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Gibberellin biosynthesis and metabolism: A convergent route for plants, fungi and bacteria

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ABSTRACT

Gibberellins (GAs) are natural complex biomolecules initially identified as secondary metabolites in the fungus *Gibberella fujikuroi* with strong implications in plant physiology. GAs have been identified in different fungal and bacterial species, in some cases related to virulence, but the full understanding of the role of these metabolites in the different organisms would need additional investigation. In this review, we summarize the current evidence regarding a common pathway for GA synthesis in fungi, bacteria and plant from the genes depicted as part of the GA production cluster to the enzymes responsible for the catalytic transformations and the biosynthetical routes involved. Moreover, we present the relationship between these observations and the biotechnological applications of GAs in plants, which has shown an enormous commercial impact.

1. Introduction

Gibberellins (GAs) are considered phytohormones and conform a large family of diterpenoids that possess the tetracyclic *ent*-gibberellane carbon skeletal structure arranged in either four or five ring systems where the variable fifth ring is a lactone. There are two main types of characterized GAs, which are shown in Fig. 1: one group that includes molecules with 20 carbon atoms (C_{20}-GAs) and another group that consists of molecules that have lost one carbon and show one lactone (C_{19}-GAs). This late group shows an acidic nature with a pKa around 4.8, where the polarity of each molecule could vary according to the number of hydroxyl and carboxyl groups, the saturation level and the presence of methylene or sugar residues (Srivastava, 2002). Although not all C_{19}-GAs are bioactive, all bioactive GAs belong to the C_{19}-GAs group and the most outstanding activity has been reported for GA_1, GA_3, GA_4 and GA_7 (Bömke and Tudzynski, 2009; Sponsel and Hedden, 2010).

Initially, the activity of GAs was related to culture filtrates of the rice pathogen *G. fujikuroi*, causative agent of the disease called *Bakanae* or Foolish Seedling Disease, where rice plants grow without control making impossible for the plant to sustain its own weight causing their fall and death; other hallmarks of the disease include infertility and empty panicles (Sawada, 1912). Later on, it was observed that auxenic cultures of *G. fujikoroi* had the ability to stimulate plant growth in different species besides rice, leading to the conclusion that the effect observed on the plant was mediated by a toxin secreted by the fungal strain (Kurosawa, 1926). In 1935, the so-called toxin was purified, received the name of Gibberellin and its biological activity was again demonstrated. After this initial purification, a yellow oily fraction was recovered, consisting of a mixture of Gibberellin A and Gibberellin B, but the mixture was not pure enough to identify the chemical nature of the components (Yabuta and Sumiki, 1938). Further purification of Gibberellin A rendered three acidic compounds named Gibberellin A_1, A_2 and A_3 (Takahashi et al., 1955). While Gibberellin A_3 was identified in Japan, the same molecule was isolated by teams from the United States and the United Kingdom, receiving the name of Gibberellic Acid. Gibberellic acid showed a profound effect on plant growth and had the ability to restore the normal phenotype on mutant dwarf plants of corn (Phinney, 1956). Further studies supported the effect of different GAs, leading to the establishment of a specific nomenclature for this family of molecules (Tamura, 1991).

More recent studies started to uncover the function of GAs at the molecular level, like the functional identification and cloning of the GAI (GA Insensitive) cDNA or the mutation of the *gai* allele, which causes insensitivity to GAs in *Arabidopsis thaliana*. Initial mechanistic studies suggested that GAs promoted GAI degradation by the proteasome in a

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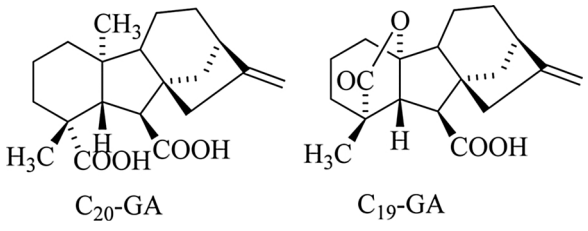

