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Mini-Reviews

Phytohormone Mutants in Plant Research

James B. Reid

Department of Plant Science, University of Tasmania, Hobart, Tasmania 7001, Australia

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Abstract. The techniques used for the production and identification of plant hormone mutants are described. The properties used to classify these mutants into the broad synthesis and sensitivity categories are discussed, and the genetic considerations needed to allow their effective use in plant hormone research examined. A brief outline of significant recent work on the gibberellin (GA), abscisic acid (ABA), auxin, ethylene, cytokinin and phytochrome mutants is provided. Suggestions for future emphasis are made, particularly relating to an examination of the tissue and ontogenetic specificity of the plant hormone genes.

One of the major developments in the study of plant hormones over the past 5 years has been the increased awareness and usage of single-gene mutants. Many excellent reviews on particular aspects of this topic have been published recently (e.g., King 1988, Koornneef 1986, MacMillan 1987, Phinney 1984). The aim of the present review is therefore to provide an overview and highlight new directions that this research might follow. The pitfalls that may reduce the potential power of the mutant approach will also be examined. No attempt has been made to discuss all the reported phytohormone mutants, especially where mutants from many species show similar responses [e.g., the gibberellin (GA) and abscisic acid (ABA) mutants].

Identification of Mutants

The first mutants that were implicated with changes in plant hormones possessed distinctive phenotypes, readily identifiable with the proposed effects of the hormone. For example, the dwarf and wilty phenotypes were associated with the GAs and ABA, respectively (Phinney 1956, 1961, Tal and Nevo 1973). Subsequent work with the dwarf and wilty mutants has shown the power of mutants as tools for unravelling the biosynthetic pathways of these groups of plant hormones, as well as their role in the control of these developmental processes (see below). This approach to identifying hormone mutants is still likely to be fruitful, since in excess of 300 phenotypically distinct single-gene mutations have been reported in each of several major crop species (e.g., maize, tomatoes, peas). This reservoir of genetic variation has largely been untapped by either developmental biologists or hormone physiologists. To select mutants of potential interest, a precise idea of the expected phenotype is required. Although this information is available for the GAs and ABA, it is not clear for the other hormones (e.g., cytokinins and auxins) even though voluminous literature on the supposed actions of these hormones is available. Equally intriguing to the concept of hormonal control of plant development is the biochemical basis for the many major developmental mutants which show no relationship to the known groups of plant hormones. Graft transmissibility has even been demonstrated for some of these developmental mutants (e.g., flowering; Murfet 1985).

Another approach to the identification of hormone mutants is to carry out mutagenesis studies using chemical mutagens or ionizing radiation. However, this technique is only as good as the scanning system designed to identify the relatively rare useful products from the vast bulk of nonuseful products. A lack of knowledge of the expected phenotype in the intact plant is again often limiting. A

significant aid to scanning is a positive selection regime, whereby only the desired mutants survive. Such a technique was elegantly designed and used by Koornneef et al. (1982) to select for ABA-deficient genotypes in *Arabidopsis thaliana*. They predicted that among nongerminating GA-deficient plants, any ABA-deficient mutant might be expected to germinate based upon the known interactions between GA and ABA in controlling seed dormancy. A mutant, *aba*, with the predicted phenotype was isolated and shown to possess reduced levels of ABA.

Unfortunately, such well-planned mutagenesis programs have been rare in the search for plant hormone mutants. A more common, but nevertheless successful technique, has been to search for mutants capable of growth on a medium that is either devoid of a necessary hormone or has abnormally high and usually toxic levels of a hormone. This technique has been successful in isolating mutants resistant to high levels of ABA in *Arabidopsis* (Koornneef et al. 1984) and auxin in *Nicotiana plumbaginifolia* (Blonstein et al. 1988). However, many such mutants do not appear to operate by blocking hormone synthesis as was originally hoped for in the search for auxin mutants. Rather their action may be quite indirect, influencing hormone response by altering the uptake or transport of the hormone to a site where it can elicit a biological response. Such mutants may be of general interest if these responses are of relevance to the development of the whole plant, but in cases where this has not been established, the usefulness of the mutants is debatable.

A simple modification of this classical method of mutagenesis is to select variants that arise during tissue culture. Significant amounts of genetic variation are produced during tissue culture and have been used as a source of variation during plant breeding but have not yet been used extensively as a technique for the production of hormone mutants.

Perhaps a more desirable method of mutant production is the use of transposons. This technique has been used in maize to analyze GA-responding *Mu*-dwarf mutants. The mutants originated among F_2 progeny from selfed lines carrying multiple copies of the transposable element known as Robertson's Mutator (*Mu*) (Phinney et al. 1986). The main advantage of transposon mutagenesis lies in the fact that the known DNA structure of the transposon should provide a probe to identify the mutated gene and consequently open up work at the molecular level, providing insights into the regulation and expression of the gene. However, multiple copies of the transposon in the genome (Phinney et al. 1986) may result in significant difficulties in executing this procedure, since determination of the insert causing the altered phenotype can be a problem. Further, the lack of suitable transposons for all species limits the usefulness of this technique.

An analogous technique is the production of mutants using the insertion of t-DNA from *Agrobacterium tumefaciens*. This has been successfully used in *Arabidopsis thaliana* by Feldmann et al. (1989) to produce a wide range of morphological mutants, including a GA-insensitive dwarf. Transformation was achieved by infecting the germinating seed with *Agrobacterium*. The technique has the advantage that genes for resistance to kanamycin can be inserted with the t-DNA, and consequently the co-segregation of kanamycin resistance with the dwarfing characteristic can be determined. However, the technique may only be suitable where a nontissue culture method of transformation is available, and the genome contains little highly repetitive DNA as in *Arabidopsis thaliana*. With these limitations it may be possible to produce mutants of interest that are readily amenable to molecular techniques.

