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GI

See *Gastrointestinal Hormone*

Gibberellins

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Gibberellins are tetracyclic diterpenes that are found in plants and fungi. A few of the 126 gibberellins identified to date are known to be active hormones that are involved in seed germination, seedling emergence, stem elongation, and flower and fruit development. The gibberellin receptor has not yet been conclusively identified.

I. INTRODUCTION

To date, 126 gibberellin A (GA) compounds from plants and fungi have been characterized. They have been named in chronological order of their discovery (GA_1 to GA_n). All GAs are diterpenes with the same tetracyclic skeleton, but they differ in the types and positions of functional groups. Although individual plant species may contain >20 different GAs, probably only one GA in each species has intrinsic biological activity and is the active hormone. The others are either precursors or deactivation products of the active hormone. GAs are present in vegetative tissues at concentrations ranging from 0.1 to 100.0 ng g^{-1} fresh weight. The concentrations may be several orders of magnitude higher in developing seeds, which historically have been an excellent source of GAs for characterization. Developing seeds have also been useful sources of biosynthetic enzymes for determining metabolic pathways. Moreover, germinating cereal grains have been a model system for studying GA action because of the role GAs play in inducing synthesis and secretion of α-amylase. Another notable action of GAs is on stem growth. Some plants treated with biologically active GAs in excess of their natural levels can be made to grow exceedingly tall. In contrast, plants treated with growth retardants that inhibit GA biosynthesis and plants in which GA biosynthesis is genetically blocked are dwarfs. GAs act in stem growth via an enhancement of both cell division and cell elongation. GAs also promote flowering, male fertility, and fruit development.

II. HISTORY

Gibberellins get their unusual name from the fungus *Gibberella fujikuroi*, from which they were first isolated. *Gibberella fujikuroi*, a pathogen of rice, causes severe stem overgrowth, commonly known as "foolish seedling disease." Early work on the characterization of growth-promoting factors produced by the fungus led to the first isolation of GAs. It became apparent in the 1950s that plants contain similar GAs to those in *Gibberella*, although at much lower levels than in the fungus. Application of GAs to a variety of dwarf or nonflorally induced plants led to striking effects on growth and flowering, paving the way for the designation of GAs as a new class of plant hormone.

III. STRUCTURE

Gibberellins are tetracyclic diterpenes with an *ent*-gibberellane ring structure (Fig. 1). They contain either 20 or 19 carbon atoms. The C_{20} GAs, e.g., GA_{12} (Fig. 1), which have the full complement of 20 carbons, are precursors of the C_{19} GAs. The C_{20} GAs do not have bioactivity per se. Although there are large numbers of C_{19} GAs, many of these do not have all the structural requirements for bioactivity, which include the presence of a 3β-hydroxyl group and absence of a 2β-substituent. Of the C_{19} GAs that fulfill these criteria, GA_1 (Fig. 1), GA_3, GA_4, and GA_7 are the most active. Their high biological activity

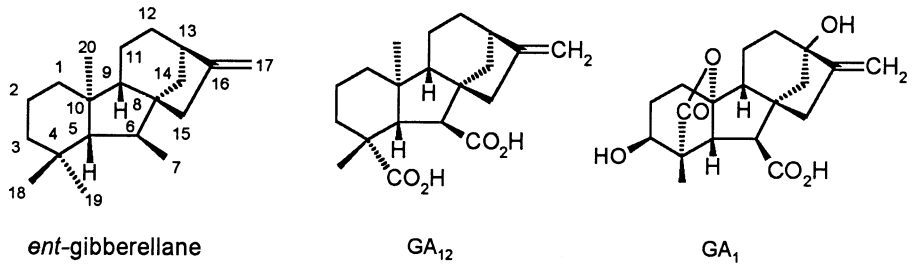

FIGURE 1 The *ent*-gibberellane skeleton, which is common to all gibberellins (GAs); GA_{12} is a C_{20} GA with the full diterpenoid complement of 20 carbons, and bioactive GA_1 is a C_{19} GA that has lost carbon 20.

undoubtedly explains why these GAs were among the very first to be isolated. In most species, GA_1 is considered to be the active hormone. However, in *Arabidopsis thaliana*, GA_4 (13-deoxyGA_1) appears to be more bioactive than GA_1, and is suggested to be the active hormone. There is also evidence that additional GAs may have activity in the control of flowering. GA_3, also known as gibberellic acid, is the major GA in *G. fujikuroi* and is produced commercially from fungal fermentations. GA_3 has many commercial uses in horticulture, agronomy, and brewing.

IV. BIOSYNTHESIS AND METABOLISM

Biosynthesis of GAs in plants can be divided into three parts. The first part occurs in plastids and involves biosynthesis of a tetracyclic hydrocarbon, *ent*-kaurene (Fig. 2). In the second part of the pathway, which occurs in the endoplasmic reticulum, *ent*-kaurene is oxidized sequentially and is converted to the first-formed GA, GA_{12} (Fig. 3). The third part of the pathway occurs in the cytosol and it can produce the multiplicity of C_{20} and C_{19} GAs known to be present in most plants.

The diterpenoid nature of GAs indicates that they are synthesized from isopentenyl diphosphate (IPP), which is the universal 5-carbon building block for all terpenoids. The classical route to IPP is the acetate/mevalonate (MVA) pathway. This pathway is located in the cytosol and leads to sesquiterpenoids (containing 15 carbon atoms) and triterpenoids (30 carbons). In the past 10 years, a second biosynthetic route to IPP has been discovered in bacteria, algae, some protozoa, and plants. This is the 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway (Fig. 2), and in eukaryotes this new pathway occurs only in plastids. IPP formed in chloroplasts and other types of plastids is further converted to monoterpenes (10 carbons), diterpenes (20 carbons), and tetraterpenes (40 carbons). Therefore, DOXP-derived IPP might be expected to be the precursor of GAs, because they are diterpenes. Although the synthesis of GAs by this route has not been demonstrated unequivocally, it is generally assumed that the DOXP pathway will be the major biosynthetic route to GA precursors.

Briefly, in the DOXP pathway, a two-carbon unit (derived by decarboxylation of pyruvate) is added to glyceraldehyde 3-phosphate to give DOXP. The reaction is catalyzed by DOXP synthase and seems to be a point of regulation in the pathway. The gene encoding DOXP synthase in several plants is expressed in a developmental and tissue-specific manner. The next reaction, catalyzed by DOXP reductoisomerase, involves the reduction and intramolecular rearrangement of the linear DOXP to give a branched structure, 2-C-methyl-D-erythritol 4-phosphate (MEP). The route from MEP to IPP is only partially defined (Fig. 2), but all reactions so far elucidated take place in plastid stroma.

