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Slender barley: A constitutive gibberellin-response mutant

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Abstract. In barley (*Hordeum vulgare* L. cv. Herta), *slender* (*sln1*) is a single-locus recessive mutation which causes a plant to appear as if it had been grown in saturating concentrations of gibberellin (GA). We have investigated two of the GA-mediated processes in slender barley, shoot elongation and the induction of hydrolytic enzymes in aleurone layers. Shoot elongation is severely retarded in normal (wild-type) barley if the biosynthesis of GA is blocked by an inhibitor, ancymidol (α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidinemethanol). However, the slender mutant continues to elongate in the presence of ancymidol. In isolated normal aleurone layers, the synthesis and secretion of α-amylase (EC 3.2.1.1), protease (EC 3.4) and nuclease (EC 3.1.30.2) are induced by exogenously applied GA_3. However, in the aleurone layers of the slender mutant these enzymes are produced even in the absence of GA but their synthesis is still susceptible to inhibition by abscisic acid. Bioassays of half-seeds of the slender mutant and their normal siblings show no detectable differences in endogenous levels of GA-like substances. We suggest that the slender mutation allows competent tissues to express fully, or over-express, appropriate GA-induced processes independent of GA. We also conclude that shoot elongation, and hydrolytic-enzyme secretion in aleurone layers, share a common regulatory element.

Key words: Abscisic acid and gene expression – Aleurone – Gibberellin and gene expression – *Hordeum* (GA mutant) – Hydrolytic enzymes, induction – Mutant (barley, GA) – Shoot elongation.

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Abbreviations: ABA = abscisic acid; GA = gibberellin; GA_3 = gibberellic acid

Introduction

Gibberellins (GA) are important in the regulation of many physiologically distinct processes in plant growth and development (for a review see Jones 1973). The most extensively studied of these processes are shoot elongation (see Jones and Moll 1983), and the induction of hydrolytic enzymes in cereal aleurone layers (see Varner and Ho 1976). The GA-elicited biochemical changes, which are responsible for cellular elongation, have yet to be identified. It has been demonstrated that GA regulates the accumulation of specific proteins and mRNAs (Chory et al. 1987), but their precise roles are yet undetermined.

The molecular biology and biochemistry of the response of the barley aleurone layer to GA is understood in some detail (Varner and Ho 1976; Jacobsen 1983). Upon imbibition the embryo synthesizes GA, which then diffuses throughout the germinating seed, and mediates the degradation of stored reserves of protein and carbohydrate in the endosperm by eliciting the synthesis and secretion of a number of hydrolytic enzymes from aleurone cells. There is no division or alongation of aleurone cells following GA treatment. Normal aleurone layers of barley are completely dependent upon the GA synthesized by the embryo for the induction of hydrolase secretion. Among the most characterized of the induced hydrolases is α-amylase. The isoenzymic forms of α-amylase are readily identifiable (Jacobsen and Higgens 1982), and copy DNAs (cDNA) for at least two of these isoenzymes are available (Rogers 1985). α-Amylase genes are activated by GA at the transcriptional level, although the mechanism of this activation is unknown (Jacobsen and Beach 1985). Another phytohormone, abscisic acid (ABA), has antago-

nistic effects on this GA-induction process (Nolan et al. 1987).

Slender, a diethyl-sulfate induced mutation in barley, was reported by Foster as a mutant of potential biochemical interest (Foster 1977). Plants homozygous for the recessive *sln-1* allele behave as if they are continually saturated with GA. The mutant phenotype is characterized by rapid extension growth in the seedling and adult stages of growth. The mature plant is limber, and unable to support itself. Basal internode elongation occurs concurrently with tillering, resulting in aerial branching. Root initials form on lower aerial nodes. Stems and leaves are narrower than normal. Heads are at least twice as long as normal, with reduced size of floral parts. The flowers are completely sterile: no pollen is produced and no seed is set upon cross-pollination. Phenocopies of the mutant can be obtained by treating normal plants with exogenous GA. These data led Foster to speculate that the mutated gene is one concerned with some aspect of GA synthesis or utilization.