Classification of Mutants

Broadly speaking, phytohormone mutants may be split into two groups, those that influence hormone levels (hereafter called synthesis mutants) and those that influence hormone response (hereafter called sensitivity mutants). The most common types of synthesis mutants are those that block steps leading to the synthesis of the active hormone, such as the GA_1-deficient dwarfs in peas and maize (Ingram et al. 1986, Phinney 1984, Reid 1986b) and the ABA-deficient wilty mutants in tomato (Tal and Nevo 1973). Overproducing mutants would also be expected and have been reported for cytokinins in the moss, *Physcomitrella patens* (Wang et al. 1981). However, the frequency of overproducing mutants is low and previous suggestions regarding this type of mutant have sometimes not been confirmed (e.g., *yg-6* in tomato; Koornneef et al. 1985, Perez et al. 1974). Mutants with low hormone levels due to increased conjugation or catabolism of the active hormone would also belong to this broad synthesis category; however, to date no mutants have been proven to operate via these mechanisms in higher plants.

The majority of hormone mutants described belong to the sensitivity category. This is not surprising since by definition only a change in the observed response to the hormone is required. Such a change may occur due to quite indirect effects (e.g., changes in general plant vigor), as well as in the

direct sequence of events leading from hormone reception to the phenotypic response under examination. The most sought after and potentially most useful subgroups of sensitivity mutants are those that influence the level or affinity of a receptor molecule. However, to date no hormone mutant has been proven to operate at this level. Such mutants might be expected to possess phenotypes identical to the hormone synthesis mutants and to show pleiotropic effects for all responses to a particular hormone group if it is assumed that a single receptor is involved. Mutants possessing these basic requirements have been described in several species (e.g., *la cry*^{s} slenders in peas, Potts et al. 1985; *D8* dwarf in maize, Fujioka et al. 1988b, Harberd and Freeling 1989; *sln* slender in barley, Chandler 1988, Lanahan and Ho 1988), but the number of other steps subsequent to the receptor which also would result in these properties is unknown.

It has been suggested that the broad sensitivity category should be subdivided into other groups; for example, mutants influencing the level of the receptor (receptivity mutants), the affinity of the receptor for the active hormone (affinity mutants), and the size of the response produced (response-capacity mutants) (Firn 1986). While the object of many research projects is to determine which of these aspects of sensitivity a mutant may influence, no reliable way is yet available to allow this subdivision to be made with certainty. Response curves do not provide the necessary information (Reid and Ross 1988b) and nor does a detailed study of the pleiotropic effects of the genes. The response-capacity category would still cover a multitude of both direct and indirect causes. Perhaps this is the most critical subdivision since if a particular developmental phenomenon is to be examined using hormone mutants usually the aim, at least in the first instance, is to examine changes in the direct sequence of events between hormone reception and the response and not secondary consequences caused by general alterations to the pattern of growth of the plant.

Genetic Considerations

Many studies using mutants have used poorly defined genetic material and consequently the results have proved misleading. The development of isogenic lines is a necessity if reliable comparisons are to be made between a mutant and its wild-type allele, especially at the molecular level. It is frequently not sufficient just to use the mutant line and its parental variety; backcrossing to the parental variety is necessary to confirm the stability and single-gene nature of the mutant. However, the production of isogenic lines requires a large input of both time and effort. For inbreeding species, a minimum of six, and preferably 12, generations of single plant selection from heterozygous plants is the simplest method, whereas for outbreeding species, repeated backcrossing to the parental variety is the normal technique. This latter technique is perhaps more desirable, since backcrossing to the same variety with a range of mutants can yield an array of lines differing by single mutations, whereas the former technique yields only the single valid comparison for each allelic pair. If isogenic lines are not available, it is still possible to obtain meaningful physiological information by comparing a single gene difference under circumstances where the remainder of the genetic background is randomized. This can be done quite rapidly by using a range of F_2 or F_3 plants from a cross between the parental and mutant lines, although closely linked genes may still cause significant problems. Even the comparison of a number of pure breeding lines possessing each allele can overcome many of the problems associated with using only two unrelated cultivars (e.g., Murfet 1988).

Frequently, little or no use is made of the genetic interactions between physiologically related genes (i.e., combinations of mutant genes are not produced). If the full physiological and biochemical potential is to be gained from mutants such recombinants must be produced (e.g., Koornneef et al. 1982, Reid 1986a). For example, if two mutants are blocking steps in the same biosynthetic pathway, the phenotype of the double mutant will indicate whether the genes are leaky or not (Reid 1986a). Further, a gene blocking early in a pathway will allow examination of the action of a gene operating at a later step in the pathway by the use of feeding experiments, unencumbered by effects of endogenous compounds in the pathway. Therefore, the need for stable isotope-labeled compounds to determine metabolic conversions and to keep concentrations of metabolites at physiological levels may be avoided. Interactions between mutant genes acting on different aspects of the same pathway (e.g., hormone synthesis and sensitivity mutants) may also provide constructive insights into gene action. For example, by combining the GA-deficient *na* gene in peas with the long-thin, slender gene combination, *la cry*^{s}, it was possible to show that the *la cry*^{s} gene combination was epistatic to gene *na*, implying that GA levels are irrelevant to elongation in plants possessing both genes *la* and *cry*^{s} (Ingram and Reid 1987, Potts et al. 1985). In addition, the phenotype of mutant combinations may be used in some instances to infer the sequence of action of mutants (Reid 1986a). Such information may be of impor-

tance if a range of insensitivity mutants is being scanned to try and identify possible receptor mutants for further study.

Genetic manipulations may also be invaluable in determining the hormone interactions between maternal and embryonic tissues. This interaction has been the subject of much speculation and experimentation. By crossing appropriate hormone synthesis mutants it is possible to place seeds possessing a particular hormone (e.g., ABA, Karssen et al. 1983) onto maternal tissue deficient in this hormone. Such genetically contrived situations have demonstrated the importance of embryonic ABA (compared with maternal ABA) in the control of seed dormancy in *Arabidopsis*.

