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Hormonal and developmental control of gene expression in wheat

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It is likely in plants, as in animal and fungal cells, that development involves the coordinated regulation of sets of genes. It is further likely that when this regulation acts on transcription that the coordination is mediated via *trans*-acting factors that recognize regulatory elements close to the responsive genes. In wheat (and barley) aleurone cells, a set of genes including those for α -amylase and for other hydrolases show increased expression at the RNA and transcriptional level in response to gibberellic acid. Based on the pattern of expression in various experimental conditions it seems likely that they are a co-regulated set, in the sense described above. However, a comparative analysis of 5' flanking regions has been made and, after the influence of relatedness between different members of gene families is accounted for, no sequence motifs can be identified that could be regulatory elements. More direct methods of analysis for such elements are described involving analysis of expression from natural or artificially constructed sequence variants.

There is a second aspect to the regulated expression of aleurone genes when they are expressed non-coordinately and not under the control of gibberellic acid in non-aleurone tissues. In some instances this is because the same gene, expressed from the same promoter, is expressed in the different tissues and suggests that there are multiple regulatory elements close to these genes that respond to different stimuli depending on the stage of development. The α -Amy2 and carboxypeptidase genes of wheat use this strategy. In other instances, however, it can be seen that the dual mode of expression is achieved when multigene families have evolved in which different subsets have a different capability of expression. This strategy is exemplified by the α -Amy1 and α -Amy3 subsets of the α -amylase gene families.

INTRODUCTION

The interaction and effect of gibberellic acid (GA) on cereal aleurone cells is one of the best studied hormonal response systems in plants. It is now known that, as a result of this response, aleurone cells reorganize into secretory cells and that there is large-scale production of hydrolytic enzymes, which are then released into the starchy endosperm for the degradation of storage reserves (see Akazawa & Miyata (1982) for review). For several of these enzymes, including α -amylase (Filner & Varner 1967), protease (of unspecified type) (Jacobsen & Varner 1967) and α -glucosidase (Hardie 1975), although not for all enzymes, the elevated levels result from *de novo* protein synthesis. This in turn is the result, at least for α -amylase and a number of unidentified products (Higgins *et al.* 1976; Baulcombe & Buffard 1983; Chandler *et al.* 1984; Rogers *et al.* 1985), of an increased level of the respective mRNA. The major level of control acts on transcription of mRNA (Jacobsen & Beach 1985; Zwar & Hooley 1986), although there is also evidence for translational regulation (Ho 1980).

[99]

Since it is now possible to identify regulatory elements of eukaryotic genes at the nucleotide level, and to use this information to track down cellular components involved in the regulatory process, there is the prospect of generating a more complete understanding of how gibberellic acid can regulate gene expression in aleurone cells. In addition, the aleurone system presents an opportunity to investigate the mechanisms involved in coordinate regulation of gene expression in plants, because the gibberellin stimulus apparently triggers the expression of many genes. These problems are examined in this paper, together with a related question, concerning the expression of the various hydrolase genes in non-aleurone cells. It is found in these latter cells that the genes are expressed, but no longer in a coordinate manner, thus requiring the deployment of one or more regulatory strategies, which are discussed.

COORDINATE CONTROL OF GENE EXPRESSION

(a) *Homologous cis-acting elements in eukaryotic gene regulation*

The early analyses of prokaryotic genes indicated that control of transcription may be achieved by the action of a *trans*-acting factor onto a sequence element located in *cis* configuration to the responsive gene or genes. As a result of this, various models have been proposed in which, with appropriate modifications, the principle was adapted to eukaryotic systems. Thus in an early form the model involved a regulatory role for repeated sequences that are found ubiquitously in eukaryotic genomes and were detected by analysis of DNA renaturation kinetics (Davidson & Britten 1979). It was envisaged that these could be elements that act either at the DNA level as transcriptional regulators or at the RNA level as post-transcriptional regulators. Although a few examples were identified in which common repeated sequences were found internally or adjacent to members of co-regulated gene sets (Sutcliffe *et al.* 1982; Zuker & Lodish 1981), later analysis has shown these findings to be without regulatory significance (Lone *et al.* 1986). Subsequently, with the realization that regulatory elements need not be as large as the repeated sequences that were resolved by renaturation kinetics (at least 100 base pairs (bp)) it became necessary to search for much smaller regulatory sequence elements. In some instances the search has proved highly successful, for instance in *Drosophila* where a common sequence motif contained within 14 nucleotides is found in the 5' region of the various genes that are expressed coordinately in response to heat stress (Pelham 1985). Mutation analysis of this sequence has confirmed its role as a regulatory element by showing that it binds to a *trans*-acting factor (Topol *et al.* 1985). Similarly, various yeast genes involved in either mating type determination (Siciliano & Tatchell 1986; Wilson & Herskowitz 1986), sugar metabolism (Guarente 1984) or production of ribosomal proteins (Woudt *et al.* 1986) have all been shown to have common regulatory elements upstream of the gene.

