Introduction
During plant development, phytohormones influence such diverse processes as seed maturation, germination and fruit ripening. The basis of phytohormone action has been a subject of intense interest to plant physiologists for many years, however, the molecular events involved have not been fully elucidated for any given response. While not all responses to hormones involve alterations in gene expression (e.g. stomatal closure in response to abscisic acid (ABA), see Hetherington & Quatrano, [1] for review), it has been demonstrated that the expression of specific sets of RNA and protein molecules can be correlated with many of the physiological responses of plants to these compounds [2-6]. These sets of products may be unique to one response or there may be overlap in the sets between several responses.

The expression of hormone-regulated genes may be regulated at several levels, including transcription [7]. The transcription machinery interacts with the DNA primarily in the 5' flanking or promoter region of the gene and the pattern of expression of a single gene or set of genes is determined by these interactions. As such, a fundamental understanding of transcriptional regulation will be an important step in elucidating the molecular basis of phytohormone action. In this paper, we discuss recent findings which identify at least some of the cis sequences involved in ABA-mediated gene regulation [8-10] and a protein factor which interacts specifically with those sequences [10]. With this central point in the ABA signal transduction pathway identified, it should now be possible to follow the response chain in both directions ultimately identifying the initial receptor for ABA, the molecular mechanism(s) involved in gene activation, and the role(s) of the proteins produced in response to this hormone.

Identification of an ABA response element (ABRE)
During late seed development in a wide variety of plants, both monocot and dicot, a set of abundant RNAs and their respective proteins is produced coincident with increases in endogenous ABA levels [11-13]. The accumulation of these products is correlated with the acquisition of desiccation tolerance of the seed embryo [14, 15]. These same RNAs and proteins can be precociously induced in embryos of earlier developmental stages through culture, in vitro, in the presence of ABA [16, 17]. In the absence of ABA, these products do not accumulate and the embryos precociously germinate into completely normal seedlings.

In wheat, one of these RNAs encodes the embryo maturation protein, Em [6]. Early work with the Em gene demonstrated that approximately 1800 bp of the 5' flanking region was sufficient to direct the induction of expression of an Em-β-glucuronidase fusion gene (Em-β-glucuronidase) in rice protoplasts in response to ABA [8]. The response was rapid, specific for ABA, and proportional to the amount of ABA added. A similar response was directed by a shorter 652 bp fragment, indicating that the shorter fragment contained all the information necessary for ABA-mediated induction. In addition, transcript mapping experiments demonstrated that the mRNA produced by the fusion in the rice protoplasts initiated at the same place as the wild-type Em transcript found in wheat seeds. This same 652 bp promoter fragment, when used for the production of transgenic Em-β-glucuronidase tobacco plants, was capable of directing the appropriate temporal and spatial expression of enzyme activity [9], i.e. only in the embryos of seeds and at a time when endogenous ABA levels normally increase during development. This activity was also inducible precociously in earlier stages of development by culture in vitro of excised embryos in the presence of ABA. As such, the results obtained using the transient expression system have been taken to be physiologically relevant, to accurately predict the response in plants and have been exploited to rapidly identify the sequences involved.

Deletion of the 5' flanking sequences has been used routinely to identify sequences involved in directing a hormone-mediated response in many systems including animals [18, 19] and plants [20,
Marcotte, Russell & Quatrano et al. (1989) demonstrated that deletion of Em 5' promoter sequences between −1800 and −168, while lowering the absolute levels of expression, had no qualitative effect on the ABA-mediated induction of GUS enzyme activity. The lower levels of expression observed when these sequences were deleted was attributed to the presence of several blocks of A/T-rich sequences. Similar sequences have been associated with quantitative levels of expression in a variety of genes, possibly as a result of interactions with high mobility group (HMG) proteins [22, 23]. In contrast, however, deletion of an additional 63 bp of 5' flanking region drastically reduced the induction phenomenon [9]. As such, at least part of an ABRE must be present within this 62 bp.

Within this region of the Em promoter, there are several sequence motifs which appear in the 5' flanking regions of other seed protein genes. One of these, the EmI box, is found in the promoter regions of all ABA-regulated genes for which sequence data is available. This sequence is present twice (as an imperfect repeat) in the Em promoter proximal to the 62 bp deletion which abolished ABA induction. Guiltinan et al. [10] showed that a 76 bp oligonucleotide containing the EmI boxes (a and b), in the context of the −90 cauliflower mosaic virus (CaMV) 35S promoter, was sufficient for ABA induction of GUS expression in the transient assay, whereas the same −90 promoter fragment is by itself uninducible. Furthermore, they went on to show that mutation of the EmIa box reduced or eliminated the induction phenomenon. Marcotte et al. (this work) have gone on to demonstrate that a 20 bp oligonucleotide containing EmIa, in the context of the −90 CaMV 35S promoter, is sufficient for ABA-mediated induction (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Activity</th>
<th>−ABA</th>
<th>+ABA</th>
<th>RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.99 g 35S promoter, GUS/35S Y</td>
<td>2.3 ± 0.8</td>
<td>3.8 ± 0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>0.99 g</td>
<td>0.5 ± 0.3</td>
<td>8.3 ± 1.0</td>
<td>17.0</td>
</tr>
</tbody>
</table>