Subsequent condensation of IPP and its isomer, dimethylallyl diphosphate, gives the monoterpene, geranyl diphosphate (GPP). Two molecules of GPP combine to give the linear diterpene, geranylgeranyl diphosphate (GGPP). At GGPP the pathway branches in several directions, with separate branch pathways leading to the carotenoids, to the phytoene side chain of chlorophyll, to the nonaprenyl side chain of plastoquinone, and to the *ent*-kaurenoids and GAs. Only the branch to the *ent*-kaurenoids and GAs is considered here.

En route to GAs, cyclization of the linear GGPP to the tetracyclic *ent*-kaurene occurs in two stages (Fig. 2). GGPP is converted first to a bicyclic compound, *ent*-copalyl diphosphate, by *ent*-copalyl diphosphate synthase (CPS). *ent*-Copalyl diphosphate is converted to the tetracyclic compound *ent*-kaurene in a reaction catalyzed by *ent*-kaurene synthase (KS). CPS is present at much lower levels than KS, and so CPS is thought to have an important "gatekeeper"

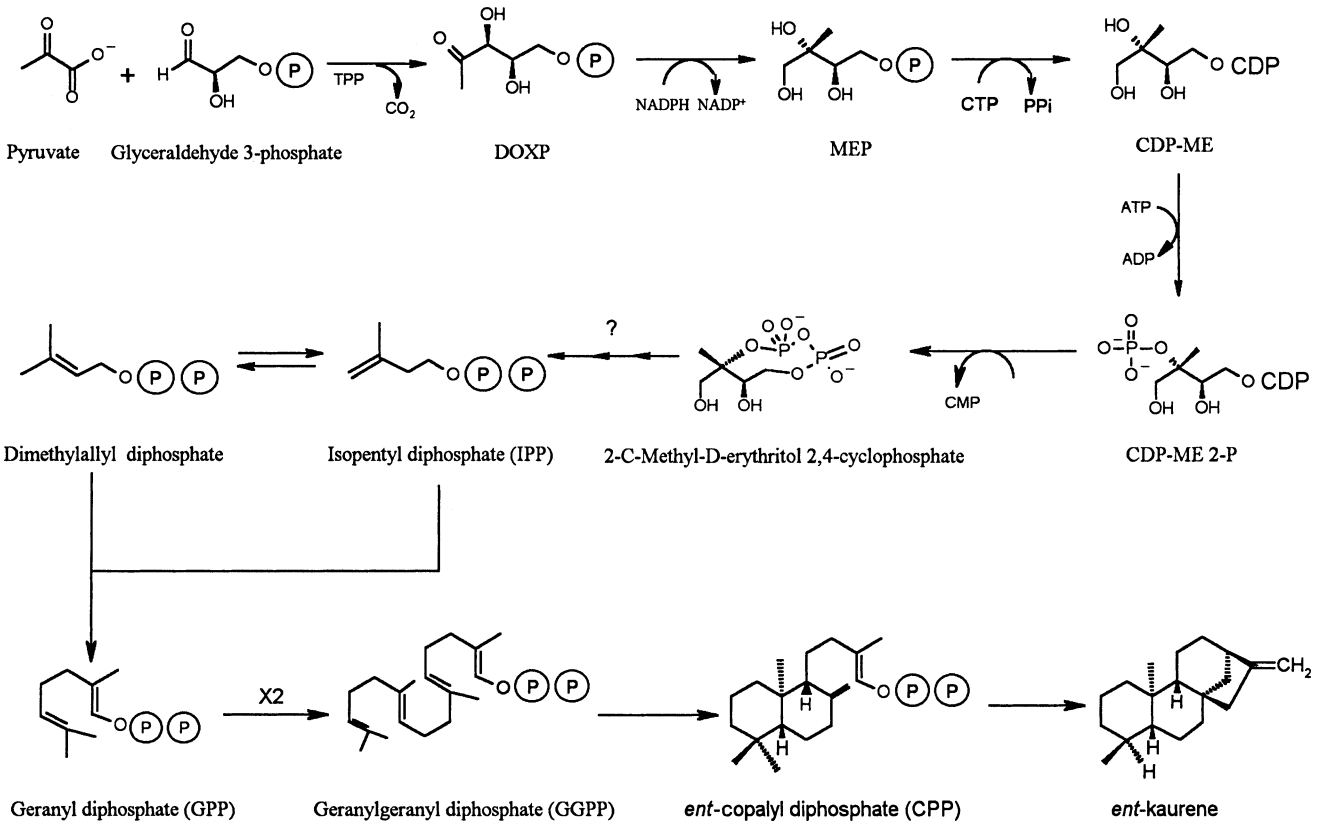

FIGURE 2 The 1-deoxy-D-xylulose 5-phosphate (DOXP) pathway to isopentenyl diphosphate, and subsequent formation of the diterpene, *ent*-kaurene. CDP-ME, 4-diphosphocytidyl-2-C-methylerythritol; CDP-ME 2-P, 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-phosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate.