The success of these studies would suggest that the notion of common *cis* elements located close to co-regulated genes is basically correct. However, investigations of genes from higher eukaryotes, animals or plants have proved less successful in terms of the identification of common regulators, especially when the studies have involved genes whose expression is modulated after a developmental transition.

There may be several reasons for this failure to observe common regulatory elements in coordinately regulated genes, two of which may be resolved by analysis of the gibberellin-responsive genes in cereal aleurones. In many instances, the criterion for the identification of

coordinately regulated genes involved the demonstration that the genes show a similar pattern of expression after a developmental transition or after application of a stimulus to an intact organism. However, only rarely is it established that the developmental transition involves a response to a single developmental trigger or that application of the stimulus does not involve the generation of multiple triggers that act at the cellular level. An example of the former situation is seen when either roots or shoots are induced to develop from tissue culture cells in response to the combined application of auxins and cytokinins. The transition of quiescent aleurone cells to a tissue that is actively secreting hydrolytic enzymes is, however, more straightforward in that this is a simple response to gibberellic acid (GA).

A second complicating feature arises in other instances where multiple, coordinately regulated genes are all members of a multigene family. Although one can be reasonably certain that the different co-regulated members respond to the same regulatory mechanism, there is difficulty in identifying common sequence elements that result from the evolutionary processes acting on the genes, as opposed to those that are functionally homologous. It is possible for example that homologies may be evolutionary debris, remnants of the sequence duplications that led to the formation of the multigene family, and have not diverged. Alternatively, sequence homologies may derive from gene conversion events operating on members of a multigene family. It is now well established that gene conversions between linked or unlinked genes, or unequal crossovers between linked genes, are an important homogenizing influence between members of multigene families, which can act independently of functional domains in or around the genes (Dover & Tautz 1986).

In our study of GA-controlled gene expression in wheat aleurones the major focus has been the different α -amylase genes. However, it has proved possible to eliminate the complications caused by evolutionary relatedness, by analysis of genes in two separate multigene families (α -*Amy1* and α -*Amy2*). These encode different types of isozyme (high *pI* and low *pI*) and are located on different chromosomes. In wheat, as in barley where homologous genes are also observed, both types of gene are subject to strong gibberellin regulation at the level of mRNA accumulation (Baulcombe & Buffard 1983; Rogers 1985). The nucleotide sequence analysis of α -*Amy1* and α -*Amy2* cDNAs from wheat, and of α -amylase cDNAs from barley, shows in fact that the differences between the gene families are maintained in the two species. For example, in some of the possible pairwise comparisons of genes within a family, genes present in two species may show more homology than paralogous genes present within a species, even in non-coding regions where the requirement to maintain open reading frames and the amino acid homology has not constrained the rate of evolution. However, in comparisons between α -*Amy1* and α -*Amy2* genes in this 3' region there is no significant homology (D. C. Baulcombe, C. Lazarus & R. Martienssen, in preparation). This therefore indicates that the evolutionary division between these two gene families is much more ancient than the evolutionary division between wheat and barley and that there has been sufficient time for the non-functional regions to diverge. The presence of the genes on separate chromosomes (Gale *et al.* 1983) and also the existence of particular features of the gene organization that are characteristic of either family indicates that homogenization between α -*Amy1* and α -*Amy2* genes is not a significant feature of their evolution. Examples of these features include a third intron, which is present in α -*Amy2* genes, in addition to the two that are conserved with α -*Amy1* genes (D. C. Baulcombe, C. Lazarus & R. Martienssen, in preparation). There are also nine extra codons at the 3' end, which are a consistent feature of α -*Amy2* genes and absent from α -*Amy1* genes (D. C. Baulcombe,

C. Lazarus & R. Martienssen, in preparation). It therefore seems likely that sequence homologies of non-coding or flanking regions in α -Amy1 and α -Amy2 genes would reflect functional conservation.

To have a further certainty that α -Amy1- α -Amy2 homology involves sequences with regulatory significance it is necessary to introduce into the comparison a third independent gene sequence that is subject also to GA control in germinating aleurones. In this instance we have used a gene that was identified originally as an anonymous copy DNA (cDNA) clone isolated from a library of clones prepared with aleurone mRNA and selected on the basis of preferential hybridization with RNA from GA-stimulated aleurones (Baulcombe & Buffard 1983). More recently the sequences of this cDNA and its genomic sequence have been determined. The protein sequence deduced from these was then compared with other sequences in the GenBank sequence library and found to show good homology with carboxypeptidase Y from yeast (D. C. Baulcombe, R. F. Barker & M. G. Jarvis, in preparation). Since this homology is extensive and includes the active site and substrate-binding domains it is highly likely that this clone represents a wheat carboxypeptidase gene, and it is referred to as such in subsequent discussion.