**ABRE-specific DNA–protein interaction**

Gene transcription is controlled by the interaction of specific proteins, transcription factors, with specific DNA sequences. The nature of these interactions determines the functional state of the promoter, i.e. induced or repressed. The modulation of gene expression by external and internal stimuli, such as environmental and developmental cues, is believed to be accomplished by alterations in these interactions. As part of an ongoing effort to dissect the components of the ABA signal transduction pathway, we have identified a protein in nuclear extracts from wheat which is capable of binding to the ABA response element [10]. This interaction is specific for the ABRE identified above, as the protein interacts with the EmIa box within the 76 bp oligonucleotide which was capable of mediating ABA induction in the transient assay. However, this same protein does not interact with the EmIb box, despite its close sequence similarity, as determined by methylation interference footprinting. In addition, competition experiments demonstrated that the 76 bp oligonucleotide containing the EmIa mutation, which was greatly attenuated for ABA induction in the transient assay, was also not an efficient target sequence for this binding activity.

A λgt11 cDNA expression library made from wheat embryo RNA was screened for the presence of fusion proteins which would interact specifically with the ABRE and two positive clones were identified (λgc12 and λgc19). Extracts from lysogens of these recombinant phage contain fusion proteins with higher molecular masses than the λgt11 control extracts, and the fusion proteins encoded by these recombinant phage have been shown to exhibit specific binding affinity for the ABRE by electrophoretic mobility shift assay (em.s.a.) and filter-binding assay [10]. Fig. 1 shows that the 76 bp oligonucleotide probe bound by lysogen extracts encoding the fusion protein is dependent on the presence of a wild-type EmIa box (compare wild type with EmIa single-mutant and EmIa/λd double-mutant probes). In contrast, the EmIb sequence does not appear to contribute to binding, either as a mutant or non-mutant site. Methylation interference experiments have established that the specificity of the fusion proteins encoded by the phage is identical to that of the protein found in wheat embryo nuclear extracts [10].

Analysis of the two positive clones revealed almost completely overlapping sequences. The deduced protein sequence contains features which have been found in a number of other transcription factors, in particular a conserved, highly basic
Plant Hormones and their Receptors

Fig. 1
DNA-binding assay: WT and mutant probes

Immobilized protein extracts (control extract, GC19 extract) were assayed for binding to wild-type (WT) and mutant probe sequences as described previously [10]. Specific nucleotide mutations are shown in lower part of Fig. Em1a, Em1b and Em1a/1b designate the box/boxes which contain mutations.

region juxtaposed with a leucine heptad repeat (leucine zipper). This class of proteins has been given the name basic-leucine zipper or bZIP, and members of this class have been described in mammals, yeasts, and plants [24]. Interaction of bZIP proteins with the DNA is mediated by the basic region, and the leucine zipper sequences are involved in the formation of protein dimers. Chromosomal mapping studies have shown that the plant sequences contained on λgc19 appear to be part of a gene family comprised of nine or two members [25]. Northern hybridization revealed only one transcript length (~1800 bases) indicating that, if these sequences represent a member of a family, all the members produce transcripts of similar length.

The partial cDNA from λgc19 was used to identify and isolate a full-length Em-binding protein cDNA (EmBP-1). Sequence analysis of this full-length clone has shown it to be similar to a number of other bZIP proteins in plants. Comparisons with the sequences for the G-box binding factor (GBF) [26], histone DNA-binding protein (HBP) [27, 28] and the common plant regulatory factor (CPRF) [29] show a very high conservation particularly in the basic region [30]. The N-terminal halves of these proteins, however, share only limited similarity, suggesting that these regions might encode functional differences. Other short, conserved regions, which are shared by several of these factors, are not necessarily common to all. As such, these significant similarities/differences may represent a basis by which to begin to group the different factors into subclasses.