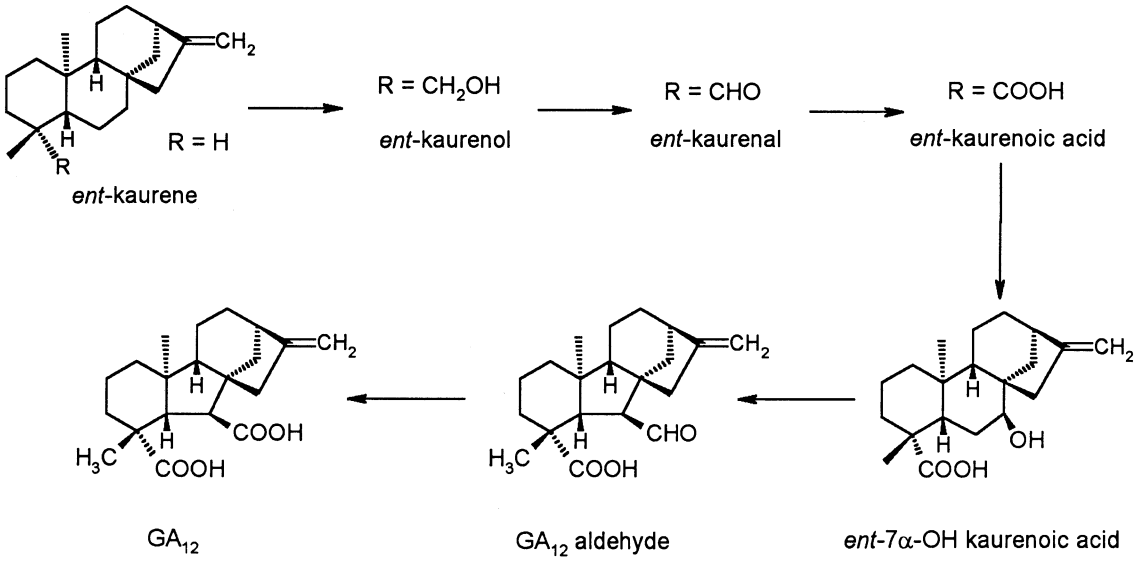

FIGURE 3 The multistep conversion of *ent*-kaurene to GA_{12}.

function on the branch of the terpenoid pathway that is committed to *ent*-kaurenoids and GAs.

The next part of the pathway (Fig. 3) involves the sequential oxidation of *ent*-kaurene at carbon 19 and is catalyzed by a membrane-bound, cytochrome P450 enzyme, *ent*-kaurene oxidase. This catalytic activity has been thought for some time to be associated with the endoplasmic reticulum, although recently *ent*-kaurene oxidase from *Arabidopsis* was reported to be localized to the chloroplast outer envelope. *ent*-Kaurene oxidase is multifunctional and can catalyze three oxidations: *ent*-kaurene → *ent*-kaurenal → *ent*-kaurenol → *ent*-kaurenoic acid (Fig. 3). The next enzyme in the pathway, *ent*-kaurenoic acid oxidase, is also a multifunctional P450 monooxygenase and is associated with the endoplasmic reticulum in *Arabidopsis* and many other species. It can catalyze the sequence: *ent*-kaurenoic acid → 7β-hydroxykaurenoic acid → GA_{12}-aldehyde → GA_{12} (Fig. 3). These reactions involve the conversion of a 6-membered B ring to a 5-membered B-ring and the formation of a carboxylic acid at carbon 7. The biosynthetic pathway leading to GA_{12} appears to be the same in all plants examined to date, but it can diverge from there on. The next reactions, comprising the last part of the pathway, occur in the cytosol.

There are multiple positions in the GA molecule for subsequent oxidation. The enzymes that catalyze these reactions are soluble 2-oxoglutarate-dependent dioxygenases. The sequential oxidation of carbon 20, until it is eventually lost as CO_2, yields a GA containing 19, instead of 20, carbon atoms (Fig. 4). The carboxyl at C-4 forms a lactone at C-10, and this γ-lactone is essential for bioactivity. Also necessary for bioactivity is the presence of a 3β-hydroxyl group or some other functionalization of carbon 3, including a 2,3 double bond. The introduction of hydroxyl groups can occur "early" or "late" in the pathway, and because the dioxygenases do not have absolute substrate specificity, parallel and interconnecting pathways may occur.

Because several of the enzymes in the pathway are multifunctional, as few as four enzymes can catalyze the 10 reactions needed to convert *ent*-kaurene to a bioactive GA. This is a much smaller number than expected. However, despite the small number of different enzymes that are needed, it is now known that some of the enzymes, particularly the dioxygenases, are encoded by small gene families. This means that there are multiple isoenzymes of some of the enzymes. In *Arabidopsis*, for instance, there are as many as five different 20-oxidases. The expression of individual members of a gene family can be tissue specific and developmentally regulated. Moreover, regulation by intrinsic and/or extrinsic factors may operate on selective isoenzymes only.

With regard to sites of synthesis, there is incontrovertible evidence that GA biosynthesis occurs in the developing seeds of many species. The stages of GA biosynthesis from GGPP have been demonstrated repeatedly in cell-free systems from endosperm preparations and other seed parts. Other sites of GA biosynthesis include actively growing tissues such as shoot tips, expanding leaves, and floral organs.

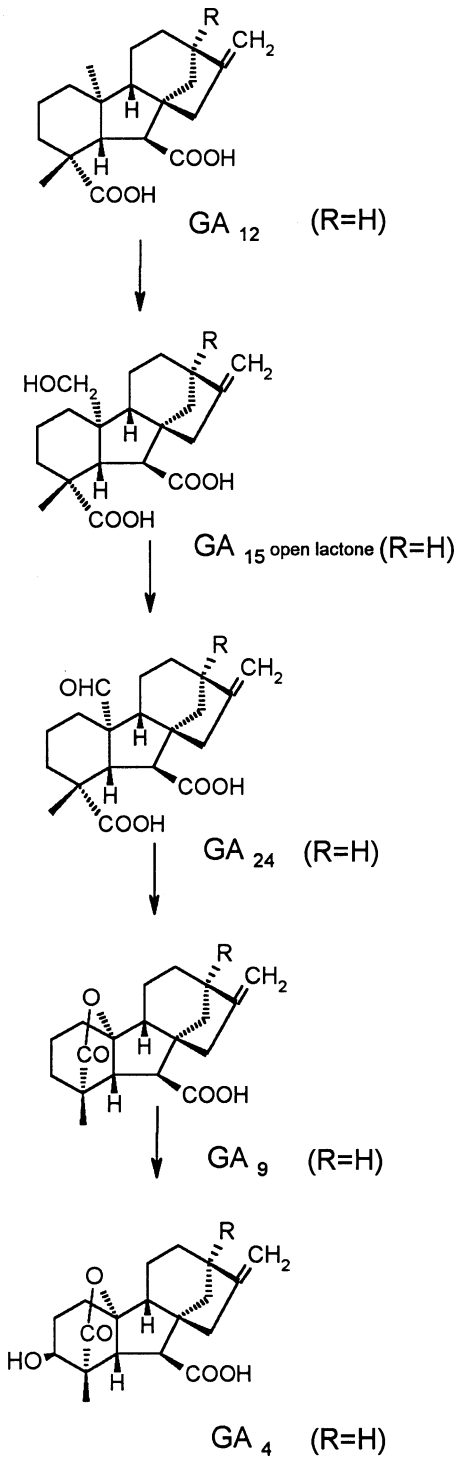

FIGURE 4 The three-step conversion of GA_{12} to GA_9 is catalyzed by a multifunctional GA 20-oxidase and results in the formation of the C_{19} GA, GA_9. Hydroxylation at carbon 3 is required to form a bioactive GA, GA_4. Any of these GAs can be a substrate for a 13-oxidase, potentially giving the following sequence: GA_{53}, GA_{44}-open lactone, GA_{36}, GA_{20}, and GA_1, in all of which R = OH.