Mutants with similar phenotypes exist in green beans and peas (Lamprecht 1948; Potts et al. 1985). Unfortunately, despite the wealth of genetic information on the slender mutant in pea, the biochemical basis of stem elongation is not well defined. Because the aleurone layers in barley are considerably more amenable to the study of the molecular action of GA, we wished to determine if the aleurone layers of slender barley exhibited the GA-saturated phenotype. By extending the phenotype to a tissue whose biochemical response to GA has been at least partly characterized, more direct questions as to the nature of the mutation may be addressed. Our results indicate that slender aleurone layers constitutively synthesize and secrete hydrolases without a requirement for GA. Furthermore, the gross morphology of slender plants is unaffected by an inhibitor of GA biosynthesis (Coolbaugh et al. 1978), indicating that the elongation response is also independent of GA levels. Thus, this mutant should be of great interest in studying the mechanism of gene regulation by GA.

Material and methods

Plant material. Barley (*Hordeum vulgare* L. cv. Herta, Cb 3041, 1979) seeds (caryopses) segregating 3 normal:1 slender were graciously supplied by Chris A. Foster, Welsh Plant Breeding Station, Aberystwyth, UK. Additional barley (cv. Himalaya) seeds (1985 harvest) were purchased from the Department of Agronomy and Soils, Washington State University, Pullman, Wash., USA.

Ancymidol treatment. Normal and slender plants were watered with a 20 mg/l solution of ancymidol (α-cyclopropyl-α-(*p*-methoxyphenyl)-5-pyrimidinemethanol; sold under the trade name "A-rest", Elanco, Indianapolis, Ind. USA) or water for a period of 21 d after the normal coleoptile was 5 cm tall and slender coleoptiles were 12.5 cm tall (5 d after sowing). In order to asses the relative heights of the plants they were placed horizontally and photographed.

Enzyme assays. Agar-plate assays were performed essentially as described by Ho et al. (1980). Seeds were cut transversely; the half-seeds containing the embryos were planted to determine their phenotype, while the embryonated half-seeds were surface-sterilized with 1% NaClO for 15 min and washed four to six times with sterile water. These half-seeds were then sliced into three pieces of roughly the same size. Slices from the same de-embryonated half-seed were placed into the same quadrant of 2% agar plantes (in $10 \cdot 10$ cm^2 square plastic plates) made in 20 mM $CaCl_2$ and 20 mM Na succinate (pH 5.0) (Ca-succinate buffer). No-hormone plates contained no hormones in the agar plate, ABA plates were made by adding ABA (to $2 \cdot 10^{-5}$ M) to the cooled agar just before pouring, and GA plates by adding gibberellic acid (GA_3, to $1 \cdot 10^{-6}$ M) to the cooled agar. To detect secreted α-amylase activity, soluble potato starch (0.2%) was added to the agar before autoclaving. α-Amylase plates were developed by flooding the plates with a solution of I_2-KI (0.72 g I_2 + 7.2 g KI/l) in 0.2 N HCl. Half-seeds which synthesized and secreted α-amylase had transparent halos around the seeds resulting from the digestion of the starch by α-amylase.

Conventional test-tube assays for hydrolytic enzymes were also performed. Normal or slender de-embryonated half-seeds were incubated for 48 h in Ca-succinate buffer containing no hormones, $2 \cdot 10^{-5}$ M ABA or $1 \cdot 10^{-6}$ M GA_3. Aliquots of this incubation media were used for enzyme assays. α-Amylase activity was determined using the procedure of Varner and Mense (1972). Nuclease assays were performed according to Brown and Ho (1986). Denatured salmon-sperm DNA (Sigma Chemical Co., St. Louis, Mo., USA) was used as the substrate in the assays. Protease activity was determined by mixing samples with 20 mM Na-succinate (pH 5.0) to a final volume of 267 µl and pre-incubated in the presence of 10 mM 2-mercaptoethanol for 30 min at 4° C; 533 µl of 0.2% (w/v) azocasein in the same buffer was added and the reaction mixtures were incubated at 30° C for 3 h. The reactions were terminated by adding 200 µl of 50% trichloroacetic acid (TCA). The reaction mixtures were kept on ice for 15 min to precipitate TCA-insoluble polypeptides. Following centrifugation of the reaction mixtures for 10 min in a Beckman Instruments (Palo Alto, Cal., USA) microfuge the relative amounts of TCA-soluble peptides hydrolyzed from the azocasein were determined by measuring A_{330}, Enzyme activities are presented as relative percentages of the most active sample.