Mutants may also be valuable in identifying common regulatory elements controlling unrelated developmental processes. This stems from the one gene:one primary action hypothesis (Beadle and Tatum 1941). A good example of this use of mutants is found in the genes conferring the slender phenotypes in peas and barley. In both species, the mutant phenotype resembles a wild-type plant treated with saturating levels of GA_1 or GA_3 (Chandler 1988, Lanahan and Ho 1988, Potts et al. 1985). However, they display this phenotype regardless of the endogenous GA level (Ingram and Reid 1987, Lanahan and Ho 1988, Potts et al. 1985). This constitutive response occurs for all GA-mediated responses recorded. In peas this includes internode elongation, rate of leaf expansion, node of first flower, flower development, leaf shape and color, and pod development (see Potts et al. 1985, Reid et al. 1983). However, the biochemical basis of these responses is not known in peas. In barley, in addition to stem elongation, well-characterized biochemical responses to GA are known in the aleurone layer of germinating seeds. In the slender mutant, *sln*, constitutive production and secretion of α-amylase, protease, and nuclease occur (Chandler 1988, Lanahan and Ho 1988). These results suggest the presence of an unknown step(s) common to the regulation of these diverse GA responses. Clearly, the regulatory pathways must diverge at some point and mutations proving this are known in barley. For example, mutation can influence α-amylase production but not the other systems (Ho et al. 1980). The common regulatory step(s) may include the hypothesized GA receptor and any number of other unknown steps. The recent identification of a second slender mutation in peas (JB Reid, unpublished observations) influencing an independent step to the well-established duplicate slender gene combination, *la cry*^{s}, argues that the common sequence involves more than one step.

The ABA antagonism of GA-induced responses in barley still occurs in the aleurone of the slender mutant indicating a clear separation of these two processes (Chandler 1988, Lanahan and Ho 1988). The identification of a gibberellin-induced factor which interacts with the 5′ upstream region of an α-amylase gene in rice aleurone (Ou-Lee et al. 1988) offers the prospect of identifying the steps in this regulation sequence and genes like *sln* will be valuable in providing the necessary genetic controls.

Selected Examples

GA Mutants

Synthesis. The first clearly identified phytohormone mutants were the GA-deficient dwarfs of maize (Phinney 1956, 1961). These mutants and similar dwarfs in peas (Ingram et al. 1983, 1984, Reid and Potts 1986) are among the best-characterized hormone mutants. They were essential to the full understanding of the GA biosynthetic pathways in these species (e.g., Ingram et al. 1984, Phinney and Spray 1982, Spray et al. 1984) and were central to the development of the view that GA_1 is the primary native GA controlling stem elongation in species possessing the early 13-hydroxylation pathway for GA biosynthesis (Fujioka et al. 1988a, Ingram et al. 1986, Phinney 1984, Ross et al. 1989). They have been reviewed recently in detail elsewhere (e.g., MacMillan and Phinney 1987, Reid 1986b) and hence the coverage here is restricted to an overview and recent additions to this story.

The GA synthesis loci in maize are d_1, d_2, d_3, d_5, and an_1 and in peas are *le*, *na*, *lh*, and *ls*. Each gene appears to block a single distinct step in the biosynthetic pathway leading to the synthesis of GA_1 (Fig. 1; see Reid 1986b for review). In peas, none of these genes appears to result in a complete block, since either double mutants (e.g., *na ls*) are shorter than the single mutant types (Reid 1986a) or more severe alleles (e.g., le^d) can be found (Ross and Reid 1987, Ross et al. 1989). This is also true for the dwarf mutants of maize (BO Phinney, unpublished data). Direct measurements of GA levels using stable-isotope internal standards also support "leakiness" in maize shoots (Fujioka et al. 1988a). Shoot elongation can be shown to correlate closely with endogenous GA_1 levels in both species (Fujioka et al. 1988a, Ingram et al. 1986, Ross et al. 1989) but not with the overall level of GAs present in the plant (Ingram et al. 1984, Fujioka et al. 1988a, Phinney and Spray 1982). The most thoroughly examined loci are the *le* locus in peas and the d_1 locus in

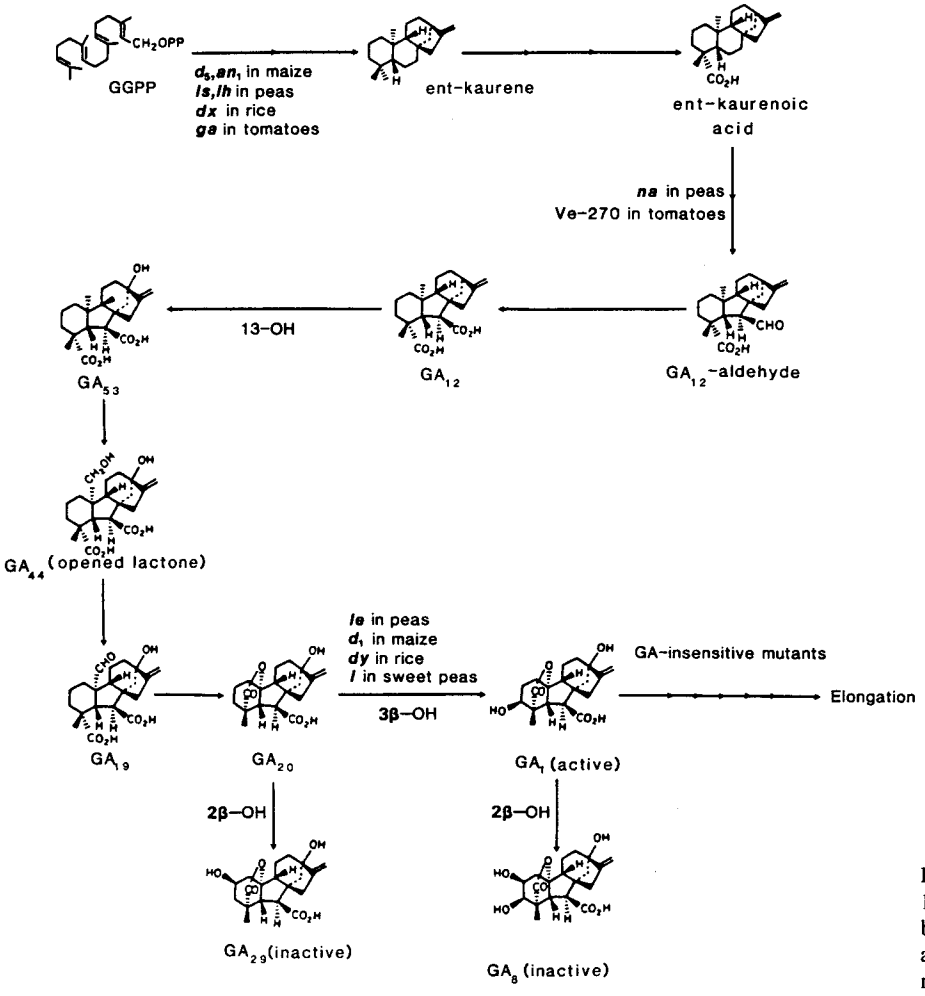

Fig. 1. The likely early 13-hydroxylation pathway for GA biosynthesis and the probable site of action of certain GA-synthesis mutants.

maize, which both block the 3β-hydroxylation of GA_{20} to GA_1 (Fig. 1). This has been demonstrated both by feeding experiments with [^{3}H, ^{13}C]GA_{20} (Ingram et al. 1984, Spray et al. 1984) and the measurement of endogenous GA levels by GC-SIM (Fujioka et al. 1988a, Ross et al. 1989).