Fig. 1. Structure of C_{20}-GAs and C_{19}-GAs. Both groups of GAs present the characteristic organization corresponding to the *ent*-gibberellane ring.

ubiquitination-dependent manner, suggesting that the biological effect of GAs was due to de-repression, instead of a simple stimulatory activity (Peng et al., 1997). GAI resulted a putative transcription factor and is now member of the DELLA family of proteins that control cell proliferation responsible for plant growth. These observations associated with further molecular and biochemical studies encouraged an enormous increase in the interest regarding the production and applications of GAs (Sun, 2008). Recent evidence concerning all these advances is further presented.

2. The gibberellin biosynthetic pathway: a gathering point for plants, fungi and bacteria

The gibberellin biosynthetic pathway has been extensively studied in plants and additional insights have also been provided by the studies performed in fungal strains. More recent studies are starting to elucidate the pathway in bacteria. Particular features depicted for the different organisms are further presented. The biochemical route for GAs synthesis starts from geranyl-geranyl diphosphate (GGPP) via isopentenyl diphosphate (IPP), which is the 5-carbon building block for all terpenoid/isoprenoid compounds (Sponsel and Hedden, 2010). In the green tissue of most plants, the basic isoprenoid unit IPP is generated by two roads: the mevalonic acid (MVA) pathway in the cytoplasm and the methyl erythritol phosphate (MEP) pathway in plastids (Eisenreich et al., 2001; Kasahara et al., 2002; Sponsel, 2001; Hedden and Thomas, 2012) and the full route can be divided into three stages according to their subcellular compartmentalization and the enzymes involved. The first stage is catalyzed by soluble enzymes located in the proplastids, leading to the production of *ent*-kaurene. In the second stage, *ent*-kaurene is oxidized to GA_{12}-aldehyde, which constitutes the general GA precursor and is further catalyzed by cytochrome P-450 mono-oxygenases at the endoplasmic reticulum. The third and final stage of the pathway is catalyzed by 2-oxoglutarate-dependent di-oxygenases in the cytosol of the cell (Sun, 2008). In fungi, the biosynthetic route involves the MVA pathway, which provides IPP for the synthesis of all terpenoids, including GAs (Fig. 2).

The first GA-specific intermediate product is generated in two cyclization steps from GGDP via *ent*-copalyl diphosphate (CPP). Sequential oxidation of *ent*-kaurene at position C-19 via *ent*-kaurenol and *ent*-kaurenal yields *ent*-kaurenoic acid, which is further oxidized to generate the *ent*-7α-hydroxykaurenoic acid. A final oxidation at position C-6β leads to the formation of the GA_{12}-aldehyde (Fig. 2). These initial steps of the pathway are similar in fungi, higher plants and bacteria, being the basic difference that in fungi only one bifunctional terpene cyclase (CPS/KS) is used for the formation of *ent*-kaurene from GGDP, while in plants and bacteria the synthesis involves two enzymes: the *ent*-copalyl-diphosphate synthase (CPS) and the *ent*-kaurene synthase (KS) (Tudzynski, 2005; Morrone et al., 2009; Hedden and Thomas, 2012; Hershey et al., 2014; Nett et al., 2017a). In higher plants, GA_{12} lies at a branch-point in the pathway, GA12-aldehyde is converted to GA12 (a non-13-hydroxylated GA) and due to the hydroxylation on C-13 (13-hydroxylation), GA_{12} is converted to GA_{53}. GA_{12} and GA_{53} are precursors for the non-13-hydroxylation and 13-hydroxylation pathways, respectively, which are then oxidized on C-20 converting these substrates into GA_9 and GA_{20} respectively (Hedden and Thomas, 2012). The final step in the formation of bioactive GAs in plants is the 3 β-hydroxylation of GA_9 and GA_{20} to generate GA_4 and GA_1, respectively (Bömke and Tudzynski, 2009). In *F. fujikuroi*, GA_{12}-aldehyde is first 3β-hydroxylated to produce GA_{14}-aldehyde, which is then oxidized at position C-7 to form GA_{14} (Tudzynski, 2005; Urrutia et al., 2001), GA_{14} is then converted to the 19-carbon gibberellin GA_4 by oxidation. GA_4, the first biologically active GA, is desaturated to GA_7, which is then converted to GA_3 by late 13-hydroxylation. GA_1 is formed in a minor side reaction by 13-hydroxylation of GA_4. Thus, the major difference between the GA biosynthetical pathways in *F. fujikuroi* and plants is the stage at which the 3β-and the 13-hydroxylations occur. In fungi, GA_{12}-aldehyde is 3β-hydroxylated to GA_{14}-aldehyde and 13-hydroxylation takes place only in the final step to form GA_3 from GA_7 (Fig. 2), whereas in plants the final step is the 3β-hydroxylation of GA_9 and GA_{20} to GA_4 and GA_1 (Bömke and Tudzynski, 2009; Hedden and Sponsel, 2015; Tudzynski, 2005). On the other hand, it was recently determined that in bacteria the GA biosynthesis occurs exactly in the same fashion that has been depicted for the non-13-hydroxylation pathway in plants (Fig. 2), where GA_{12}-aldehyde is converted to GA_{12} via C7 oxidation, then GA_{12} is oxidized at C20 to form GA_{15}, GA_{15} is oxidized to form GA_{24}, subsequently the loss of C20 and the formation of the lactone ring gives rise to the formation of GA_9 (Méndez et al., 2014; Nett et al., 2017a,b), GA_9 undergoes a 3β-hydroxylation process to form the bioactive GA_4 (Nagel et al., 2017; Nett et al., 2017a).