The evidence for GA biosynthesis in these tissues comes from studying the expression of genes that encode key enzymes in the GA biosynthetic pathway. The use of reporter genes for these studies has been particularly instructive. In many cases, the site of GA synthesis correlates with the site of GA action. Although localized movement of GAs may occur—for instance, within regions of the shoot apex or between different seed parts—there is little evidence that long-distance transport is required.

Alteration in GA biosynthesis in response to environmental factors is one important way that light, for example, can have an impact on plant growth and development. GA biosynthesis is affected by both light quantity and quality. In *Arabidopsis* and spinach a stem-expressed 20-oxidase is up-regulated in shoots after exposure to long days, coincident with stem elongation and prior to flowering. 3-Oxidation in lettuce and *Arabidopsis* seeds is enhanced in red illumination and is important for germination. In both of these examples, up-regulation of an enzyme catalyzing a rate-limiting step in the biosynthetic pathway leads to an increase in the level of bioactive GA.

V. USE OF MUTANTS

In the past, there have been concerted efforts to identify all GAs in a given plant species, to determine the GA metabolic pathways within the plant, and to characterize a comprehensive series of mutants. The GA-responsive stem length mutants of corn, pea, and *Arabidopsis* have been particularly useful. For each of these species a series of mutants that showed a graded severity of phenotype, from extreme dwarf to semidwarf, were either generated or obtained from natural sources (e.g., *Arabidopsis*; Fig. 5). Each of these mutants was shown to be blocked at a specific stage in GA biosynthesis between *ent*-kaurene and the active hormone. Treatment of a particular mutant with an *ent*-kaurenoid or GA beyond the metabolic block can restore normal growth. If a mutant is blocked for 3β-hydroxylation (*ga4* in *Arabidopsis*, *d1* in corn, *le* in pea,), 3-deoxy GAs cannot restore growth, whereas GA_4 or GA_1 can, providing evidence that the active GA must be 3β-hydroxylated.

In addition to having an extreme dwarf phenotype, the *ga1*, *ga2*, and *ga3* mutants of *Arabidopsis* are also male-sterile. Flower buds fail to open and no seeds are set (Fig. 5). Treating these mutant plants with GA not only restores normal stem elongation but also restores fertility. Moreover, seeds of these

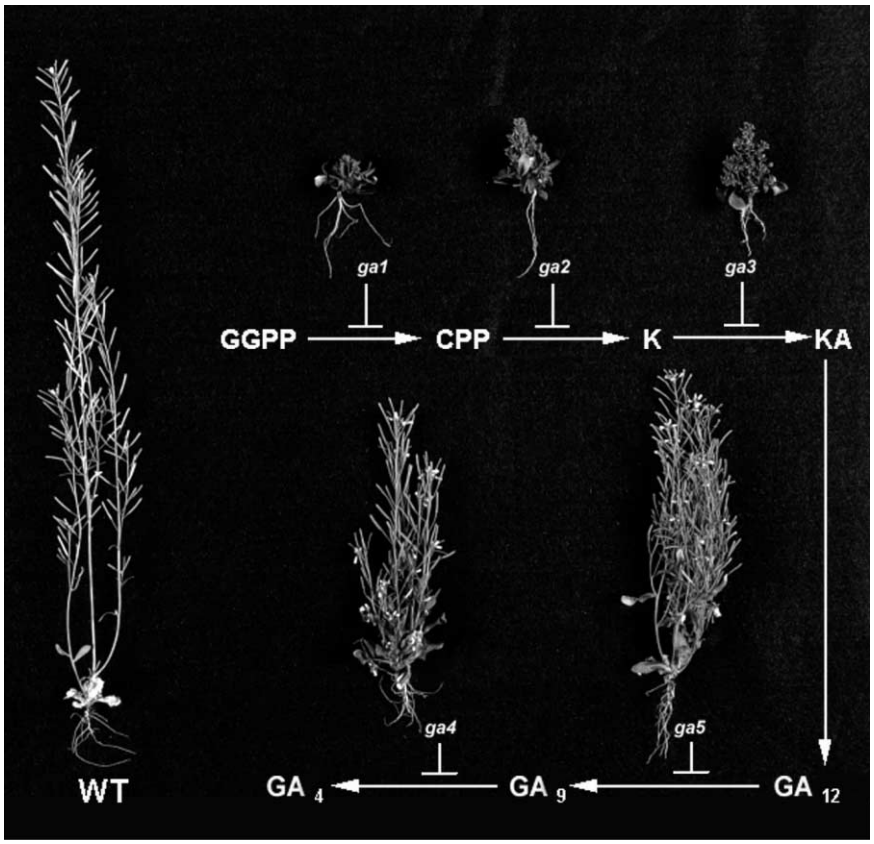

FIGURE 5 *Arabidopsis thaliana*. The Landsberg *erecta* ecotype (wild type) and five mutants, *ga1–ga5*. The position of the metabolic block in the GA biosynthetic pathway is shown for each mutant. All plants are photographed at 7 weeks. Only wild-type, *ga4*, and *ga5* plants have flowered and set seed. The *ga1*, *ga2*, and *ga3* mutants are male-sterile dwarf plants.

three GA-deficient mutants of *Arabidopsis* cannot germinate unless they are treated with GA, underscoring the requirement for GAs in several different phases of a plant's life cycle.

Using GA biosynthesis mutants, it has been shown that stem length is proportional to the content of bioactive GA. This has been clearly demonstrated for pea stem length and GA_1 content. Thus, factors that control the pool size of GA_1 or other active GA in the target tissue will be important for determining growth. Control points include the regulation both of biosynthesis of the active hormone and of its subsequent deactivation. In *Arabidopsis*, the 20-oxidases and 3-oxidases are regulated by feedback. The action of GA in promoting growth down-regulates transcription of genes that encode enzymes occurring earlier in the GA pathway. The precise mechanism whereby this regulation occurs is not known. Deactivation involves 2β-hydroxylation and may also involve conjugation to glucose. The 2β-hydroxylase is regulated by a feedforward mechanism.