(b) *Regulatory elements close to GA-regulated genes have not yet been identified*

At the first level of analysis for regulatory elements that allow these three genes (α -Amy1, α -Amy2 or carboxypeptidase) to respond to GA, the nucleotide sequences in the 500–1000 nucleotides of 5' flanking region were compared with the use of a computer program designed to detect local sequence homologies (Kanehisa 1982). A definition of a necessary probability of match as greater than 10^{-5} produced no matches present in all three genes under consideration (D. C. Baulcombe, C. Lazarus & R. Martienssen, in preparation). There might be several explanations for this.

1. That GA activates several regulatory mechanisms, each of which acts on a different set of genes. To evaluate this possibility the expression of α -amylase and carboxypeptidase genes was compared under several various physiological conditions and with different experimental material from the standard Chinese Spring wheat. In each instance, irrespective of the incubation time, GA concentration, presence of abscisic acid or genetical modification of the gibberellin sensitivity of the plant material with the *Rht3* genes, there was a parallel effect on the α -amylase and carboxypeptidase gene expression (Baulcombe & Buffard 1983; Baulcombe *et al.* 1984; D. C. Baulcombe, unpublished). It is therefore unlikely that these genes are responding to different stimuli.

2. That the regulatory sequence elements are outside the regions that have been compared. Although in the few examples of both animal and plant genes where regulatory elements have been defined these are located within a few hundred nucleotides of the transcriptional start region, there is a recent report of a regulatory element located 6.1 kb upstream of the transcriptional start point (Theisen *et al.* 1986). For wheat α -amylase genes (α -Amy1), however, it is unlikely that regulatory elements are so far away because a comparison of two genes has shown that homology breaks down further than about 700 bp from the transcriptional start (R. Martienssen & D. C. Baulcombe, in preparation). As discussed above, the homologies of closely related genes are not confined to regulatory elements, but may well be expected to include them and so it is likely that the regulatory elements would be internal to the region up to 700 bp away from the gene.

3. That there are regulatory elements comprising non-contiguous nucleotides. It is quite feasible that *trans*-acting regulatory factors would not necessarily interact with contiguous nucleotides. This would be true, for example, if the contact points were all located on one side of the DNA double helix, or if the factor recognised a secondary structure feature adopted by the DNA in chromatin. The former possibility is demonstrated by the interaction between λ -phage repressor and the binding site in the target operator (O_r) (Ptashne *et al.* 1980). In eukaryotic systems the role and significance of secondary structure features in the regulation of gene expression is implied by studies of sites hypersensitive to DNase I that may be correlated with the activity of the genes (Reeves 1984).

(c) *Analysis of naturally occurring genetic variation as a means of defining regulatory sequences*

There is a high degree of natural polymorphism in wheat both for the number and properties of α -amylase isozymes (Ainsworth *et al.* 1985). One approach to the analysis of functional regions in or around the α -amylase genes exploits this by analysis of the basis of the polymorphism at the DNA sequence level and its effect on gene expression. A precedent for this type of approach is in the work of Vodkin *et al.* (1983) showing that the presence of an insertion element-like sequence in the lectin gene of soybean caused expression to be abolished.

The analysis of DNA sequence polymorphism in the wheat α -amylase genes is complicated by the presence of multiple copies of the gene. However, the multigene families can be subdivided and the groups identified by using probes from sequences flanking the genes. In one example of this approach, the subfamily identified by a 5' flanking sequence probe is polymorphic between Chinese Spring and Capelle-Desprez cultivars of wheat. Restriction enzyme mapping and DNA sequence analysis of genomic and cloned genomic DNA has shown that the polymorphism is the result of a composite insertion in the Chinese Spring allele, approximately 500 bp upstream of the transcriptional start region involving DNA that is dispersed and repeated in the wheat genome (R. Martienssen & D. C. Baulcombe, unpublished). The insertion contains two types of dispersed repeat sequence. One of these is represented many hundreds of times in the wheat genome and is present at each end of the insertion in an inverted repeat configuration. There is in addition a duplication of 9 bp of the α -amylase flanking sequence close to the termini of these repeats in Chinese Spring wheat, which is absent from related genes that do not contain the insertion. Since terminal inverted repeats and short target site duplications are features of well characterized transposons in maize and *Antirrhinum* (Freeling 1984) it seems likely that this polymorphism has arisen from the insertion of a transposon-like element into the Chinese Spring α -amylase gene, although it is not known whether the extant insertion is still capable of movement, or whether sequences essential for that function have been lost.

Within the insertion, and between the terminal inverted repeats, there is a second type of sequence element, which by virtue of its dispersed repetition is evidently also capable of movement. However, the mechanism of such movement is not yet clear. This element is reiterated approximately 50-fold in the wheat genome and has an internal organization comprising multiple direct repeats of a short sequence motif, which suggest analogy with the so-called mini-satellite sequences found in the human genome (Jeffreys *et al.* 1985).

Further analysis is needed to establish whether this insertion has an effect on the regulation or expression of the adjacent gene.