The elucidation of the gibberellin synthesis pathway in plants, fungi and bacteria indicates that three pathways have evolved independently, although they are similar in their intermediates, the relevant enzymes are either from different enzymatic classes or belong to different families within the same class and share very low overall sequence identity (Nett et al., 2017a; Nagel and Peters, 2017a,b).

3. Genes encoding the enzymes responsible for GA biosynthesis

The identification of several genes involved in the biochemical route responsible for gibberellin biosynthesis provided further insights into these pathways and their regulation. However, some of the enzymes are encoded by multiple genes, each subjected to particular control expanding genetic complexity for the biochemical program. Here we present the particular features reported so far for the genetic code responsible for GA biosynthesis in plants, fungi and bacteria.

3.1. Gibberellin biosynthetic genes uncovered in plants

The availability of the full *Arabidopsis* genome sequence in 2000 enabled the identification and characterization of the majority of the genes implicated in the GA biosynthetic pathway in this organism (Bömke and Tudzynski, 2009). Additional information came from the cloning of Gibberellin 13-hydroxylases from rice (Magome et al., 2013), allowing the identification of genes for all the enzymes involved in gibberellin biosynthesis in plants (Table 1).

A total of seven enzymes belonging to three different classes are required for the biosynthesis of bioactive GAs from GGDP: two terpene synthases, two cytochrome P450 monooxygenases, and 2-oxoglutarate-dependent dioxygenases [2ODDs] (Hedden et al., 2001). The enzymes catalyzing early reactions in the pathway generally are encoded by single genes, while those involved in later steps, particularly dioxygenases, are encoded by gene families (Hedden, 2003). The two-step conversion of GGDP to *ent*-Kaurene is catalyzed by the *ent*-copalyl diphosphate synthase (CPS) and the *ent*-Kaurene synthase (KS) and both enzymes are encoded by a single locus in *Arabidopsis* (*GA1* and *GA2*, respectively) (Yamaguchi et al., 1998; Regnault et al., 2014) and rice (OsCPS1 and OsKS1, respectively) (Yamaguchi, 2008; Prisic et al., 2004). On the other hand, only one gene coding for KS has been identified in pumpkin (*Cucurbita máxima* L.) (Yamaguchi et al., 1996), while in mature leaves of *Stevia rebaudiana* two *KS* gene copies were