Other mutants that have been characterized have altered responses to GAs. These mutants, which can be identified by their short or very tall phenotypes, are unlike the previously described mutants because their stem height often bears no direct correlation to their level of endogenous GA_1 (or GA_4). This is because the feedback control of the biosynthetic pathway requires GA-induced growth. In the absence of growth and the consequent absence of feedback regulation, the transcription of genes encoding 3-oxidase and 20-oxidase enzymes is up-regulated, which results in very high GA levels in severely dwarfed individuals. In contrast, other mutants can be unusually tall, even if the levels of endogenous GAs are reduced to almost zero by chemical or genetic means. Both of these types of mutants are useful tools with which to dissect the signal transduction pathway, and are discussed later.

VI. MODE OF ACTION IN CEREAL GRAIN

The cereal aleurone system has been the most well-investigated site of GA action. During germination, GA_1 from the embryo moves to the aleurone, which is a layer or layers of cells that envelope the starchy endosperm (Fig. 6). Within the aleurone layer, GA from the embryo induces the synthesis and secretion of hydrolytic enzymes, including several isoforms of α-amylase. α-Amylase catalyzes the breakdown of starch in the nonliving starchy endosperm, yielding usable forms of fixed carbon to nourish the growing seedling (Fig. 6). Cereal grains would therefore appear to be excellent material from which to isolate a GA receptor, and attempts to do so are ongoing.

The site of GA perception appears to be the plasma membrane of aleurone cells. The evidence for this is twofold. First, GA_4 that has been covalently linked to agarose beads to prevent its uptake into the cell can still induce α-amylase production. Second, GA injected directly into aleurone cells has no bioactivity. Two GA-binding proteins that specifically bind bioactive GAs have been isolated from aleurone cells, but unequivocal evidence that either or perhaps both (as a dimer) are the GA receptor is not yet available. Despite this serious gap in our understanding of GA perception, there is now a considerable amount of evidence for downstream signaling events that transduce the GA signal into the well-defined responses of α-amylase production and secretion. There are some small differences in GA signaling in grains of different genera (barley, rice, wheat, and wild oats are all used extensively), and the following description draws together experimental data from several different systems.

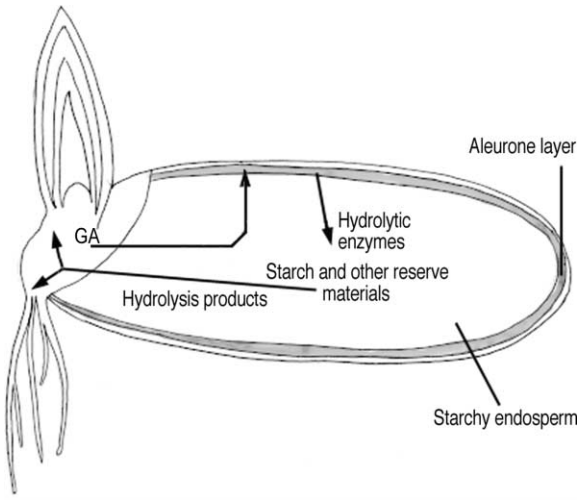

FIGURE 6 Cereal grain showing that GA from the embryo moves to the aleurone. *De novo* synthesis of amylase and other hydrolytic enzymes occurs in the aleurone. These enzymes break down reserve materials in the starchy endosperm in order to nourish the growing seedling.

Components of the GA signal transduction chain have been identified in the plasma membrane, cytosol, and nucleus (Fig. 7). Several lines of evidence indicate that bioactive GA, perceived at the plasma membrane and bound to a hypothetical receptor protein, interacts with a membrane-localized heterotrimeric G-protein. G-protein agonists can induce α-amylase gene expression in wild oat protoplasts in the absence of GA. Moreover, guanosine triphosphate (GTP)-γ-S, which binds G_α subunits and holds them in an activated form, stimulates α-amylase expression, whereas guanosine diphosphate (GDP)-β-S, which holds the subunits in an inactivated form, prevents GA induction of α-amylase gene expression. Additional evidence linking a putative heterotrimeric G-protein with GA signal transduction comes from the observation that the *dwarf1* (*d1*) mutant of rice, which has impaired sensitivity to GA, has a defective G_α subunit.

Based on identification of both Ca^{2+}-dependent and -independent events, there appears to be a dichotomy in the GA signal transduction pathway within the cytoplasm of barley aleurone cells (Fig. 7). One branch of the signaling pathway leads to rapid increases in intracellular calcium concentration, particularly in the peripheral cytoplasm, whereas the other branch leads to induction of α-amylase gene expression. Sustained lowering of Ca^{2+} levels for several hours does not affect α-amylase gene expression but inhibits α-amylase secretion. On the other hand, syntide 2 (an inhibitor of phosphorylation) inhibits GA induction of α-amylase but does not inhibit the rapid increase in Ca^{2+} concentration that is observed with GA treatment. These results show that effects of GA on α-amylase gene expression are independent of the Ca^{2+} effects, although intracellular Ca^{2+} concentration is important in α-amylase secretion. In the *d1* mutant of rice, previously mentioned because it defines a mutation in a G_α subunit of a heterotrimeric G-protein, both Ca^{2+}-dependent and -independent responses to GA_3 are perturbed at all but the highest dose of GA_3. This global disruption of GA responses would reinforce the position of a G_α-protein early in the GA signal transduction pathway (Fig. 7).

The mechanism for elevating intracellular Ca^{2+} concentration is presently unknown, but it has been suggested to involve increased flux of Ca^{2+}