(d) Analysis of genes introduced into a foreign genetic background

One of the most powerful techniques in molecular biology for the analysis of gene regulation involves the transfer of genes as DNA either intact or in a modified form into the nucleus of new cells by using transformation techniques. Unfortunately, the ability to transform DNA into cereal cells is currently confined to the use of dedifferentiated and non-regenerable tissue culture cells via procedures involving direct uptake of DNA mediated by polyethylene glycol (Lorz *et al.* 1985) or by electroporation (Fromm *et al.* 1986). Since we are interested in the regulated expression of genes in aleurone cells, the transformation approach is not yet appropriate.

It is possible to introduce genes of cereals into dicotyledonous plants, but the degree of success in terms of expression is limited. So far only two examples have been reported: (i) the zein genes (from *Zea mays*) have been expressed in callus cells of sunflower (Matzke *et al.* 1985) and (ii) the chlorophyll *a/b* binding protein gene from wheat has been expressed and found to show the correct organ-specific and light-regulated expression in tobacco (Lamppa *et al.* 1985). Similarly, the genes for storage proteins of legumes are expressed at high level and with the correct developmental specificity in tobacco (Sengupta-Gopalan *et al.* 1985). These data suggest therefore that there is not necessarily a bar to expression of genes in a foreign background, and so the wheat α -amylase (α -*Amy2*) genes, including several kilobase pairs of upstream promoter and downstream sequence, have been introduced into tobacco. Analysis of three independent transformants has not shown expression, either in leaves where α -*Amy2* genes are not expressed at a high level in wheat, or in germinating F₁ seeds (A. Huttly & D. C. Baulcombe, unpublished). The reason for this is not clear, but may result from the absence of an appropriate *trans*-acting factor in tobacco. Alternatively there may be another feature of α -amylase gene expression, for example processing or transcriptional termination, which does not occur efficiently in tobacco and which prevents the correctly regulated initiation of transcription from being detected. To test these possibilities it will be necessary to introduce chimeric constructions into plants containing intronless coding sequences and transcriptional terminators known to function in tobacco.

A further approach to this problem may involve the use of methods to obtain transient expression of DNA after the use of electroporation to introduce the DNA into the cells. This approach has been used successfully to introduce and express new DNA sequences into protoplasts of maize and tobacco cells (Fromm *et al.* 1985). In our experiments the recipient cells will be aleurone protoplasts (Hooley 1982). These may be isolated in high yield and retain viability and GA responsiveness for many days. However, the question mark concerning these experiments involves the uncertainty of whether the non-chromosomal DNA will adopt a conformation that would allow the appropriate regulation.

NON-COORDINATE CONTROL OF GENE EXPRESSION

It would not be expected that α -amylase, carboxypeptidases and other hydrolytic enzymes produced in the germinating grain would be expressed uniquely at that stage of development. In fact, this is confirmed by analysis of enzyme activities and isozyme production and by analysis of mRNAs from different tissues (Lazarus *et al.* 1985). These all indicate that the genes are expressed variously in leaves and in developing grain in addition to the germinating grain.

In wheat, lines carrying the *Rht3* gene, which confers constitutive reduced sensitivity to gibberellin, have been used to show that expression at these alternative stages of development is no longer under the control of gibberellic acid (Gale & Ainsworth 1984; D. C. Baulcombe, unpublished). It is therefore necessary to explain how a gene product is produced at various times in development at different levels and under the influence of difference gene regulatory mechanisms. Figure 1 outlines three strategies by which this may be achieved. In the first, labelled 'multiple genes' and in the simple case of two different modes of expression, there would be two genes each containing a similar coding sequence, positioned next to promoters that respond to different *trans*-acting regulators. This is exemplified in plants by expression of members of the glutamine synthase gene family in different tissues of *Phaseolus* (Cullimore *et al.* 1984) and also by the expression of genes for maize phosphoenolpyruvate carboxylase (Hudspeth *et al.* 1986).

