatsu, T., King, R.W., Helliwell, C.A. and Koshioka, M. (2005) The integration of photoperiod to gibberellin signalling in plant responses to abiotic stress. Plant Cell Physiol. 46: 1343–1350.

Itoh, H., Tatsumi, T., Sakamoto, T., Otomo, K., Toyomasu, T., Kitano, H., et al. (2004) A rice semi-dwarf gene, Tan-Ginbozu (D35), encodes the
gibberellin biosynthesis enzyme, ent-kaurene oxidase. Plant Mol. Biol. 54: 533–547.

Itoh, H., Ueguchi-Tanaka, M., Sentoku, N., Kitano, H., Matsuoka, M. and Kobayashi, M. (2001) Cloning and functional analysis of two gibberellin 32-hydroxylase genes that are differentially expressed during the growth of rice. Proc. Natl. Acad. Sci. USA 98: 8909–8914.

Jacob, D. and Brian, J. (2020) The short and intricate life of the suspensor. Physiol. Plant. 169: 110–121.

Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, L. et al. (2005) KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Curr. Biol. 15: 1560–1565.

Kamya, Y. and Graebe, J.E. (1983) The biosynthesis of all major pea gibberellins in a cell-free system from Pisum sativum. Phytochemistry 22: 681–689.

Kamya, Y., Takahashi, N. and Graebe, J.E. (1986) The loss of carbon-20 in C20-gibberellin biosynthesis in a cell-free system from Pisum sativum L. Planta 169: 524–528.

Kaneko, M., Itoh, H., Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Ashikari, M., et al. (2003) Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants? Plant J. 35: 104–115.

Kasahara, H., Hanada, A., Kuzuyama, T., Takagi, M., Kamya, Y. and Yamaguchi, S. (2002) Contribution of the mevalonate and mehylery-thritol phosphate pathways to the biosynthesis of gibberellins in Arabidopsis. J. Biol. Chem. 277: 45188–45194.

Katsarou, K., Wu, Y., Zhang, R.X., Bonar, N., Morris, J., Hedley, P.E., et al. (2016) Insight on genes affecting tuber development in potato upon potato spindle tuber viroid (PSTVd) infection. PLoS One 11: e0150711.

Kawai, Y., Ono, E. and Mizutani, M. (2014) Evolution and diversity of the 2-oxoglutarate-dependent dioxygenase superfamily in plants. Plant J. 78: 328–343.

Kawade, H. (2006) Biochemical and molecular analyses of gibberellin biosynthesis in fungi. Biosci. Biotechnol. Biochem. 70: 583–590.

Keeling, C.I., Dullat, H.K., Yuen, M., Ralph, S.G., Jancsik, S. and Bohlmann, J. (2006) Biochemical and molecular analyses of gibberellin biosynthesis in developing pumpkin seedlings. Plant J. 45: 533–544.

Keller, D.B. (2015) What would be the observable consequences if phospho-lipid bilayer diffusion of drugs into cells is negligible? Trends Pharmacol. Sci. 46: 537–540.

Kim, D.H., Yamaguchi, S., Lim, S., Oh, E., Park, J., Hanada, A., et al. (2008) SOMNUS, a CCCH-type zinc finger protein in Arabidopsis, negatively regulates light-dependent seed germination downstream of PILS. Plant Cell 20: 1260–1277.

Kingsley, C., Keese, B., Nitschke, S., Blundell, C.A. and Evans, L.T. (2008) Selective deactivation of gibberellins below the shoot apex is critical to flowering but not to stem elongation of Loliuun. Mol. Plant 1: 295–307.

Kielsing, C.I., Dullat, H.K., Yuen, M., Ralph, S.G., Jancsik, S. and Bohlmann, J. (2010) Identification and functional characterization of monofunctional ent-copalyl diphosphate and ent-kaurene synthases in white spruce reveal different patterns for diterpene synthase evolution for primary and secondary metabolism in gymnosperms. Plant Physiol. 152: 1197–1208.

Kell, D.B. (2015) What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible? Trends Pharmacol. Sci. 36: 15–21.

Lee, D.J. and Zeevaart, J.A.D. (2007) Regulation of gibberellin 20-oxidase1 expression in spinach by photoperiod. Plant Cell 19: 107–119.

Lee, D.J. and Zeevaart, J.A.D. (2007) Regulation of gibberellin 20-oxidase1 expression in spinach by photoperiod. Plant Sci. 127: 624–629.

Liang, M.Z., Deng, L.X., Liu, J.F., He, A.N. and Chen, L.B. (2008) Interaction of β-gibberellins at the shoot apex. Plant Cell 20: 1260–1277.

Lange, M.J.P. and Lange, T. (2016) Ovary-derived precursor gibberellin A9 is essential for female flower development in cucumber. Development 143: 4425–4429.

Lange, M.J.P., Liebrandt, A., Arnold, L., Chmielewska, S.M., Felsberger, A., Freier, E., et al. (2011) Functional characterization of gibberellin oxidases from cucumber, Cucumis sativus L. Phytochemistry 90: 62–69.