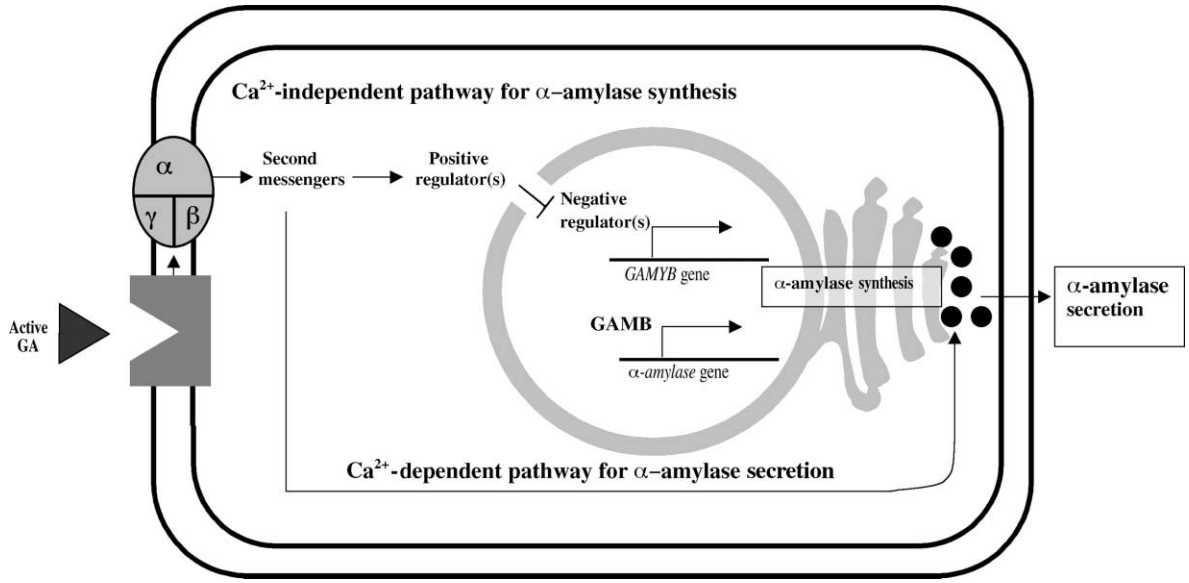

FIGURE 7 Simplified scheme to show some putative players in GA signal transduction in a generic cereal aleurone cell. The GA receptor is thought to be membrane localized, and the signal may be transduced through a heterotrimeric G-protein, the α-subunit of which is encoded by the *D1* locus in rice. The transduction pathway bifurcates into Ca^{2+}-dependent and Ca^{2+}-independent branches. The latter branch is under the control of both positive (GID1 in rice, GSE in barley) and negative (SLR in rice, SPY and SLN in barley, Rht1 in wheat) regulators. In the presence of a GA signal the negative regulation is removed, allowing GAMYB transcription factors to be formed, α-amylase genes to be transcribed, and α-amylase protein to be synthesized and secreted.

from the apoplast and/or a release of Ca^{2+} from an intracellular storage pool. GA also causes an elevation of calmodulin (CaM) levels in the aleurone. Targets for the elevated levels of Ca^{2+} and CaM in the cytoplasm may be transporters, channels, or other membrane proteins at several cellular locations, some of which may be directly involved in the secretory process.

Use of guanylyl cyclase inhibitors prevents a transient increase in cyclic GMP (cGMP) normally seen in barley aleurone layers 2 h after GA treatment. These inhibitors also reduce the accumulation of α-amylase mRNA and that of the gibberellin-associated MYB transcription factor, GAMYB. For this and other reasons, it has been suggested that cGMP may be a component of the transduction chain, provisionally placed downstream of the G-proteins and upstream of MYB. Interestingly, there is evidence in tomato for phytochrome A activation of a heterotrimeric G-protein, which in turn uses cGMP and Ca^{2+} in separate transduction chains to bring about anthocyanin production and chloroplast development.

Reversible phosphorylation is also involved in the GA signal transduction chain. The aforementioned syntide 2, which essentially blocks phosphorylation by competing with natural substrates, inhibits GA induction of gene expression. In contrast, okadaic acid, which is a phosphatase inhibitor, negates the effects of GA on Ca^{2+} accumulation, on gene expression, and on α-amylase secretion. This inhibitor therefore must act early (i.e. before the transduction chain branches) or, if it acts later it, it must act at two sites to inhibit both branches of the signal transduction chain.

The induction of gene expression by GA has been studied in detail. Within their promoter regions, amylase genes contain a GA response element (GARE) to which GA-inducible nuclear proteins bind. The GAMYB proteins have been identified as GA-inducible transcription factors that bind to the conserved sequence TAACA/GA of GAREs and transactivate amylase genes. The same transcription factors may also transactivate other GA-inducible genes. Because the expression of *GAMYB* precedes the expression of amylase genes and does not require protein synthesis, it is suggested that *GAMYB* represent primary response genes. There is a possibility that GA acts to repress a repressor of GAMYB (this is discussed again briefly in the next section). Additional nuclear proteins can bind other sequences adjacent to the GARE that comprise a so-called GARC (GA response complex). The TATCCAC box

in the promoter of amylase 1 is a suspected site of interaction. The identity of these cis-acting elements is as yet unknown, but they are envisaged to act as enhancers.

In summary, GA signaling in aleurone cells (represented schematically in Fig. 7) involves a putative membrane-bound receptor that may interact with a heterotrimeric G-protein to bring about a cascade of events within the cytoplasm. These may include, but are not limited to, an enhancement of guanylyl cyclase and an increase in cGMP, an elevation of intracellular Ca^{2+}, and the induction of transcription factors that facilitate α-amylase gene expression. α-Amylase is one of several hydrolytic enzymes that move to the starchy endosperm and catalyze the mobilization of reserve materials. The resulting breakdown products are essential for nourishment of the emerging seedling. Cereal grains in which amylase production is perturbed have impaired seedling emergence. Because cereals provide the world's population with a majority of its caloric needs and over half its protein, the importance of GA-induced amylase production in cereal aleurone cannot be overstated.

VII. REGULATION OF STEM GROWTH BY GAs

One of the most easily recognized effects of GA is on stem elongation, and a number of stem length mutants with altered sensitivity to GA have been characterized. The mutants fall into two broad phenotypic categories: those that are dwarf and have reduced responsiveness to applied GAs and those that are as tall or taller than wild-type plants, irrespective of how much GA they contain. Biochemically, the mutants also fall into two main categories: those that have a mutation in a negative regulator of GA signaling and those that have a mutation in a positive regulator of GA signaling. The exact nature of a mutation (i.e. whether it is a loss- or gain-of-function mutation) will have an impact on the phenotype. The mutant loci discussed here are listed in Table 1.

It is now apparent that many plants contain a mutated form of a gene initially defined by the *GAI* (*gibberellin insensitive*) locus in *Arabidopsis*. *GAI* encodes a putative transcription factor that is a negative regulator of GA signaling. In wild-type plants, GA can derepress this regulator, allowing GA responsiveness. If a deletion occurs in a particular region of the *GAI* gene, identified by a DELLA motif in the amino acid sequence, then the alteration causes a semidominant gain-of-function mutation. This type of mutation (designated as the *gai1-1* allele) prevents GA from being able to derepress the negative regulator, or, to put it in other words, a mutation in the DELLA region makes *GAI* a constitutive repressor of the GA response. The resulting phenotype is a very dwarf plant that is GA insensitive.