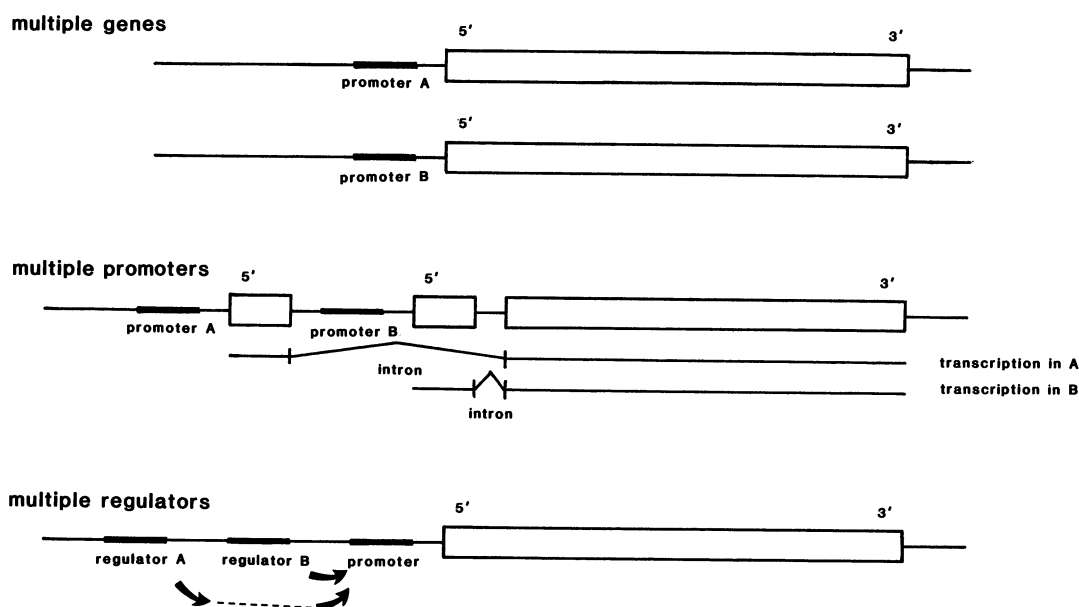


FIGURE 1. Strategies for the alternative expression of a gene product in different cell types. The diagrams illustrate three ways in which a gene may be expressed at two different developmental stages (A and B). Exons are shown as boxed regions between the 5' start and the transcriptional termination (3') region. Introns, where shown, are the non-exon regions between these points. Other features of the gene (promoters, regulators) are shown as thickened lines.

A second strategy, labelled 'multiple promoters' in figure 1, requires only a single gene, probably with an intron close to the 5' end, positioned next to two promoters that are used alternatively. The examples of this include mouse α -amylase, which is expressed in the liver, pancreas and salivary gland (Young *et al.* 1981), alcohol dehydrogenase of *Drosophila* (Benyajati *et al.* 1983) and the mammalian immunoglobulin genes (Rogers & Wall 1984). These are all similar in that the alternative promoters direct the transcription of different 5' exons, which are then spliced on to the main part of the mRNA molecule. The example of the immunoglobulin genes has some similar features to those predicted for the differential expression of α -amylase and carboxypeptidase genes, as the protein products are targeted to different subcellular locations in the different modes of expression (Rogers & Wall 1984). The immunoglobulins

are secreted when expressed in T lymphocytes but are located in the cell membrane in B lymphocytes. Similarly, the α -amylase and carboxypeptidase proteins are secreted from aleurones, but it seems unlikely that this would occur either in leaves or developing grains. Carboxypeptidase has been located in the central vacuole of leaf cells (Zuber & Matile 1968) and α -amylase is most likely to occur in the same compartment as cellular starch, namely the plastids, although this has yet to be demonstrated.

The third model that may be relevant to the expression of aleurone genes in other tissues is labelled 'multiple regulators' in figure 1. This predicts that a single copy of the gene would be expressed always from the same promoter, but that the control of this promoter would be from different regulatory elements in the different cell types. This model, although it may account for the expression of many different types of gene, actually has only a few precedents in eukaryotic systems. For example, in a higher eukaryote it has been established that expression of the lysozyme gene in chicken macrophages involves an enhancer element that is distinct from the regulatory elements important for expression under oestrogen control in the oviduct (Thiesen *et al.* 1986). It is likely also that interferon genes have two regulatory regions controlling non-induced expression or expression induced by poly(I)-poly(C) (Zinn *et al.* 1983). Conceptually similar is an example in yeast of genes responsive to the products of the yeast mating type loci. Each of these *trans*-acting peptides has a different regulatory receptor in the upstream region of responsive genes (Siliciano & Tatchell 1986; Wilson & Hershowitz 1986).

The carboxypeptidase genes are present only once in each of the three homoeologous genomes that make up the hexaploid genome of wheat (D. C. Baulcombe, R. F. Barker & M. G. Jarvis, in preparation). This rules out the possibility of the multiple gene option. To allow the remaining models to be distinguished, the technique of S1 nuclease transcript mapping was used with a probe from the genomic sequence that spans the 5' start of the aleurone mRNA. This confirmed that in leaf and developing grain RNA exactly the same mRNA start was used as in aleurone cells and therefore suggested that the multiple regulator strategy ensured differential expression of these genes in multiple tissues (D. C. Baulcombe, R. F. Barker & M. G. Jarvis, in preparation).

In contrast, the α -amylase genes have a much more complex hierarchical organization, which allows the 'multiple genes' option to be used although probably in conjunction with the 'multiple regulators' on some of the genes. The top level of the organizational hierarchy is the division of the genes into the three multigene families located on the group 5 (*Amy3*), group 6 (*Amy1*) and group 7 (*Amy2*) chromosomes (D. C. Baulcombe, R. Martienssen & R. F. Barker, in preparation; Lazarus *et al.* 1985). The *Amy3* genes in fact comprise only a single gene on each of the group 5 chromosomes and so there is no chance for further subdivision in this gene family. These genes exemplify the 'multiple genes' option as they are expressed only in the developing grain, in contrast to the α -*Amy1* and α -*Amy2* genes, which show the highest level of expression in the germinating aleurone.