Analysis of newly synthesized proteins. The protein-labeling procedure and electrophoresis procedure used has previously been described in detail (Nolan et al. 1987). The phenotypes of the aleurone layers were predetermined using a small sliver of aleurone layer to perform an α-amylase plate assay. To determine relative rates of α-amylase synthesis and secretion under various hormonal conditions, five sterile slender half-seeds or five normal half-seeds were first imbibed for 2 d on filter paper soaked with Ca-succinate buffer. The aleurone layers were removed and placed in 1 ml fresh Ca-succinate buffer containing either no hormones, or $2 \cdot 10^{-5}$ M ABA, or $1 \cdot 10^{-6}$ M GA_3, and incubated with agitation for 8 h. The layers were then further incubated for 3 h with 9.4 MBq/ml (specific activity >37 TBq/mmol) of a mixture of [^{35}S]methionine and [^{35}S]cysteine (*trans*

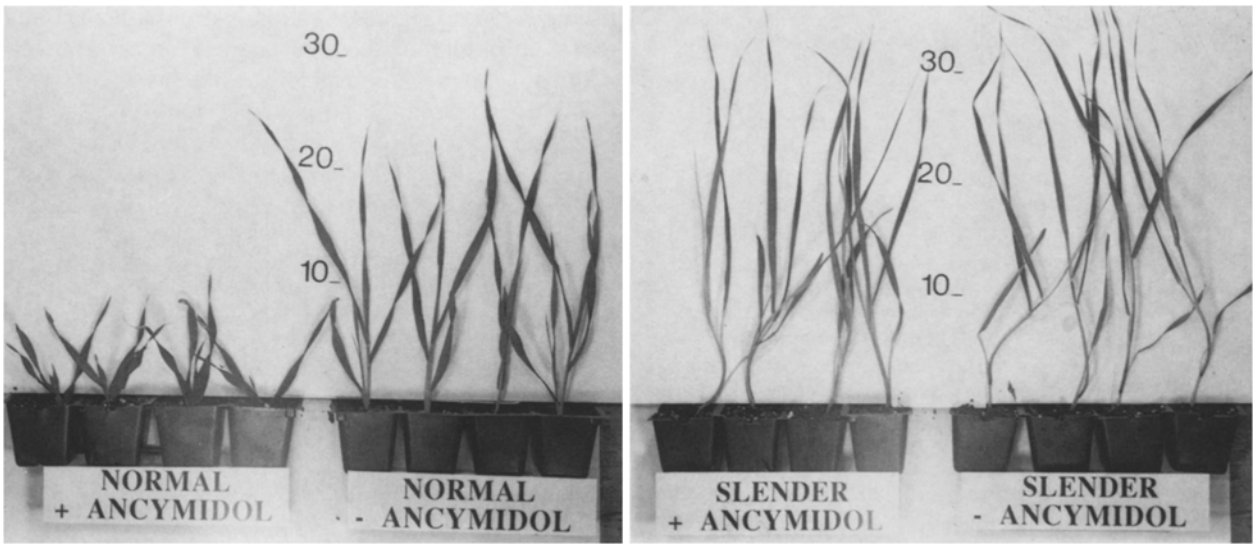

Fig. 1. Effect of ancymidol, a GA-biosynthesis inhibitor, on shoot elongation in normal and slender barley plants. Five days after germination plants were watered without ancymidol (− ancymidol) or with a solution of ancymidol (+ ancymidol) for 21 additional days. Immediately following this treatment the plants were photographed. Height scale is in centimeters

^{35}S-label from ICN, Irvine, Cal., USA). Equal TCA-precipitable counts as determined by the method of Mans and Novelli (1960) from the medium were run on denaturing Na-dodeoxylsulphate-polyacrylamide gels (SDS-PAGE) as described by Laemmli (1970). After electrophoresis the gels were impregnated with 2,5-diphenyloxazole and dried as described by Jen and Thach (1982). The dried gels were then exposed to Kodak XAR-5 film (Eastman-Kodak, Rochester, N.Y., USA) at −80° C.

To determine relative rates of α-amylase synthesis under various hormonal conditions, layers were prepared and treated as above except that during imbibition of the half-seeds the appropriate hormone was included in the media. The period of labeling was reduced to 1 h. After labeling the layers were rinsed in 1 mM each of non-radioactive methionine and cysteine, and ground with sea sand in 100 µl of 10 mM leupeptin, a thiol protease inhibitor (Boehringer Mannheim Biochemicals, Indianapolis, Ind., USA), in a chilled mortar. An equal volume of 30% glycerol, 10 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol-HCl (Tris)-HCl, pH 6.8, 0.01% bromophenol blue (Bio Rad, Richmond, Cal., USA) was then mixed with the homogenate. Insoluble debris were removed by centrifugation in a Beckman microfuge for 5 min. Equal amounts of TCA-precipitatable counts were run on SDS-polyacrylamide gels; SDS-PAGE, PPO impregnation, gel drying, and exposure were as described above.