However, recent work suggests that both species possess GAs in addition to the usual members of the early 13-hydroxylation pathway (albeit in trace amounts). This could potentially complicate an otherwise elegant story. In maize, GA_5 has been identified from vegetative shoots of wild-type seedlings but not from the mutant d_1 (Fujioka et al. 1988a), suggesting that GA_5 may be a byproduct of the 3β-hydroxylating enzyme that converts GA_{20} to GA_1. GA_3 has been also identified at low levels (much lower than the level of GA_1, Fujioka et al. 1988b) and is produced from GA_{20} via GA_5 (MacMillan 1989). Consequently, a limited amount of the biological activity in maize may be attributed to GA_3 rather than GA_1.

Both maize (Fujioka et al. 1988b) and pea (Potts 1986) shoots have been shown also to produce low levels of GAs belonging to the non-13-hydroxylation pathway (e.g., GA_{15}, GA_{24}, GA_9). The levels are substantially below the levels of the comparable 13-hydroxylated GAs (Fukioka et al. 1988b), and the presence of this pathway may only indicate that a limited amount of GA_{12}-aldehyde or GA_{12} does not undergo 13-hydroxylation to GA_{53}-aldehyde or GA_{53} prior to further metabolism. The biological significance of this pathway is probably negligible, since even GA_4, the biologically active 3β-hydroxylated member of this pathway, has lower biological activity in maize and pea bioassays than GA_3 (Crozier et al. 1970). Further, GA_4 has only been detected in the nonresponding *D8* mutant of maize where 3β-hydroxylated GAs appear to accumulate (Fujioka et al. 1988b).

Although the significance of GA_1 for the control of internode elongation was primarily based on results from dwarf mutants of maize and peas (for

reviews see Phinney 1984, Reid 1986b) substantial evidence suggests that this mechanism is common to many monocots and herbaceous dicots. This includes evidence from GA_1-deficient mutants in rice (Suzuki et al. 1981), *Brassica rapa* (Rood et al. 1989), tomato (Zeevaart 1984), and sweet pea *Lathyrus odoratus* (Ross et al., in preparation), and studies with a GA synthesis inhibitor in wheat (Lenton et al. 1987). These systems have not yet been examined in sufficient detail to determine whether small levels of other biologically active GAs may also be present as occurs in the maize system.

Sensitivity. GA-sensitivity mutants are more frequent than GA synthesis types and are probably the most common group of phytohormone mutants. For example, more than 20 GA nonresponding mutants have been mapped in maize (Fujioka et al. 1988b). This group of mutants showed an altered response to biologically active GAs, such as GA_1 and GA_3. Insensitivity to applied GAs is a very general test and hence the category is broad and includes mutants that have quite indirect effects on GA sensitivity, such as gene *lm* in peas [which results in abnormal root development, Reid and Ross (1988a)] through to putative GA receptor mutants, such as D_8 in maize (Fujioka et al. 1988b, Harberd and Freeling 1989) and the slender mutant in peas and barley (Chandler 1988, Lanahan and Ho 1988, Potts et al. 1985). The sequence of events from GA reception to elongation is not known in any species but the judicious use of the GA-sensitivity mutants should allow such steps to be elucidated (see Reid 1989). For example, microtubule and microfibril orientation have been reported to be modified by GA and are of importance for cell elongation (e.g., Mita and Katsumi 1986). The identification of mutants influencing these processes would clearly be of significance. Likewise, debate exists over the changes induced by GA to cell wall characteristics (Cosgrove and Sovonick-Dunford 1989). The examination of the cell wall properties of a range of GA-sensitivity mutants, such as exist in maize (Fujioka et al. 1988b) and peas (Reid 1989), may help clarify this situation by providing mutants which specifically influence certain wall characteristics. Mutants which influence internode elongation by specifically altering either epidermal cell length (e.g., *lm* and *lkb*, Reid and Ross 1988a, 1989) or the number of cells per internode (Murfet 1989) have been described in peas making it difficult to envisage that cell division is immaterial to cell elongation as is suggested by a purely physical view of stem elongation (see Cosgrove and Sovonick-Dunford 1989). These examples are sufficient to show that sensitivity mutants may provide the tools to help unravel hormone action by identifying the individual biochemical processes involved.

Examination of GA levels and metabolism in GA-insensitive mutants has provided a diverse set of results. In the eight GA-sensitivity mutants examined in peas, no major qualitative changes in the levels of GA-like substances have been observed (e.g., Potts et al. 1985, Reid and Potts 1986, Reid and Ross 1988a, 1989). However, relatively small quantitative differences cannot be ruled out until measurements with internal standards are conducted. The metabolism of $[^3H]GA_1$ appears similar in five of these mutants (Steane et al. 1989). In wheat, the dwarfing gene *Rht3* leads to increased levels of GA_1 (Stoddart 1984) but the rate of GA_1 turnover does not appear to be altered and alterations in GA_1 metabolism do not appear to account for the dwarf, GA-insensitive phenotype produced (Stoddart 1984, 1989). The nonresponding mutant, D_8, in maize also accumulates substantially higher levels of 3β-hydroxylated GAs, such as GA_1, GA_8, GA_4, and GA_3, than comparable wild-type plants (Fujioka et al. 1988b). This genotype is a true phenocopy of the GA-deficient synthesis mutants in maize, such as d_1, d_2, d_3, and d_5. This is not the case for the other nonresponding dwarf mutants of maize or peas and argues for an action of D_8 at or soon after GA perception. Genetic results are also consistent with this hypothesis (Harberd and Freeling 1989). However, the significance of the elevated levels of 3β-hydroxylated GAs is not clear and should not be interpreted to mean that GA_1 metabolism is associated with perception and action until further studies are carried out. Information on the metabolism of $[^3H]GA_1$ in D_8 maize has yet to appear in print. These diverse results for GA levels and metabolism in GA-sensitivity mutants probably only reflect the diversity of steps at which the various mutants block the response to GA_1.