The same strategy is also evident in the α -*Amy2* gene family where only a subset of the genes is expressed in the developing grain. This subset has been identified by isozyme analysis and by hybridization (Gale & Ainsworth 1984), with the use of DNA probes from different cloned copies of α -*Amy2* genes (A. Huttly & D. C. Baulcombe, unpublished). However, the α -*Amy2* genes active in the developing grain are the same genes active in the germinating aleurone and, as the same promoters initiate transcription at the same points in both tissues (A. Huttly

& D. C. Baulcombe, unpublished), are an example of the multiple regulator model (figure 1). The α -*Amy1* gene family shows subdivisions that are just as clear as those in the α -*Amy2* family based on hybridization and DNA sequence analysis. It is not yet clear, however, that these correspond to divisions associated with differential expression.

CONCLUSIONS

In terms of molecular and developmental biology, our understanding of how α -amylase and carboxypeptidase gene expression is regulated in wheat plants is still at an early stage. It is known that in the cell lineage derived from fertilization of the polar nuclei, which leads to development of triploid endosperm and aleurone cells, there is a transcriptional activation of these genes in response to cellular stimulation by gibberellin.

In diploid ($2N$) cell lineages there are different, gibberellin-insensitive gene activation processes leading to expression of the same genes, from the same promoters (carboxypeptidase or α -*Amy2*) or of related genes (α -*Amy3*). As yet nothing is known about *trans*-acting elements that in aleurone cells are predicted to ensure the coordinate expression of the genes. However, these are problems that are becoming amenable to analysis by expression *in vivo* of DNA sequences that have been introduced by transformation or transient uptake methods.

At the next stage it will be necessary to ask how these controls fit into the more general scheme of plant development. It is known, for example, that immature aleurone cells are induced to respond to GA by drying but only if the caryopses are older than 20 days post-anthesis (Cornford *et al.* 1986). It will be interesting to determine, for example, whether the factors that control aleurone gene expression are regulated themselves at these two key stages 20 days post-anthesis or at drying. Alternatively, it may be, for example, that there is a change in the chromatin status of the responsive genes at these times such that they only develop the facility to respond to the factors after a transition at one of these stages.

A similar problem viewed from a different angle involves the gibberellin response in leaves and aleurone cells. Although the final response is different in these two cell types, it is clear from the similar sensitivity to *Rht3* genes that the GA signal-response pathway has common features in both cells. By tracing this pathway back from the gene to the GA, the point at which the gibberellin response diverges in the two cell types could be determined. Identification of the component at this point will thus enable more central questions of development to be answered.

A second question will also need to be addressed concerning the subcellular location of the gene products in different cell types. If, as seems likely, the α -amylase and carboxypeptidase proteins secreted from aleurone cells are targeted to plastid or tonoplast membranes respectively when expressed in non-aleurone cells, it will be necessary to explain how this is achieved. The transcript mapping that showed that the mRNA terminus is the same in different cell types implies that the same N-terminal signal peptide is always present and therefore that selectivity of targeting occurs after the proteins have been transferred to vesicles derived from the rough endoplasmic reticulum. This is not difficult to envisage for the transfer of carboxypeptidase to the tonoplast. However, if α -amylase does transfer to the plastids it is more difficult to understand how this would occur. Normally the transfer of nuclear gene products into plastids is not thought to involve vesicles but instead a mechanism involving cleavage of a transit peptide sequence from the N-terminus of the protein. Transit peptides have a structure quite

distinct from signal peptides. A recent report of tobacco mosaic virus coat protein in plastids (Reinero & Beachy 1986), however, does suggest that protein transfer into plastids may occur independently of a transit peptide mechanism.