Gibberellin bioassays. Gibberellin extracts were prepared according to Ho et al. (1981). Samples (1.5 g each) of sterile half-seeds of slender, normal sibling, or Himalaya barley half-seeds, or whole seeds were imbibed for 2 d on Ca-succinate buffer soaked filter paper. Following this imbibition the tissue was homogenized with a Brinkmann polytron at full speed for 2 min in methanol. The homogenate was shaken for 1 h, on ice, and the insoluble debris removed by centrifuging at 12000·*g* for 10 min. The supernatant was dried by bubbling nitrogen gas through the sample. The residue was dissolved in 15 ml of 0.25 M Na-phosphate buffer, pH 8.5. (At this point several internal extraction controls for the extraction procedure were prepared by adding no GA_3 or various concentrations of GA_3 to 15 ml of phosphate buffer.) The GA extracts, in phosphate buffer, were extracted twice with 15 ml of ethyl acetate. The pH of the aqueous phase was adjusted to 2.5 with 2 N HCl, and the aqueous phase was extracted twice with 15 ml of ethyl acetate. The 30-ml acidic ethyl-acetate fraction was dried under a stream of nitrogen gas. The residue was dissolved in 0.5 ml of Ca-succinate buffer and insoluble matter removed by centrifugation in a Beckman microfuge for 2 min. Two sterile aleurone layers from Himalaya barley, which had been prepared from 4-d-imbibed half-seeds, were incubated in each of the 0.5-ml GA extracts for 24 h. Aleurone layers were also incubated in Ca-succinate buffer containing no GA_3, or known concentrations of GA_3. After 24 h, 0.3 ml of the incubation media were removed from each sample and assayed for total α-amylase activity (see *Enzyme assays*). Two GBq of trans ^{35}S-label was added to each of the samples (now 0.2 ml) of aleurone layers and they were incubated for an additional 1 h. Protein extracts were prepared and analyzed as in *Analysis of newly synthesized proteins.*

Results

Inhibition of GA biosynthesis does not change the phenotype of slender plants. We determined the dependence of the slender phenotype on GA concentrations by assessing the effect of ancymidol, a GA-biosynthesis inhibitor, on plant development. Slender and normal plants were grown for 21 d, watered with either water or a solution of ancymidol (20 mg/l). The results, shown in Fig. 1, demonstrate the effect of inhibition of GA biosynthesis on plant height. The heights of normal plants were significantly decreased following inhibition of GA biosynthesis. The heights of slender plants, however, were unaffected by inhibition of GA biosynthesis. This observation indicates that the pheno-

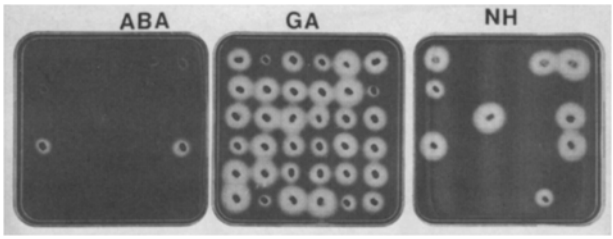

Fig. 2. α-Amylase agar-plate assay results showing the response of normal and slender barley half-seeds to various hormone treatments. Progeny seeds from selfed heterozygous barley plants carrying the slender gene (*Sln1/sln1*) were de-embryonated and cut transversely into three pieces of roughly the same size. A slice from each seed was put into the same quadrant of starch plates containing ABA, GA_3, or no hormones (*NH*). The plates were incubated for 2 d and then developed (see *Materials and methods*). The GA plate shows GA-induced α-amylase secretion from a heterogenous population of half-seeds. The NH plate depicts hormone-independent secretion of α-amylase by about 25% of seeds tested. The ABA plate demonstrates the ability of ABA to inhibit the hormone independent α-amylase secretion from slender half-seeds

type of slender plants is independent of endogenous GA levels.