Lange, T., Hedden, P. and Graebe, J.E. (1994) Expression cloning of a gibberellin 20-oxidase, a multifunctional enzyme involved in gibberellin biosynthesis. Proc. Natl. Acad. Sci. USA 91: 8552–8556.

Lange, T., Kappler, J., Fischer, A., Frisse, A., Padeffke, T., Schmidtke, S., et al. (2005) Gibberellin biosynthesis in developing pumpkin seedlings. Plant Physiol. 139: 213–223.

Lange, T., Schweimer, A., Ward, D.A., Hedden, P. and Graebe, J.E. (1994) Separation and characterization of three 2-oxoglutarate-dependent dioxygenases from Cucurbita maxima L. endosperm involved in gibberellin biosynthesis. Plant J. 195: 98–107.

Lee, D.J. and Zeevaart, J.A.D. (2007) Regulation of gibberellin 20-oxidase1 expression in spinach by photoperiod. Plant Cell 127: 624–629.

Lemke, C., Potter, K.C., Schulte, S. and Peters, R.J. (2019) Conserved bases for primary and secondary metabolism in gymnosperms. Phytochemistry 169: 524–533.

Lee, J.J. and Petrov, J.D. (2007) Selection of precursors of gibberellin 20-oxidase1 expression in spinach by photoperiod. Plant Cell 19: 107–119.
Tatsukami, Y. and Ueda, M. (2016) Rhizobial gibberellin negatively regulatesTakehara, S., Sakuraba, S., Mikama, B., Yoshida, H., Yoshimura, H., Itoh, A., et Thomas, S.G., Blazquez, M.A. and Alabadi, D. (2016) DELLA proteins: master regulators of gibberellin-responsive growth and development. Annu. Plant Rev. 49: 189–228.

Thomas, S.G., Phillips, A.L. and Hedden, P. (1999) Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation. Proc. Natl. Acad. Sci. USA 96: 4698–4703.
Yamaguchi, S., Saito, T., Abe, H., Yamane, H., Murofushi, N. and Kamiya, Y. (1996) Molecular cloning and characterization of a cDNA encoding the gibberellin biosynthetic enzyme ent-kaurene synthase B from pumpkin (Cucurbita maxima L.). Plant J. 10: 203–213.

Yamaguchi, S., Sun, T., Kawaide, H. and Kamiya, Y. (1998) The GA2 locus of Arabidopsis thaliana encodes ent-kaurene synthase of gibberellin biosynthesis. Plant Physiol. 116: 1271–1278.

Yumane, H., Satoh, Y., Nohara, K., Nakayama, M., Murofushi, N., Takahashi, N., et al. (1988) The methyl ester of a new gibberellin, GA73—the principal antheridiogen in Lygodium japonicum. Tetrahedron Lett. 29: 3959–3962.

Yoshida, H., Hirano, K., Sato, T., Mitsuda, N., Nomoto, M., Maeo, K., et al. (2014) DELLA protein functions as a transcriptional activator through the DNA binding of the INDETERMINATE DOMAIN family proteins. Proc. Natl. Acad. Sci. USA 111: 7861–7866.

Zhang, H., Li, M., He, D.L., Wang, K. and Yang, P.F. (2020) Mutations on ent-kaurene oxidase 1 encoding gene attenuate its enzyme activity of catalyzing the reaction from ent-kaurene to ent-kaurenoic acid and lead to delayed germination in rice. PLoS Genet. 16: e1008562.

Zhang, Y.F., Su, P., Wu, X.Y., Zhou, J.W., Zhao, Y.J., Hu, T.Y., et al. (2019) The gibberellin 13-oxidase that specifically converts gibberellin A9 to A20 in Tripterygium wilfordii is a 2-oxoglutarate-dependent dioxygenase. Planta 250: 1613–1620.

Zhang, Y.Y., Zhang, B.C., Yan, D.W., Dong, W.X., Yang, W.B., Li, Q., et al. (2011) Two Arabidopsis cytochrome P450 monooxygenases, CYP714A1 and CYP714A2, function redundantly in plant development through gibberellin deactivation. Plant J. 67: 342–353.

Zhou, F., Wang, C.Y., Gutensohn, M., Jiang, L., Zhang, P., Zhang, D.B., et al. (2017) A recruiting protein of geranylgeranyl diphosphate synthase controls metabolic flux toward chlorophyll biosynthesis in rice. Proc. Natl. Acad. Sci. USA 114: 6866–6871.

Zhou, K. and Peters, R.J. (2009) Investigating the conservation pattern of a putative second terpene synthase divalent metal binding motif in plants. Phytochemistry 70: 366–369.

Zhou, K., Xu, M.M., Tiernan, M., Xie, Q., Toyomasu, T., Sugawara, C., et al. (2012) Functional characterization of wheat ent-kaurene(-like) synthases indicates continuing evolution of labdane-related diterpenoid metabolism in the cereals. Phytochemistry 84: 47–55.

Zhu, J.C., Mafu, S., Peters, R.J. (2014) To gibberellins and beyond! Surveying the evolution of (di)terpenoid metabolism. Annu. Rev. Plant Biol. 65: 259–286.