ABA Mutants

Synthesis. The well-characterized wilty mutants in tomato, *flc, sit,* and *not* (e.g., Neill and Horgan 1985, Tal and Nevo 1973), potato, *dp* (Quarrie 1982), pea, *wil* (Wang et al., 1984a), and *Arabidopsis thaliana, aba* (Koornneef et al. 1982) all appear to result from reduced levels of ABA. Likewise, the viviparous mutants of maize, *vp-5, w-3,* and *vp-7* appear to possess reduced ABA levels due to blocks in carotenoid biosynthesis (Moore and Smith

1985). The data from these mutants have shown that ABA controls aspects of seed dormancy, germination, and water relations. This was possible even in the absence of suitable ABA synthesis inhibitors or a detailed knowledge of the biosynthetic pathway. These results and the phenotypic effects and genetic interactions of these mutants have been reviewed extensively (e.g., Koornneef 1986, Reid 1987) and provide an excellent example of the power of the mutational approach to the study of plant development.

Recent work with the ABA-deficient mutants has concentrated on the elucidation of the ABA biosynthetic pathway. Two proposals have been widely examined: first, direct synthesis of ABA by the cyclization and oxidation of farnesyl pyrophosphate (C_{15} pathway), and second, an indirect pathway via the breakdown of a carotenoid, such as violaxanthin (C_{40} pathway). Recent work by several groups supports the indirect pathway (e.g., Zeevaart et al. 1986). For example, the viviparous mutants in maize possess inhibited carotenoid biosynthesis, as well as reduced ABA levels (Moore and Smith 1985) suggesting that a carotenoid intermediate may be necessary for ABA synthesis. Work with carotenoid synthesis inhibitors supports this conclusion (Henson 1984). If a C_{40} intermediate is cleaved to yield a C_{15} precursor of ABA, a C_{25} (single cleavage) or C_{10} (double cleavage) byproduct should be produced. Taylor et al. (1984) have found a potential C_{10} byproduct, 2,7-dimethyl-2,4-octadienedoic acid (ODA) by examining the ABA-deficient tomato mutants. They found that ODA levels were high in the *flc* and *sit* mutants, especially when water stressed, compared with isogenic wild-type plants. This may suggest that *flc* and *sit* may block ABA synthesis after the cleavage of the C_{40} precursor. ODA levels were low in *not* plants compared with nonmutant controls, again especially when the plants were water stressed (Taylor 1987), suggesting that *not* may block ABA synthesis at or prior to cleavage of the C_{40} precursor. Lindforth et al. (1987) have subsequently shown that in the *flc* and *sit* plants 2-*trans*-ABA alcohol accumulates in the same way as ODA and to a level which approximately accounts for the reduced levels of ABA. This may suggest that 2-*trans*-ABA alcohol is an intermediate in ABA synthesis. Parry et al. (1988) examined the conversion of [^{13}C]xanthoxin, a possible C_{15} cleavage product of violaxanthin, to ABA. They found that in wild-type and *not* plants substantial conversion occurred but only limited conversion took place in *flc* and *sit* plants. These results are consistent with the actions of *flc*, *sit*, and *not* as suggested by Lindforth et al. (1987); however, details of the intermediates in the pathway are still not clear and the precise actions of the three genes are still not defined (Lindforth et al. 1987, Parry et al. 1988). Even the general basis of the pathway is still debated since Milborrow (1989) has argued from labeling experiments that xanthoxin is not an ABA precursor nor ODA a byproduct of ABA synthesis.

Sensitivity. ABA-insensitive mutants have been isolated in several species, including *Arabidopsis thaliana* (Koornneef et al. 1984), barley (Ho et al. 1980, Raskin and Ladyman 1988), and the fern, *Ceratopteris* (Hickok 1985). Such mutants are usually selected by their lack of response to applied ABA. Some exhibit insensitivity for all ABA responses examined (e.g., *abi-1* and *abi-2* interfere with water relations, seed dormancy, and seedling growth in *Arabidopsis*, Koornneef et al. 1984), and thus superficially resemble ABA-deficient types. Other mutants have been identified which influence only specific ABA responses. For example, *abi-3* in *Arabidopsis* only possesses reduced seed dormancy (Koornneef et al. 1984), whereas the "cool" barley mutant fails to close its stomata after ABA treatment (Raskin and Ladyman 1988). These results suggest that at least some common steps occur for such wide-ranging effects of ABA on seed dormancy, seedling growth, and stomatal closure. Further, the presence of at least two such loci in *Arabidopsis* (Koornneef et al. 1984) argues for more than just a single common receptor step. It would seem unlikely that the mutants influencing only one physiological response represent receptor mutants (compare with Raskin and Ladyman 1988). Rather they may cause a lesion in the transduction chain subsequent to its primary branch point.

Auxin Mutants

Synthesis. Although extensive searches have been carried out for auxin-synthesis mutants, no such mutants have been identified in higher plants at the whole plant level. The absence of auxin-synthesis mutants has been explained either because they are lethal or due to a lack of information on the appearance of such mutants (King 1988). The former reason seems unlikely to explain the lack of mutants since most known hormone-synthesis mutants are "leaky" to varying degrees (e.g., Ingram et al. 1986, Neill and Horgan 1985, Reid 1986a). However, elegant work by Blonstein et al. (1988) using leaf protoplasts from *Nicotiana plumbaginifolia* identified clones requiring auxin for growth. These

auxin auxotropic variants could not be regenerated to complete plants, although grafting to wild-type *N. tabacum* plants did allow limited development. They formed a bush of small shoots suggesting weakened apical dominance, slow expansion of leaves, and small, brown spots on older leaves prior to desiccation of the whole leaf and death without abscission. This may indicate the phenotype to be expected for auxin-auxotrophic plants and therefore provide the necessary selection critera. Leaky auxin-synthesis mutants have been identified in the moss *Physcomitrella patens* where they result in reduced caulonemata and gametophore production (Ashton et al. 1979). A mutant with similar morphological effect in *Funaria hygrometrica* may be caused by increased degradation of indole-3-acetic acid (IAA) (Bhatla and Bopp 1985). A "giant" mutant of *Lemna gibba* with elevated levels of free IAA has been reported (Slovin and Cohen 1988), but the genetic control is unknown and the significance of the elevated IAA levels is unclear.