Progeny seed from selfed heterozygous plants synthesize and secrete hydrolytic enzymes without exogenous GA_3. To determine the individual phenotypes of a mixed population of progeny seeds from a heterozygous selfed plant, plate assays for several hydrolytic enzymes were employed. Exogenous GA_3 is not necessary for the synthesis and secretion of α-amylase from a fraction of the seeds. This is illustrated in Fig. 2 by the cleared zones around one quarter of the half-seeds on the no-hormone plate. The ratio of seeds requiring GA_3 for α-amylase secretion to those that did not was 3:1 (104:34 total tested), as would be expected of the progeny from a selfed plant heterozygous for a completely recessive single-locus mutation. Aleurone layers are triploid tissues as the rest of the endosperm, and the possible genotypes of the aleurone layers are *Sln/Sln/Sln*, *Sln/Sln/sln*, *Sln/sln/sln*, or *sln/sln/sln*. It was conceivable that the *sln1* allele might have displayed a dosage effect in aleurone tissue. However, this was not the case as only one quarter of the seeds displayed a non-normal (i.e. slender) phenotype. An additional one or two quarters of the seeds would have had a slender, or intermediate phenotype if *sln1* was a partially recessive allele. By germinating the corresponding embryo-containing half-seeds the vegetative slender phenotype was found to co-segragate with the slender aleurone phenotype (data not shown). Finally, ABA significantly inhibited secretion of α-amylase activity in those seeds which did not require exogenous GA_3 (Fig. 2, "ABA"). Thus ABA exerts its antagonistic effect on the process of α-amylase synthesis and secretion in the mutant.

Synthesis and secretion of α-amylase are independent of exogenous GA_3 in slender aleurone layers. By comparing lane 1 with lane 2 in Fig. 3A we concluded that synthesis of α-amylase in normal (*Sln/-*) aleurone layers requires exogenous GA_3. In slender (*sln/sln/sln*) aleurone layers however, α-amylase is synthesized in the absence of exogenous GA_3 (compare lane 4 with 5, Fig. 3A). The results in Fig. 3B demonstrate that the relative rates of α-amylase synthesis and secretion are independent of exogenous GA_3 in slender aleurone layers (compare lanes 5 and 6 in Fig. 3B). Normal aleurone layers are dependent upon exogenous GA_3 for the synthesis and secretion of α-amylase. Since GA_3-

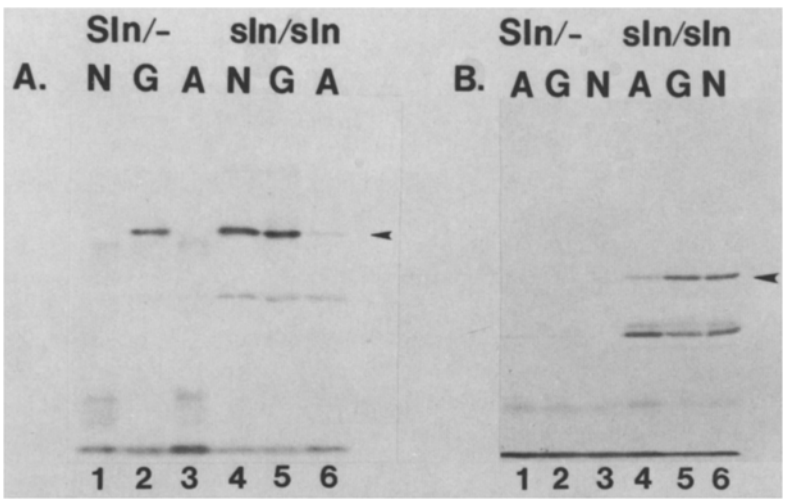

Fig. 3A, B. Profiles of newly synthesized and secreted proteins in normal and slender barley aleurone layers. Proteins labeled with [^{35}S]methionine/cysteine from normal (*Sln/−/−*) or slender (*sln/sln/sln*) half-seeds were separated on SDS-polyacrylamide gels (see *Materials and methods*). **A** shows labeled proteins extracted from aleurone layers, and **B** is labeled proteins from media used to incubate the layers (secreted proteins). *A*, *G*, and *N* refer to ABA, GA_3, or no hormone in the incubation media of the aleurone layers. *Arrows* indicate α-amylase bands (relative molecular mass = 44 kilodaltons)

induced α-amylase secretion from normal layers would not be expected after only an 8-h GA_3 treatment, it is expected that no secreted α-amylase would be detected in the media (Fig. 3B, lane 2). Non-denaturing gel analysis of labeled proteins from normal and slender aleurone layers showed that all of the α-amylase isoenzymes were affected similarly in the mutant (data not shown). These data also confirmed the fact that ABA inhibits the synthesis and secretion of α-amylase by slender aleurone layers (Fig. 3A, lane 6; and Fig. 3B, lane 4).