GAI orthologues present in maize (*dwarf8*, *D8*), wheat (*reduced height1*, *RHT1*), barley (*slender*, *SLN*), and rice (*slender rice*, *SLR*) all encode a similar protein. So too does the *RGA* (*repressor of ga1-3*) gene in *Arabidopsis*, which was isolated as a suppressor of the GA-deficient phenotype of *ga1-3* mutants. The *d8* and *Rht1* semidominant mutations, like *gai1-1*, have a deletion in the DELLA region and are dwarf nonresponders to GA. Historically, this mutation has had a profound effect on the well-being of millions of people, because it is the mutation responsible for the development of semidwarf cereals adapted to the semitropics by Dr. Norman Borlaug in the Green Revolution of the 1960s. Interestingly, it has been shown that mutations in *GAI* and its orthologues in barley (*SLN*) and rice (*SLR1*) can give either slender or dwarf phenotypes, depending on the locations of the mutations. For example, in rice the slender phenotype is exhibited by plants in which a mutation occurs in the nuclear localization sequence of *SLR1*. In these recessive loss-of-function mutants, the GA response is constitutively derepressed, giving a very tall phenotype. Alternatively, a truncation in the DELLA region of *SLR1* (produced by transforming wild-type plants with *pSLRtr*) results in a semidominant gain-of-function mutation, which gives a GA-insensitive dwarf phenotype. This unusual situation in which the two mutations in the same gene can lead to opposite phenotypes has been demonstrated for *sln* and *gai* mutants too. An intragenic suppressor of *GAI* gives a recessive mutation, and these plants (*gai*) have a nondwarf phenotype that is in direct contrast to the severely dwarf phenotype of the *gai1-1* mutants (Table 1). It is assumed that this *gai* loss-of-function mutant does not have a slender phenotype because of the presence of a functional repressor of gibberellin (RGA) protein.

Recessive mutations at another locus, involving *Spindly* (*SPY*), which was first defined in *Arabidopsis*, partly suppress the *gai* phenotype. This indicates that *SPY* may also be a negative regulator of GA signal transduction. It has been proposed that in the absence of GA, SPY activates GAI and RGA. In the presence of GA, SPY would be inactivated, or GAI and RGA might be modified to prevent interaction with SPY. The outcome would be the removal of negative

TABLE 1 Genetic Loci Known, from Mutant Analysis, to Affect GA Response

Species	Locus[a]	Mutant	Type	Phenotype	Proposed action of wild-type gene product
Arabidopsis	*Spindly*	*spy*	Recessive	Tall	Negative regulator of GA signaling
Barley	*Spindly*	*Hvspy*	Recessive	Tall	Negative regulator of GA signaling
Arabidopsis	*RGA*	*rga*	Recessive	Semidwarf	Negative regulator of GA signaling
Arabidopsis	*GAI*	*gai1-1*	Semidominant	Dwarf	Negative regulator of GA signaling
Arabidopsis	*GAI*	*gai*	Recessive	Wild type	Negative regulator of GA signaling
Maize	*Dwarf8*	*d8*	Semidominant	Dwarf	GAI orthologue
Wheat	*Reduced ht1*	*Rht1*	Semidominant	Dwarf	GAI orthologue
Barley	*Slender*	*sln1*	Recessive	Tall	GAI orthologue
Rice	*Slender rice*	*slr1-1*	Recessive	Tall	GAI orthologue
Rice	*Slender rice*	*pSLRtr*	Semidominant	Dwarf	GAI orthologue
Rice	*Dwarf1*	*d1*	Recessive	Dwarf	Positive regulator of GA signaling
Rice	*GID*	*gid*	Recessive	Dwarf	Positive regulator of GA signaling
Barley	*GSE*	*gse*	Recessive	Dwarf	Positive regulator

[a]Abbreviations: *RGA*, *repressor of ga1-3*; *GAI*, *gibberellin insensitive*; *GID*, *gibberellin-insensitive dwarf*; *GSE*, *gibberellin sensitive*.

regulation of the signaling pathway, allowing a GA response. It has recently been shown that SPY also affects plant development in addition to, but separate from, being involved in GA signaling. These additional roles for SPY have yet to be defined.

Studies utilizing green fluorescent protein (GFP) have shown that RGA is localized in the nucleus. However, given that neither RGA nor GAI possesses DNA-binding domains, it is likely that they interact with an additional factor (possibly a GAMYB transcription factor) that can bind to DNA. In barley, the gene product of *SLN*, which is the barley orthologue of *GAI* and thus a negative regulator of GA signaling, appears to be degraded in the presence of GA. This degradation of SLN is required before the expression of GAMYB in aleurone cells can be enhanced by GA. This result ties in a negative regulator of GA signaling (SLN), which was first defined through a mutant phenotype in stems with a known transcription factor (GAMYB) in the signaling pathway in aleurone. The GA-induced disappearance of RGA from *Arabidopsis* nuclei has also been reported, and again is consistent with the direct or indirect removal of a negative regulator by GA.

Compared to negative regulation, less is known about positive regulation of GA response pathways. Two loss-of-function (recessive) mutations, *d1* and *gid1* (*gibberellin-insensitive dwarf1*) in rice have been described. The mutant phenotypes are dwarf or semidwarf. As mentioned earlier, *D1* encodes a G_{α}-subunit, implicating it early in signal transduction in aleurone cells. The function of *GID* has been examined in double mutants. Plants with an *slr1 gid1* genotype are phenotypically slender, indicating that *slr1* is epistatic to *gid1*. This is consistent with a role for SLR1 downstream of GID1. In preliminary results it has been shown that in wild-type plants SLR1, visualized by fusion of the gene to one encoding GFP, is localized to nuclei and disappears with GA treatment. In *gid1 SLR1-GFP* plants, the GFP signal is retained in the nucleus, implying that a functional GID1 protein is required for the GA-dependent removal of the negative regulation imposed by SLR1.

A putative positive regulator, encoded by the *GSE* (*gibberellin sensitivity*) locus, has been described in barley. A recessive loss-of-function mutation gives a dwarf phenotype with some reduction in sensitivity to GAs for both leaf elongation and amylase synthesis. In double mutants, *sln1* is epistatic to *gse*, implying that SLN1 is located downstream of GSE in the signal transduction chain.