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REFERENCES

- Akazawa, T. & Miyata, S. 1982 Biosynthesis and secretion of α -amylase and other hydrolases in germinating cereal seeds. In *Essays in biochemistry* (ed. P. N. Campbell & R. D. Marshall), vol. 18, pp. 41–78. London: Academic Press.
- Ainsworth, C. C., Doherty, P., Edwards, K. G. K., Martienssen, R. A. & Gale, M. D. 1985 Allelic variation at α -amylase loci in hexaploid wheat. *Theor. appl. Genet.* **70**, 400–406.
- Baulcombe, D. C. & Buffard, D. 1983 Gibberellic-acid-regulated expression of α -amylase and six other genes in wheat aleurone layers. *Planta* **157**, 493–501.
- Baulcombe, D., Lazarus, C. & Martienssen, R. 1984 Gibberellins and gene control in cereal aleurone cells. *J. Embryol. exp. Morph.* **83**, 119–135.
- Benyajati, C., Spoerel, N., Haymerie, H. & Ashburner, M. 1983 The messenger RNA for alcohol dehydrogenase in *Drosophila melanogaster* differs in its 5' end in different developmental stages. *Cell* **33**, 125–133.
- Chandler, P. M., Zwar, J. A., Jacobsen, J. V., Higgins, T. J. V. & Inglis, A. S. 1984 The effects of gibberellic acid and abscisic acid on α -amylase mRNA levels in barley aleurone layers studies using an α -amylase cDNA clone. *Pl. molec. Biol.* **3**, 407–418.
- Cornford, C., Black, M., Chapman, J. & Baulcombe, D. 1986 Expression of α -amylase and other GA-regulated genes in the aleurone tissue of developing wheat grains. *Planta*. (In the press.)
- Cullimore, J. V., Gebhardt, C., Saarelainen, R., Mifflin, B. J., Idler, K. B. & Barker, R. F. 1984 Glutamine synthetase of *Phaseolus vulgaris* L.: organ-specific expression of a multigene family. *J. Molec. appl. Genet.* **2**, 589–599.
- Davidson, E. H. & Britten, R. J. 1979 Regulation of gene expression: possible role of repetitive sequences. *Science, Wash.* **204**, 1052–1059.
- Dover, G. A. & Tautz, D. 1986 Conservation and divergence in multigene families: alternatives to selection and drift. *Phil. Trans. R. Soc. Lond. B* **312**, 275–289.
- Filner, P. & Varner, J. E. 1967 A test for de novo synthesis of enzymes; density labeling with H_2O^{18} of barley α -amylase induced by gibberellic acid. *Proc. natn. Acad. Sci. U.S.A.* **58**, 1520–1526.
- Freeling, M. 1984 Plant transposable elements and insertion sequences. *A. Rev. Pl. Physiol.* **35**, 277–298.
- Fromm, M., Taylor, L. P. & Walbot, V. 1985 Expression of genes transferred into monocot and dicot plant cells by electroporation. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5824–5828.
- Fromm, M. E., Taylor, L. P. & Walbot, V. 1986 Stable transformation of maize after gene transfer by electroporation. *Nature, Lond.* **319**, 791–793.
- Gale, M. D. & Ainsworth, C. C. 1984 The relationship between α -amylase species found in developing and germinating wheat grain. *Biochem. Genet.* **22**, 1031–1036.
- Gale, M. D., Law, C. N., Chojecki, A. J. & Kempton, R. A. 1983 Genetic control of α -amylase production in wheat. *Theor. appl. Genet.* **64**, 209–316.
- Guarente, L. 1984 Yeast promoters: positive and negative elements. *Cell* **36**, 799–800.
- Hardie, D. G. 1975 Control of carbohydrase formation by gibberellic acid in barley endosperm. *Phytochemistry* **14**, 1719–1722.
- Higgins, T. J. V., Zwar, J. A. & Jacobsen, J. V. 1976 Gibberellic acid enhances the level of translatable mRNA for α -amylase in barley aleurone layers. *Nature, Lond.* **260**, 166–168.
- Ho, D. T.-H. 1980 Hormonal and genetic regulation of α -amylase synthesis in barley aleurone layers. In *Genome organisation and expression in plants* (ed. C. J. Leaver), pp. 147–158. New York and London: Plenum Press.
- Hooley, R. 1982 Protoplasts isolated from aleurone layers of wild oat (*Avena fatua* L.) exhibit the classic response to gibberellic acid. *Planta* **154**, 29–40.
- Hudspeth, R. L., Glackin, C. A., Bonner, J. & Grula, J. W. 1986 Genomic and cDNA clones for maize phosphoenolpyruvate carboxylase and pyruvate, orthophosphate dikinase: expression of different gene-family members in leaves and roots. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2884–2888.
- Jacobsen, J. V. & Beach, L. R. 1985 Control of transcription of α -amylase and rRNA genes in barley aleurone protoplasts by gibberellin and abscisic acid. *Nature, Lond.* **316**, 275–277.
- Jacobsen, J. V. & Varner, J. E. 1967 Gibberellic acid-induced synthesis of protease by isolated aleurone layers of barley. *Pl. Physiol.* **42**, 1596–1600.