Nuclease and protease activities are also expressed in slender aleurone layers without the requirement for exogenous GA_3. To rule out the possibility that the slender mutation affects only expression of α-amylase, the expression of secreted protease and nuclease activities were also assessed. To determine the activities of the secreted hydrolytic enzymes, conventional enzyme assays were performed on media which had been used to incubate five half-seeds which were previously identified as slender or normal. Half-seeds were incubated in solutions of GA_3, ABA, or no hormones. Aliquots of the incubation media were assayed for total α-amylase, protease, and nuclease activities (see *Materials and methods*). As shown in Fig. 4 secretion of nuclease, α-amylase, and protease activities is dependent upon exogenous GA_3 in normal aleurone layers whereas slender aleurone layers do not require exogenous GA_3 to secrete these hydrolytic activities. It should be noted that nuclease induction is considerably slower than either α-amylase or protease induction. Normally, secreted nuclease activity is not detected until after 48 h of GA-induction and maximal rates of nuclease secretion are not attained until at least well after 48 h of GA_3 treatment (Brown and Ho 1986; Chrispeels and Varner 1967). It is not expected that normal layers will have secreted appreciable amounts of this activity after only 2 d of GA_3 treatment. Conversely, α-amylase secretion reaches maximal levels of synthesis and secretion by 12–16 h of GA_3 treatment (Nolan et al. 1987; Chrispeels and Varner 1967). As in the agar plate assay of α-amylase activity, ABA exerts inhibitory effects upon the secretion of all three hydrolytic activities from slender aleurone layers.

Gibberellin bioassays show slender and normal aleurone layers contain similar amounts of endogenous GA-like substances. The phenotype of slender aleurone layers could be the consequence of a number of factors. It is conceivable that slender half-seeds

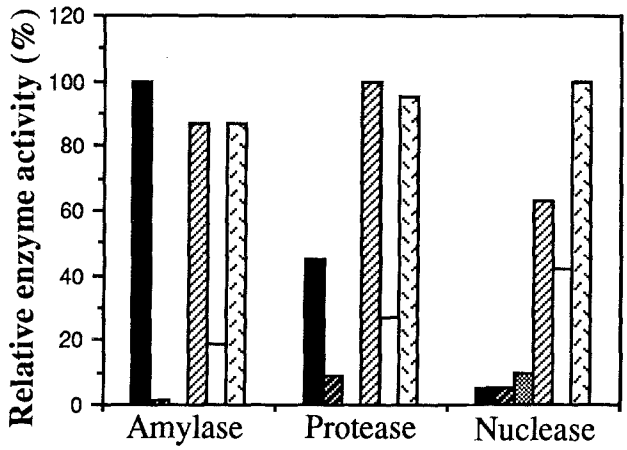

Fig. 4. Hydrolytic enzyme activities secreted from normal and slender aleurone layers of barley. Normal and slender half-seeds were incubated in medium containing either GA_3, ABA, or no hormones (*NH*) for 48 h. Incubation media were assayed for α-amylase, protease, and nuclease activity. Assays were performed and relative activities determined as described in *Materials and methods* ■ norm. GA, ▨ norm. NH, ▩ norm. ABA, ▨ sl GA, □ sl ABA, ▧ sl NH

contain high levels of endogenous GA or are capable of synthesizing GA. Upon imbibition this GA could then induce the aleurone cells to synthesize and secrete hydrolytic enzymes. It is also possible that slender aleurone layers have lost their dependence on GA for induction, and will synthesize and secrete the hydrolytic enzymes constitutively. To distinguish between these possibilities the levels of GA-like compounds in slender seeds and various control tissues were determined using a bioassay for GA-like activity.

Gibberellin extracts from slender half-seeds, various other tissues or known amounts of GA_3 were used to induce normal Himalaya barley aleurone layers to synthesize α-amylase (see *Materials and methods*). By qualitatively comparing the ratio of newly synthesized α-amylase to the amount of other newly synthesized protein in the different samples, the relative amounts of GA-like substances can be estimated (Fig. 5). Since equal trichloroacetic-acid-precipitable counts of each sample were loaded, direct comparisons of amounts of α-amylase could not be made. Alternatively, by determining the activities of α-amylase secreted into the medium by the Himalaya aleurone layers, the levels of the GA-like substances in the extract could be estimated (Fig. 6). Similar conclusions were reached using either method of determination. The no-GA controls (Figs. 5, 6; lanes 1 and 2) have no GA-like activity. Positive extraction controls (Figs. 5, 6; lanes 3, 4 and 5) demonstrate that the majority of the GA in the samples was