Overall, the picture that is emerging for GA signaling in both stems and aleurone is of a series of negative regulators (e.g., SPY, and GAI and its orthologues, namely, RGA, SLR, SLN, Rht1, D8,) that keep the GA response pathway repressed in the absence of GA. GA removes the repression, either by acting directly or though one or more positive regulators (e.g., GID1) to inactivate or degrade the negative regulator(s) (Fig. 7).

A GA-binding protein has not been unequivocally identified in vegetative tissue, nor have many ultimate target genes of the GA signal been identified. Compared to the downstream events in the aleurone system, stem elongation and flowering responses are more complex to analyze. Nevertheless, progress has been made with several systems, notably one

employing deep-water rice and its response to flooding. Deep-water rice is grown in Southeast Asia, where severe flooding can occur during the monsoon season. Rapid stem extension of up to 25 cm day^{-1} after submergence is a consequence of an increased ratio of GA to abscisic acid. Applied GA, in the absence of flooding, can mimic the increases in cell division and the enhanced cell extension normally seen after submergence. Rice genes that are regulated by GA include those encoding proteins that function in the cell cycle and those involved in "loosening" the cell wall to allow elongation to occur. Expansins are a class of proteins that are closely correlated with the walls of elongating cells, but as yet have an unknown function. Other genes that might encode components in the transduction pathway have been sought in rice using a variety of approaches. The expression of a gene that codes for a leucine-rich repeat receptor-like protein kinase is up-regulated by GA in actively dividing and elongating cells. Also rapidly up-regulated in meristematic cells by GA treatment is the gene *Growth Regulating Factor1*, which encodes a putative transcription factor or activator. Further progress in elucidating the function of this and other GA-regulated genes in deep-water rice can be expected in the near future, and it will enhance greatly our understanding of the mode of action of GAs in stem growth.

VIII. SUMMARY

The GA group is a daunting array of 126 individual compounds known to be present in plants and fungi. Much is known about their biosynthesis and metabolism, and structure/activity considerations lead us to believe that only a handful of GAs are active hormones in their own right. Their repertoire of activities includes promotion of signal events in the life cycle of a plant such as seed germination, seedling emergence, stem growth, flowering, and fruit development. Despite our inability to identify conclusively a GA receptor, much is now known about how the GA signal is transduced into the well-defined responses of amylase production and secretion in germinating cereal grains, and into less well-defined responses such as cell elongation.

Glossary

aleurone Layers of cells surrounding the starchy endosperm in cereal grains; on stimulation by gibberellins during germination, the cells synthesize hydrolytic enzymes to break down starch and other storage materials in the starchy endosperm.

C_{20} gibberellins Diterpenes with the full diterpenoid complement of 20 carbon atoms; the first of the products formed in the GA biosynthetic pathway.

C_{19} gibberellins Diterpenes that, through metabolism, have lost carbon 20 and so contain only 19 carbon atoms.

GA-insensitive dwarf mutants Plants in which there is a defect in the ability to detect or respond to bioactive GA; cannot be normalized by application of GA.

GA-sensitive dwarf mutants (GA biosynthesis mutants) Plants in which one of the enzymatic steps in the GA biosynthetic pathway is blocked, resulting in reduced levels of GA; can be normalized by application of a GA normally produced in the pathway after the metabolic block.

See Also the Following Articles

Abscisic Acid • Auxin • Brassinosteroids • Cytokinins • Ethylene • Jasmonates • Salicylic Acid

Further Reading

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Glucagon Action

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Glucagon is a 29-amino-acid peptide hormone secreted from the alpha cells of the pancreas. The main target organ for glucagon is the liver, where it increases hepatic glucose production and release. Glucagon achieves this by having coordinated actions to increase gluconeogenesis and glycogenolysis and to inhibit glycolysis and glycogen synthesis, with the net effect of directing hepatic flux toward synthesis and release of glucose. These actions are opposed by insulin. In health, glucagon and insulin secretion and action are carefully coordinated to provide an integrated control of hepatic glucose metabolism.

I. INTRODUCTION

The mechanisms of glucagon action are outlined in this article; the focus is on the established hepatic effects of glucagon, but there is also discussion of some of the putative actions of glucagon on nonhepatic organs. Some of the conditions in which the physiology of glucagon action is disturbed are described. Several biological actions of glucagon have been exploited therapeutically and therefore some possible diagnostic and therapeutic uses for exogenous glucagon in clinical practice are detailed.

II. GLUCAGON RECEPTOR

Like other peptide hormones, the intracellular actions of glucagon are initiated by binding to a cell surface receptor, which triggers a series of intracellular events mediated by second-messenger pathways. The glucagon receptor (GR) was first cloned in 1993 and is encoded on chromosome 17q25. The GR is a member of a large family of guanosine triphosphate (GTP)-binding protein-coupled receptors, all with seven transmembrane domains. Within this larger family of receptors, the glucagon receptor belongs to a subset of highly homologous receptors that includes receptors for peptides closely related to glucagon, i.e., glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), secretin, and growth hormone-releasing hormone (GRH). The subset also includes receptors for peptides unrelated to glucagon, such as corticotropin-releasing hormone (CRH), parathyroid hormone (PTH), and calcitonin.

III. SIGNAL TRANSDUCTION

Glucagon binding to the cell surface GR triggers two complementary second-messenger pathways that act intracellularly to mediate the downstream actions of glucagon, the cyclic AMP (cAMP) pathway and the calcium-mediated pathway.

A. cAMP Pathway

The best characterized mechanism for the transduction of glucagon binding to the GR into intracellular actions of glucagon involves formation of the second messenger, cAMP. Activation of the cAMP pathway is mediated by a GTP-dependent regulatory protein (G-protein) mechanism. Binding of glucagon to the GR results in an interaction with the stimulatory G-protein complex (GS). GS is a complex composed of α-, β-, and γ-subunits. In its trimeric state, the GS complex has the nucleotide guanosine diphosphate (GDP) bound to its α-subunit and is inactive. Glucagon binding to the GR acts to release the α-subunit of the GS by a process involving exchange of GDP for guanosine triphosphate. The liberated α-subunit is then active and able to stimulate the enzyme adenylyl cyclase, which converts ATP into the second messenger, cAMP. This process is limited by the endogenous GTPase activity of the α-protein, which will break down GTP to GDP, allowing reassociation of the inactive trimer.