The similarities between the basic DNA-binding domains of these proteins are not surprising because of the similarity in the target sequences for these proteins. All bind to a sequence which contains a CACGTGG or closely related core [26–29]. Because of these similarities, we investigated the possibility of cross-competition for binding to the EmBP-1 factor. Using 20 bp target sequences, the Em1a sequence was the most efficient competitor for the binding activity present in wheat nuclear extracts and the Em1b sequence was the least effective. While the 20 bp target sequences from the ribulose bisphosphate carboxylase and chalcone synthase promoter regions were not as efficient competitors as the Em1a sequence, all were capable of competing for binding (data not shown).
As mentioned earlier, changes in the transcriptional activity of genes are attributed to alterations in the interactions of proteins with specific target sequences on the DNA. However, the mere presence of a particular protein factor may not be sufficient for binding and thereby activation. Data presented by Datta & Cashmore [31] suggests that protein modifications, e.g. phosphorylation/dephosphorylation, may be involved in the activation of some transcription factors. To begin to address this possibility within the context of EmBP-1, we have analyzed different tissues and developmental stages to determine if the presence of the mRNA for EmBP-1 can be correlated with the presence of Em mRNA. Using specific polymerase chain reaction primers to generate products diagnostic of the presence of these two mRNAs, we have been able to demonstrate that the mRNA for EmBP-1 is present in most tissues of the wheat plant, but that this presence does not necessarily correlate with presence of the Em mRNA (data not shown). As expected, both the Em and EmBP-1 mRNAs are detectable in Stage-3 wheat embryos cultured in the presence of ABA. However, the EmBP-1 mRNA is also present in roots and leaves while the Em mRNA is not. This result is consistent with a model in which the EmBP-1 factor must be activated in some way, possibly through protein modification or interaction with accessory proteins the expression of which is developmentally or spatially regulated. One must be cautious, however, in the interpretation of these results as they address only expression at the mRNA level and not the presence of the corresponding protein. Extension of this analysis to the protein level will await the generation of antibodies specific for EmBP-1.

Conclusions
These and similar studies are identifying the molecular components involved in the transcriptional regulation of gene expression in response to a variety of signals. It is only through a thorough knowledge of these most basic cellular processes that it will be possible to address more complex developmental problems. What is beginning to emerge is the realization that general themes in regulation have been highly conserved during the evolution of divergent species of animals and plants. This conservation of structure and function within the plant kingdom is exemplified by the similarity of factors and target sequences involved in responses to such diverse signals as light, hormones and stress. The identification of components of these signal transduction pathways will eventually provide an understanding and appreciation of the mechanisms whereby a whole organism assimilates information into a co-ordinated physiological response.

The results discussed here provide evidence for the existence of an ABRE in the 5' flanking region of the Em gene from wheat. The reduction of the required sequences to a 20 bp oligonucleotide and the ability of that sequence to direct ABA-induced expression highlights the versatility of a transient expression system for the rapid analysis of sequence requirements. The identification of a nuclear protein which interacts specifically with those sequences may provide additional insight into the molecular mechanisms involved in ABA signal transduction. Having identified a central point in the ABA signal transduction pathway, it should now be possible to pursue the pathway in two directions, leading ultimately to the identification and characterization of an ABA receptor and also providing some insight into the function of the proteins produced in response to ABA.

This work was supported by grants from the U.S.D.A. Competitive Research Grants Office (90-37262-5281 to W.R.M. and 89-37262-4456 to R.S.Q.).


Expression of auxin-responsive genes in soybean and transgenic tobacco
Tom J. Guilfoyle, Gretchen Hagen, Yi Li, Melissa A. Gee, Timur N. Ulmasov and Gary Martin
Department of Biochemistry, University of Missouri, 117 Schweitzer Hall, Columbia, MO 65211, U.S.A.

Introduction
Auxins are plant hormones that are thought to control or modulate processes such as cell division, cell expansion, xylogenesis, tropisms, apical dominance and rooting. The mechanisms of action for auxins in these processes remain to be elucidated. One approach to gain insight into these mechanisms has been to determine if and how auxins modify gene expression during auxin-induced growth and developmental responses. This approach has led to the identification of a number of auxin-responsive mRNAs and the genes which encode these mRNAs [1, 2]. Here, we report on the organ-specific and tissue-specific expression of two classes of auxin-responsive mRNAs from soybean, GH3 and SAURs (small auxin-up RNAs). We also report on results with the GUS (i.e., the uidA gene of Escherichia coli which encodes β-glucuronidase) reporter gene, which has been fused to GH3 or SAUR promoters and transferred to tobacco via Agrobacterium tumefaciens T-DNA. The SAUR promoter has also been used to drive expression of the A. tumefaciens ipt (isopentenyl transferase) gene in transgenic tobacco.

Expression of GH3 and SAUR mRNAs in soybean
GH3 and SAUR cDNA clones were isolated from soybean cDNA libraries with probes prepared from mRNA of etiolated seedlings which were either untreated or treated for 1–2 h with the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D) [3, 4]. The auxin-responsive cDNA clones were selected by differential hybridization signals, and the auxin-responsiveness of the corresponding mRNAs was confirmed by RNA blot hybridization. The increases in abundance of GH3 and SAUR mRNAs following auxin application to seedlings or excised seedling organs were shown to be regulated, at least partially, at the level of transcription [5, 6]. Nuclear run-on transcription experiments in vitro showed that applied auxins induced the transcription of GH3 and SAUR genes within 5–10 min after hormone treatment [5, 6]. Increases in GH3 and SAUR mRNAs were detected by RNA blotting within 15 and 2.5 min after auxin treatment, respectively [3, 4].

Abbreviations used: SAURs, small auxin-up RNAs; 2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indole-3-acetic acid; NAA, α-naphthylene acetic acid.