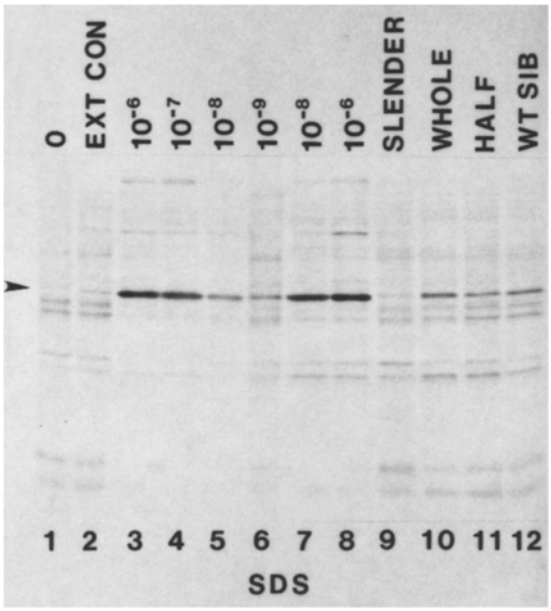

Fig. 5. Bioassay of extractable GA-like substances in slender and normal aleurone layers of barley: SDS-PAGE. SDS-PAGE of [^{35}S]methionine/cysteine-labeled proteins isolated from Himalaya barley aleurone layers incubated for 24 h in extracts of GA-like substances, or various control concentrations of GA_3 (see *Materials and methods*). Sample *1*, no GA control; *2*, no GA extraction control; *3*, *4*, *5*, GA extraction controls, 10^{-6}, 10^{-7}, 10^{-8} M of GA^3, in the order given; *6*, *7*, *8*, GA "standards", in the order 10^{-9}, 10^{-8}, 10^{-6} M of GA_3; *9*, *10*, *11*, *12*, extracts from, in this order slender, whole Himalaya seeds, Himalaya half-seeds, and normal half-seeds. *Arrow* indicates α-amylase bands

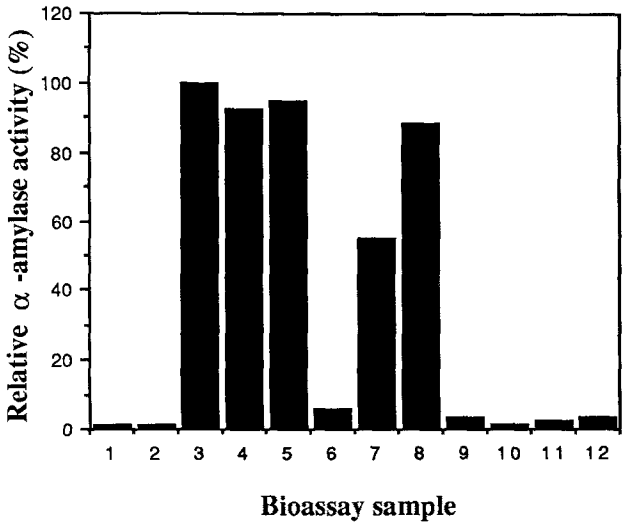

Fig. 6. Bioassay of extractable GA-like substances in slender and normal aneurone layers of barley: α-amylase activity. Relative α-amylase activities secreted from Himalaya barley aleurone layers incubated in extracts of GA-like substances, or various control concentrations of GA_3 for 24 h (see *Materials and methods*). Samples *1–12* same as lanes *1–12* in **Fig. 5**

extracted and retained biological activity. In Figs. 5 and 6 lanes 6, 7, and 8 are internal standards (GA_3 was added directly to the Ca-succinate buffer from a stock solution). By comparing lane 9 with lanes 10, 11, and 12 the conclusion can be drawn that extracts from slender half-seeds contain GA-like concentrations as low as, or lower than either extracts from whole Himalaya seeds imbibed for 2 d, Himalaya half-seeds, or their normal siblings. In a separate bioassay, extracts from whole Himalaya seeds that were imbibed for 4 d had GA-like activity equivalent to $5 \cdot 10^{-9}$ M GA_3 (data not shown).

Discussion

We have extended the analysis of the phenotype of slender barley to aleurone layers. Slender aleurone layers synthesize and secrete at least three hydrolytic enzymes without the requirement for GA treatment. Furthermore, the level of GA-like substances in slender half-seeds does not exceed that in normal half-seeds. We suggest that slender is a constitutive GA-response mutant, i.e. those GA-induced activities which would normally be expressed only in response to GA, are expressed independent of GA levels.