Torti et al. (1988) have shown that the level of both free and bound IAA are substantially reduced in the defective endosperm mutant *de-B18* of maize. Applied napthaleneacetic acid (NAA) overcomes the reduction in endosperm weight caused by the mutation. The mutation did not influence the germination and vitality of the seedling suggesting that auxin in maize seedlings may be synthesized in situ and not transported from seeds.

A tryptophan-synthesis mutant in *Arabidopsis, trpl-1,* may also result in reduced IAA levels (Last and Fink 1988), since tryptophan is a precursor of IAA (Law 1987). Plants possessing *trpl-1* are slow growing and possess crinkled leaves, a bushy habit, and at least some infertile flowers. This phenotype of *trpl-1* plants is consistent with reduced IAA levels and is similar to the known auxin-resistant mutant *axr-1* (Last and Fink 1988).

Endogenous auxin levels have been raised by genetic manipulation in transgenic plants. For example, the auxin gene from the t-DNA of *Agrobacterium* was incorporated (coding for tryptophan monooxygenase) into petunia under the control of a strong, constitutively expressed promotor (the cauliflower mosaic virus 19S promoter) (Klee et al. 1987). A tenfold increase in auxin levels (compared with wild-type plants) resulted in plants with almost complete apical dominance, greater internode length, leaf curling, increased xylem and phloem production, and parthenocarpic fruit (Klee et al. 1987). With the aid of tissue-specific promoters such transgenic plants offer prospects to explore the effects of auxin overproduction in specific tissues.

Sensitivity. Auxin-sensitivity mutants are more common than synthesis mutants. The diageotropic (*dgt*) mutant of tomatoes appears to be one example, since it shows a reduced response to applied auxin for both hypocotyl elongation and ethylene production (Kelly and Bradford 1986). It was suggested that it may influence the primary site of auxin perception or action rather than the response (Kelly and Bradford 1986) but at present the data appear insufficient to justify this conclusion. This mutant was originally described as an ethylene-synthesis mutant due to its low production of ethylene after auxin treatment and the ability of applied ethylene to phenotypically normalize certain responses (Zobel 1973). Scott (1988) has shown that the lack of auxin responses in the *dgt* mutant does not impair its GA responses.

Other auxin-sensitivity mutants tend to be categorized as auxin resistant (e.g., Blonstein et al. 1988, Estelle and Sommerville 1987, Maher and Martindale 1980, Muller et al. 1985), in that they are capable of growth when cultured with elevated auxin levels. The primary action of these mutants at the biochemical level is not clear, although they share certain phenotypic responses (e.g., altered root growth and geotropism, Blonstein et al. 1988). The roots of the agravitropic mutant (*agr*) in barley are also resistant to applied IAA but not to NAA or 2,4-dichlorophenoxyacetic acid (2,4-D) (Tagliani et al. 1986). Some of these mutants also appear to influence shoot development (e.g., apical dominance and shoot length) suggesting that they may influence common steps in the sequence of events between auxin levels and development (e.g., Estelle and Sommerville 1987).

Ethylene Mutants

Synthesis. As with the GAs, applied ethylene has clearly defined effects on intact plants. For example, it influences elongation, increases apical dominance, abscission, leaf senescence, ethylene production, and fruit ripening (see Roberts et al. 1987). These clear effects, combined with a well-defined biosynthetic pathway and the availability of biosynthesis inhibitors would seem an ideal situation for the selection of ethylene mutants. However, unlike the GAs and ABA this is not the case. For example, even though many genes influencing fruit ripening have been characterized in tomatoes, none seems to act by directly altering ethylene syntheis (Roberts et al. 1987). The reason for this lack of mutants is not clear but may stem from the fact that many of

the phenotypic responses to ethylene observed in seedlings require elevated levels. The removal or blockage of ethylene production in wild-type plants does not result in dramatic effects. Most mutants might be expected to reduce synthesis rather than elevate ethylene levels even where a branched biosynthetic pathway occurs. A comparison with GA mutants illustrates this point, since GA-deficient mutants are common but overproducers are rare.

However, two mutants with elevated ethylene levels have been reported. The *lk* mutant in peas results in a severely dwarfed, GA-insensitive plant which possesses swollen stems and petioles (especially in the dark) and increased apical dominance (Ross and Reid 1986). Thus, this has some similarities to ethylene-treated peas. Further, *lk* plants elongate in response to applied AVG (an inhibitor of ethylene production); its GA response is also increased, although in neither case to the level of comparable wild-type plants. Under certain circumstances *lk* plants produce elevated levels of ethylene (Ross and Reid 1986), but it is not clear if this is the primary action of the gene or a secondary effect resulting from the dramatic effects of the *lk* mutation. The inability to fully restore the wild-type phenotype by AVG argues for a secondary effect.

The *Epi* gene in tomato has likewise been shown to result in ethylene overproduction and confers increased stem and petiole thickness, epinasty, and a compact growth habit (Fujino et al. 1988). This mutant possesses elevated ACC levels (the precursor of ethylene) but it does not revert to a phenocopy of the parental variety after treatment with ethylene synthesis or action inhibitors (Fujino et al. 1989). Consequently, determination of the primary action of this gene must also wait until further results are available (Fujino et al. 1988).

Sensitivity. A dominant ethylene-insensitive mutant, *etr,* has been reported in *Arabidopsis* (Bleecker et al. 1988). The mutant inhibits a wide range of normal ethylene responses, such as reduced organ elongation and feedback suppression of ethylene synthesis, enhanced peroxidase activity, seed germination, and leaf senescence. The results suggest that *etr* controls a step common to all these responses. In vivo binding of ethylene is also reduced by the *etr* mutation suggesting that *etr* may influence the ethylene receptor itself, although identification of the gene product is still required. In *Arabidopsis* this appears achievable by the cloning of the *etr* gene, since chromosome walking appears to be a feasible strategy (Meyerowitz 1987).