- Jeffreys, A. J., Wilson, V. & Thein, S. L. 1985 Hypervariable 'minisatellite' regions in human DNA. *Nature, Lond.* **314**, 67–73.
- Kanehisa, M. I. 1982 Los Alamos sequence analysis package for nucleic acids and proteins. *Nucl. Acids Res.* **10**, 183–196.
- Lamppa, G., Nagy, F. & Chua, N.-H. 1985 Light-regulated and organ-specific expression of a wheat Cab gene in transgenic tobacco. *Nature, Lond.* **316**, 750–752.
- Lazarus, C. M., Baulcombe, D. C. & Martienssen, R. A. 1985 α -Amylase genes of wheat are two multigene families which are differentially expressed. *Pl. molec. Biol.* **5**, 13–24.
- Lone, Y.-C., Simon, M.-P., Kahn, A. & Marie, J. 1986 Sequences complementary to the brain-specific 'identifier' sequences exist in L-type pyruvate kinase mRNA (a liver-specific messenger) and in transcripts especially abundant in muscle. *J. biol. Chem.* **261**, 1499–1502.
- Lorz, H., Baker, B. & Schell, J. 1985 Gene transfer to cereal cells mediated by protoplast transformation. *Molec. gen. Genet.* **199**, 178–182.
- Matzke, M. A., Susani, M., Binns, A. N., Lewis, E. D., Rubenstein, I. & Matzke, A. J. M. 1984 Transcription of a zein gene introduced into sunflower using a Ti plasmid vector. *EMBO J.* **3**, 1525–1531.
- Pelham, H. 1985 Activation of heat-shock genes in eukaryotes. *Trends Genet.* **1**, 31–36.
- Ptashne, M., Jeffrey, A., Johnson, A. D., Maurer, R., Meyer, B. J., Pabo, C. O., Roberts, T. M. & Sauer, R. T. 1980 How the λ repressor and Cro work. *Cell* **19**, 1–11.
- Reeves, R. 1984 Transcriptionally active chromatin. *Biochim. biophys. Acta* **782**, 343–393.
- Reinero, A. & Beachy, R. N. 1986 Association of TMV coat protein with chloroplast membranes in virus infected leaves. *Pl. molec. Biol.* **6**, 291–302.
- Rogers, J. & Wall, R. 1984 Immunoglobulin RNA rearrangements in B lymphocyte differentiation. *Adv. Immunol.* **35**, 39–56.
- Rogers, J. C. 1985 Two barley α -amylase gene families are regulated differently in aleurone cells. *J. biol. Chem.* **260**, 3731–3738.
- Rogers, J. C., Dean, D. & Heck, G. R. 1985 Aleurain: a barley thiol protease closely related to mammalian cathepsin H. *Proc. natn. Acad. Sci. U.S.A.* **82**, 6512–6516.
- Sengupta-Gopalan, C., Reichert, N. A., Barker, R. F., Hall, T. C. & Kemp, J. D. 1985 Developmentally regulated expression of the bean β -phaseolin gene in tobacco seed. *Proc. natn. Acad. Sci. U.S.A.* **82**, 3320–3324.
- Siliciano, P. G. & Tatchell, K. 1986 Identification of the DNA sequences controlling the expression of the MAT α locus of yeast. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2320–2324.
- Sutcliffe, J. G., Milner, R. J., Bloom, F. E. & Lerner, R. A. 1982 Common 82-nucleotide sequence unique to brain RNA. *Proc. natn. Acad. Sci. U.S.A.* **79**, 4942–4946.
- Theisen, M., Stief, A. & Sippel, A. E. 1986 The lysozyme enhancer: cell-specific activation of the chicken lysozyme gene by a far-upstream DNA element. *EMBO J.* **5**, 719–724.
- Topol, J., Ruden, D. M. & Parker, C. S. 1985 Sequences required for *in vitro* transcriptional activation of a *Drosophila hsp 70* gene. *Cell* **42**, 527–537.
- Vodkin, L. O., Rhodes, P. R. & Goldberg, R. B. 1983 A lectin gene insertion has the structural features of a transposable element. *Cell* **34**, 1023–1031.
- Wilson, K. L. & Herskowitz, I. 1986 Sequences upstream of the STE6 gene required for its expression and regulation by the mating type locus in *Saccharomyces cerevisiae*. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2536–2540.
- Woudt, L. P., Smit, A. B., Mager, W. H. & Planta, R. J. 1986 Conserved sequence elements upstream of the gene encoding yeast ribosomal protein L25 are involved in transcription activation. *EMBO J.* **5**, 1037–1040.
- Young, R. A., Hagenbuchle, O. & Schibler, U. 1981 A single mouse α -amylase gene specifies two different tissue-specific mRNAs. *Cell* **23**, 451–458.
- Zinn, K., DiMaio, D. & Maniatis, T. 1983 Identification of two distinct regulatory regions adjacent to the human β -interferon gene. *Cell* **34**, 865–879.
- Zuber, H. & Matile, P. H. 1968 Acid carboxypeptidases: their occurrence in plants, intracellular distribution and possible function. *Z. Naturf.* **23b**, 663–665.
- Zuker, C. & Lodish, H. F. 1981 Repetitive DNA sequences cotranscribed with developmentally regulated *Dictyostelium discoideum* mRNAs. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5386–5390.
- Zwar, J. A. & Hooley, R. 1986 Hormonal regulation of α -amylase gene transcription in wild oat (*Avena fatua* L.) aleurone protoplasts. *Pl. Physiol.* (In the press.)