In peas, the slender phenotype results from a particular combination of two genes, *la* and *cry*^{s} (Potts et al. 1985). Slender pea plants contain amounts of endogenous GA comparable to their normal siblings, and inhibition of GA biosynthesis will not influence their phenotype. The phenotype of slender peas is therefore also independent of GA concentrations. In pea, the phenotype arising from the slender gene combination is epistatic to deficiencies in GA biosynthesis (Potts et al. 1985). There is another allele of the locus responsible for the slender phenotype in pea which causes less severe slender characteristics, *cry*^{c}. Perhaps this allele allows partial repression of the GA-controlled elongation response.

Since α-amylase, protease, and nuclease activities are affected similarly in slender aleurone layers, the mutation may disrupt an early regulatory event in the GA-controlled induction process. A strict genetic interpretation of a recessive mutant which confers constitutive expression to a class of genes, is the loss of a repressor function. Examples of constitutive mutants which affect response mechanisms are common in *Escherichia coli* (Riabaud and Schwartz 1984) and yeast (Nogi et al. 1984). For example, certain mutants of the *gal 80* gene in yeast confer constitutive transcription of the genes in the pathway of galactose utilization,

Gal 1, 7 and *10* (Nogi et al. 1984). The gal 80 protein is known to inhibit transcription from the promoters of the *Gal 1, 7* and *10* genes. In humans, loss of genes thought to be responsible for the suppression of cellular protooncogenes in differentiated cell types leads to tumour formation (e.g. retinoblastoma; (Kakkis and Calame 1987; Lee et al. 1987). Such mutants are, as is the slender mutation of barley, recessive.

Constitutive hormone responses could also be explained by changes in the hormone receptor proteins. Constitutive derivatives of the mammalian glucocorticoid receptor have been reported (Hollenber et al. 1987; Miesfeld et al. 1987). Genes for constitutive glucocorticoid receptor protein resulted from in-vitro deletion mutagenesis. These modified receptors display hormone-independent transcription of mouse mammary tumor virus (MTV) and the gene encoding α-1-acid glycoprotein (AGP). Constitutive receptor mutations, however, would be dominant.

Our results with slender barley reinforce the hypothesis that the processes of GA-regulated hydrolase synthesis and secretion in aleurone layers and GA-induction of cellular elongation share a common regulatory element (Ho et al. 1981). Other genetic evidence exists which supports such a connection, at the molecular level, between GA regulation of enzyme induction in barley aleurone layers and shoot elongation. Many dwarfing mutations in plants block the synthesis of GA. Such a mutant will respond to exogenously applied GA by an increase in height. However, dwarfs also exist which do not respond to exogenously applied GA by growing taller. The aleurone layers of some of these mutants (*Rht 3* in wheat, Ho et al. 1981) also do not respond to GA (i.e. there is no secretion of hydrolytic enzymes). Certain other mutants, such as the Apam dwarf cultivar, of barley, is a GA-non-responsive dwarf but its aleurone layers remain responsive to GA in terms of α-amylase induction (Bougler et al. 1982). Finally, mutants exist which are tall yet their aleurone layers do not synthesize and secrete α-amylase in response to GA (G52 in barley; Ho et al. 1980).

We propose a branched pathway for GA action to account for these observations. It is generally believed that GA interacts with a receptor to produce a cellular state in which a secondary messenger, or the receptor-hormone complex itself, affects the transcription of certain genes. The various mutants indicate that two different classes of genes are regulated by GA; one class is expressed in green tissues, to facilitate elongation, another class in aleurone cells, i.e. genes encoding hydrolases. The similarity between the response to GA by the two tissues could be at the level of the hormone-receptor interaction or any number of subsequent steps in the signal-transduction process. Any basis for the eventual difference in gene expression in the two tissues would be purely speculative, but the existence of such a divergence is indicated by the mutants mentioned above.

The observation that ABA retains its inhibitory effect on hydrolase secretion in slender indicates that ABA may act at a point after the action of the *sln1* gene product. Alternatively, the mechanism of ABA suppression of hydrolase synthesis may be a distinct pathway of inhibition and not involve any component of the GA-induction pathway.

The slender mutation in barley will undoubtedly aid in deciphering the mechanism of gene regulation by GA in aleurone layers. Historically, integrated approaches involving genetics, biochemistry and molecular biology have led to greater understanding of complex regulatory mechanisms or biosynthetic pathways. Currently, we are concentrating our efforts on the effects that a number of different mutations have upon the expression of GA-regulated genes in aleurone layers.

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