Cytokinin Mutants

No single-gene cytokinin mutants have been proven in intact higher plants. This is possibly because the expected phenotype is unclear. From the purported actions of the cytokinins a deficiency or insensitivity might be expected to result in enhanced leaf senescence and apical dominance. Although mutants influencing these processes have been reported (e.g., Blixt 1972, Thomas 1987), a thorough examination for the involvement of cytokinins has not usually been undertaken.

Differences in both the metabolism and sensitivity to cytokinins have been demonstrated at the tissue culture level. Mok et al. (1980) have evidence of a single gene difference in *Phaseolus vulgaris* which controls the cytokinin dependency of hypocotyl callus cultures. Variation in the metabolism of cytokinins between callus cultures from *P. vulgaris* and *P. lunatus* has also been demonstrated (Turner et al. 1985). In *Nicotiana tabacum,* the dominant genes *Hl-1* and *Hl-2* result in a cytokinin-autotropic phenotype for tissue cultures (i.e., they do not require cytokinins for growth as do cultures from the leaf of wild-type plants, Meins and Foster 1986). Plants possessing *Hl-1* do not overproduce *trans*-zeatin riboside or certain other cytokinins even though gene *Hl-1* is suggested to have oncogenic functions similar to the isopentyl transferase (*ipt*) locus of the Ti plasmid (Hansen et al. 1985, 1987). This may indicate that *Hl-1* enhances the sensitivity of certain plant tissues to cytokinins. The mutant however does not markedly alter overall plant growth.

Cytokinin mutants are well established in mosses. The cytokinin overproducing mutants of *Physcomitrella patens* possess a 100-fold increase in isopentanyladenine and zeatin levels (Wang et al. 1981). They possess increased numbers of gametophores and resemble wild-type plants treated with cytokinin (Wang et al. 1984b). This clearly demonstrates that cytokinins play a key role in controlling gametophore production. Somatic hybridization has indicated at least three genes can lead to the overproduction of gametophores and cytokinin (see Wang 1987). Cytokinin-deficient mutants may also occur. They do not form gametophores but can be induced to do so by adding cytokinin (Wang et al. 1984b). A mutant with the appropriate phenotype for a cytokinin-insensitive mutant has also been found. It produces normal levels of cytokinins and is similar to the wild-type except that it lacks gametophores (Wang et al. 1984b, 1987). Such elegant studies stress the biological significance of the cytokinins in mosses.

Unfortunately, we are left without such a clear

role for the cytokinins in the control of the growth of higher plants. For example, Medford et al. (1989) have introduced a chimeric, heat-inducible promotor-isopentenyl transferase gene into tobacco and *Arabidopsis*. They found elevated cytokinin levels even without heat shock, and these were associated with the release of axillary buds, reduced stem length, and root development. After heat shock, substantial increases in the cytokinin content occurred (e.g., 52-fold increase in zeatin), but no further alterations in growth and differentiation occurred, arguing against cytokinin levels exerting a marked effect on the pattern of differentiation in whole plants. Additional work with the cytokinin biosynthesis genes from *Agrobacterium tumefaciens* may resolve this issue. Further details of these genes and their actions are beyond the scope of this paper but have recently been reviewed in detail by Morris (1986), Horgan (1987), and Klee et al. (1987).

Phytochrome Mutants

Phytochrome mutants have been included in this review for two reasons. First, alterations in phytochrome levels or function frequently result in modified responses to the phytohormones with the consequence that the same mutant may be classified as both a hormone and a phytochrome mutant. The *lv* mutant in peas is a good example. It was initially characterized as a GA-hypersensitive mutant due to its enhanced response to applied GA_1 (Reid and Ross 1988b). Further work showed that it could be classified as a phytochrome mutant since it lacked the normal end-of-day FR response and exhibited only a weak response to R light compared with the isogenic parental line (Nagatani et al. 1989b). R/FR reversibility of elongation was also substantially reduced in *lv* plants. The *yg-6* mutant in tomatoes is another example (Koornneef et al. 1985, Perez et al. 1974). Second, phytochrome mutants may be classified into synthesis and sensitivity types in the same way as the phytohormone groups. This latter group includes the so-called transduction mutants, as well as "reponse" mutants (see Adamse et al. 1988a). Transduction mutants are suggested to act in the direct sequence of events leading from the photoreceptor to the physiological response, whereas reponse mutants influence a single-specific response (Adamse et al. 1988a). As with the phytohormone mutants indirect effects can also impinge on the size of a specific response. Consequently, unless the biochemical effects have been determined it may be impossible to distinguish true response mutants from more general indirect effects.

Synthesis. The most thoroughly examined phytochrome synthesis mutant is the au^w mutant in tomatoes which results in a long hypocotyl, a marked reduction of chlorophyll levels, and strongly reduced chloroplast development, anthocyanin synthesis, seed germination (Koornneef et al. 1985), and photoregulation of chlorophyll a/b-binding protein (*cab*) synthesis (Sharrock et al. 1988). Both spectrophotometric (Koornneef et al. 1985) and immunological (Parks et al. 1987) anaylses have shown that the phytochrome content of dark grown au^w plants is reduced to below 5% of the wild-type. In the light, an approximately 50% reduction was observed (Adamse et al. 1988c). These results have been interpreted to suggest that the au^w gene prevents the accumulation of light-labile phytochrome (probably phytochrome I) (Parks et al. 1987) but does not influence the accumulation of light-stable phytochrome (probably phytochrome II) (Lopez-Juez et al. 1989). Recent work suggests that phytochrome mRNA is produced in dark-grown au^w plants and is functional in vitro (Sharrock et al. 1988). A less severe allele, *yg-6*, has also been found at the *au* locus (Koornneef et al. 1986). Phytochrome-deficient mutants, *hy-1* and *hy-2*, have also been found in *Arabidopsis* (Koornneef et al. 1980) and the *yg-2* mutant in tomatoes possess a similar phenotype to au^w (Koornneef et al. 1985).

These mutants have provided information directly relevant to several key physiological questions. For example, it has long been assumed that P_{fr} was the active form of phytochrome. The au^w, *hy-1*, and *hy-2* mutants, which would possess reduced P_r levels in the dark, grow long in darkness like the wild-type plants. If P_r was active, the mutants should be shorter than the wild-type in the dark (Quail et al. 1987). The mutants have also suggested which type of phytochrome controls a particular response. The au^w mutant exhibits many abnormal phytochrome-controlled responses (presumably due to a lack of light-stable phytochrome). However, the normal increase in elongation after an end-of-day FR treatment is present suggesting that physiologically light-labile phytochrome may not be controlling this response. Rather it is suggested that light-stable phytochrome, which appears to be present in au^w plants, may control this process (Adamse et al. 1988c, Lopez-Juez et al. 1989).

The phytochrome mutants also offer the easier application of molecular techniques to plant development than do the phytohormone mutants, since

phytochrome is a protein for which the sequence is known. Monoclonal antibodies have also been raised against phytochrome and a start has been made to identify the genes producing it (Quail et al. 1987). More than one gene appears to be present (Quail et al. 1987), and the strong possibility exists of differential expression from tissue to tissue and between ontogenetical stages. There is little information on such genetic systems for phytohormone synthesis. Different pathways for GA biosynthesis have been shown between developing seeds and vegetative tissue in a number of species (e.g., *Phaseolus vulgaris,* Endo et al. 1989) and certain GA synthesis genes have been shown to operate only in one tissue type (e.g., *Le* does not result in GA_1 production in developing seeds and *na* does not block GA synthesis in developing seeds of peas, Potts and Reid 1983). However, whether this implies that separate enzymes, and hence genes, operate in different tissues or simply that the same genes are regulated in a tissue-specific way has yet to be defined. The work with phytochrome mutants may show the way forward when the products of the hormone synthesis genes are eventually isolated.

Sensitivity. Two phytochrome-sensitivity mutants that may influence the transduction chain are the *lh* mutant in cucumber and the *lv* mutant in peas (Nagatani et al. 1989a,b). They possess very similar phenotypes including elongated stems when grown in white or red light, slightly lower chlorophyll levels, and the absence of the normal end-of-day FR response (Adamse et al. 1987, 1988b, Nagatani et al. 1989b). Consequently, both genotypes appear as if they do not fully deetiolate. Both mutants appear to possess normal levels of phytochrome I and II in both the light and dark when examined spectrophotometrically or immunologically (Nagatani et al. 1989b), although initial spectrophotometric measurements of the *lh* mutant suggested a possible reduction in phytochrome II levels (Adamse et al. 1988b). The presence of an end-of-day FR response in the au^w mutant of tomatoes (Adamse et al. 1988c) and its absence in the *lh* mutant of cucumbers and the *lv* mutant of peas has been used to suggest that physiologically light-stable phytochrome controls this response and that *lh* and *lv* are mutants in the transduction chain from this light-stable phytochrome. (Nagatani et al. 1989a,b). A third possible phytochrome-insensitive mutant is the *hy-5* mutant in *Arabidopsis* (Koornneef et al. 1980).

The *hp* mutant in tomatoes, which results in high levels of anthocyanins (Adamse et al. 1988a), reduced plant height in red and yellow light (Mochizuki and Kamimura 1985) and elevated chlorophyll levels in fruit tissue (Adamse et al. 1988a), may be a hypersensitive phytochrome mutant, since its phenotype is in many respects the reverse of the phytochrome deficient *auw* mutant.

Phytochrome-sensitivity mutants that influence only one or a few phytochrome responses are also known. For example, the photoperiod response genes *sn, dne,* and *hr* in peas (Murfet 1985) result in the loss of the phytochrome-controlled long day response (Reid and Murfet 1977). Although the response to light quality may appear to differ in these genotypes (Reid 1982), these mutants probably do not directly influence phytochrome levels or the transduction chain since they appear essentially normal for other phytochrome-controlled processes. As suggested by Adamse et al. (1988a) these mutants influence a specific phytochrome response and may therefore be categorized as response mutants to separate them from the transduction mutants.

Conclusions

One of the most striking features of phytohormone mutants is the similarity of phenotypes for similar mutants in widely divergent species (e.g., ABA or GA deficiences). This similarity argues for similar control systems in higher plants. At the biochemical level, the site of action of mutants is also similar. For example, of 14 well-defined mutants that block GA_1 synthesis, four block the conversion of GA_{20} to GA_1, six appear to block between geranylgeranyl pyrophosphate and kaurene, and two block just prior to GA_{12}-aldehyde (Fig. 1). The limited number of steps controlled by the mutants may suggest that there are a limited number of steps in the pathways which may regulate the biosynthesis of the active hormone. Such steps and the genes controlling them deserve special attention.

The mutants that have been studied at the biochemical level are those which produce specific, and often marked phenotypic effects. Many mutants which influence hormone biosynthesis may be missed using the selection criteria outlined earlier if they do not result in obvious phenotypic effects. Such mutants may not influence steps that are limiting the production of the biologically active hormone, or the hormone itself may not markedly alter differentiation in the whole plant (e.g., Medford 1989). However, these mutants are still of potential interest and in the future, direct scanning for them

using rapid immunological techniques may provide valuable data.

Virtually all hormone analyses, including those on mutants, have used tissue that either varies ontogenetically or contains a range of tissue types. Consequently, site-specific alterations in hormone levels have rarely been examined. Even relatively crude analyses (e.g., Potts and Reid 1983) have shown that hormone synthesis genes may show marked tissue specificity. Whether this implies that these genes are regulators of structural genes or that gene families exist which control hormone production in different tissues has not even been examined. However, such age and tissue-specific questions deserve detailed examination. The production of transgenic plants with tissue-specific promotors attached to hormone-synthesis genes is possibly not far away and may answer many of these questions and overcome many of the deficiencies with the plant hormone concept.

Finally, at a more classical level, the detailed pleiotropic effects of the presently known phytohormone mutants deserves further attention. Each group of plant hormones has been suggested to have an enormous range of effects, but work with mutants has normally concentrated on only one or a few of these effects. Much evidence, albeit possibly negative, may still be gained by the judicious use of the presently known mutants. They still provide one of the most promising tools to study hormone physiology in higher plants.

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