Plant Molecular Biology **19**: 1–14, 1992.

Transcriptional and post-transcriptional regulation of gene expression in plants

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Key words: plant genes, transcription, RNA stability, translation, signal transduction

Introduction

In the past few years virtually every aspect of plant gene expression has been covered by thoughtful, in-depth reviews. In this article, it is not my goal to repeat, combine or update those reviews. Rather, I will attempt to give as general as possible an overview of plant gene expression. In order to reach this goal I have selected a small number of examples to illustrate what, in my opinion, are important concepts in plant gene expression.

Many concepts in plant gene expression derive from animal, yeast and bacterial systems. This interaction between plant and non-plant research has been and continues to be extremely rewarding. I will emphasize these general concepts, but at the same time highlight where plant gene expression is different or our understanding more advanced.

Plant nuclear genes are like other eukaryotic genes

Over the past years a great number of plant genes have been cloned, and if one general conclusion can be drawn from the accumulated data it is that plant genes are very much like animal or yeast genes. Plant genes use the same genetic code, are split by introns, and use regulatory mechanisms that are similar in principle. However, that is not to say that genes can be shuffled at will between plants and animals. Introns in plant nuclear genes, for instance, are removed by the same lariat-type mechanism as in animals, but the sequence requirements for splice site recognition are subtly different. Similar things can be said about plant promoters. There are many variations on the general theme but the basics remain the same.

Not too surprisingly, the first genes to be cloned were those that are highly expressed. What could one learn from such a cloned cDNA? Let us take the case of the small subunit of Rubisco, a very abundant well-characterized enzyme. Cloning provided the amino acid sequence not only of the mature protein, but also of the N-terminal transit sequence, which targets the protein to the proper compartment, the chloroplast stroma. Comparison of many such transit sequences can shed light on what makes a transit sequence specific for the chloroplast and not for the mitochondrial compartment. What is important for chloroplast targeting is apparently not so much a particular sequence but rather the overall structure, or in this case 'random coil': i.e. the absence of any structure [36].

Southern blotting and genomic cloning showed that many proteins are encoded in the plant chromosomes by multiple gene copies. In pea the small subunit of Rubisco is encoded by five genes, each with two introns [17]. In all plants analysed to date there are 4–12 gene members per genome. In pea these *rbcS* genes code for identical mature proteins, but in other organisms the corresponding proteins have small sequence variations. The *rbcS* sequence variations are minor and there is no evidence that they are correlated with differ-

ent functions of the proteins. Of course, in many instances isozymes with different functions are well-known, a good example being enzymes such as glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for which both cytoplasmic and chloroplast forms exist [10, 61, 81]. The cytoplasmic form is involved in glycolysis, whereas the chloroplast enzyme catalyses the reverse reaction in the Calvin cycle. This picture of two isozymes has been greatly refined with the aid of the cloned genes. In maize the chloroplast enzyme is a heterodimer encoded by the *gapA* and *gapB* genes. The cytoplasmic enzyme is homomeric and encoded by several *gapC* genes. In cases such as GAPDH, but also many others, the relatively straightforward standard methods of molecular biology have enabled physiologists and biochemists to obtain highly precise information not only on the gene families but more importantly on the peculiarities of the encoded isozymes. The individual enzymes can often be overexpressed in *Escherichia coli* or yeast and subsequently purified and analyzed for structure and enzymatic functions.

Thus, gene cloning methods have provided a wealth of data on proteins that would have been impossible to obtain with classical biochemical or physiological methods.

Nuclear genes are primarily regulated at the level of transcription

To understand the function of a protein it is necessary not only to characterize enzymatic activities, it is equally important to know where and when the protein is present. Let us turn again to the example of the small subunit of Rubisco [22, 26, 46, 94, 96]. The major Rubisco activity is present in green leaves and *in vitro* translation/immunoprecipitation showed a correlation between the prevalence of translatable mRNA and Rubisco protein. Subsequent studies using *rbcS* cDNA showed that mRNA levels in etiolated seedlings rise after a red light pulse. The red light effect is far-red-reversible, establishing the involvment of the photoreceptor phytochrome in the modulation of mRNA levels. So-called nuclear run-off experiments indicated that these modulations of mRNA levels arise primarily from modulations of the rate of transcription and not from post-transcriptional processes such as differential mRNA stability.

The *rbcS* genes within a given plant show minor sequence divergence. Yet the expression of individual genes varies considerably [22, 46, 96]. In petunia expression varies only quantitatively, i.e. there are highly expressed genes and lowly expressed genes, but all genes appear to be expressed in the same organs at the same relative levels and at the same time in development. In tomato there are both differences in the transcript levels *and* in the patterns of expression. Such differential gene expression can be observed in many gene families, often more dramatically than in the *rbcS* family. Another example are again the GAPDH encoding genes. The maize *gapA* and *gapB* genes which code for the chloroplastic forms are induced by light whereas *gapC* genes for cytosolic GAPDH are not light-induced. Genes for glycolytic enzymes are usually induced during anaerobiosis, because the low energy efficiency of fermentation requires an increased flux through the glycolytic pathway. It is interesting that of the cytosolic GAPDH genes only *gapC1* is anaerobically induced, whereas *gapC2* mRNA levels remain constant [61, 81].

The *cis*-acting elements

In the previous section we have seen that even closely related genes may have very different patterns of expression. What makes a gene expressed the way it is? This question can be answered by mutational analysis. In complex eukaryotes such as plants this can only be done by starting with the cloned gene, mutating it *in vitro* and returning the mutated gene to the plant. The required gene transfer techniques are described in an accompanying paper. Suffice it to say here that basically there are two approaches. One relies on the introduction of DNA into protoplasts and the assay of mRNA or protein within a few days [85]. These

systems are fast and semi-quantitative, but have the drawback that some characteristics, such as tissue specificity, cannot be scored. More recently, novel techniques such as particle bombardment [28, 70] and microinjection (G. Neuhaus, pers. comm.) have been developed to obtain transient expression in differentiated tissues. The second approach utilizes transgenic plants, plants that are identical to wild-type plants except for the fact that they have a mutated gene integrated into the genome. Transgenic plants take more time to raise and analyse but have the advantage that they allow us to study the gene in its natural environment: the intact plant [1, 46].

Mutational analyses of the type described above have defined two classes of DNA sequence important for transcription of a gene (Fig. 1). First there is the TATA box, or a functionally related sequence that binds the RNA polymerase complex and determines at what site transcription will start, about 30 bases downstream. Mutations in the TATA box interfere with proper transcription initiation. Constructs consisting of a TATA box fused to a reporter gene usually give low to undetectable transcript levels. It must be noted that very little is known about plant TATA boxes or genes without TATA boxes. This is in strong contrast with the flood of publications on the upstream elements. There is some evidence that the TATA box, or sequences in the close vicinity, may be important for the light-regulated expression of pea *rbcS* genes [47, 49, 66].

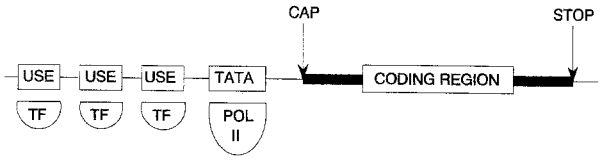

Fig. 1. Schematic view of a plant nuclear gene. The 'coding region' is the DNA sequence between the ATG translational initiation codon and the TAA, TGA or TAG translational stop codons. The coding region may be interrupted by introns, sequences that are present in the DNA and in the primary RNA transcript, but are removed by splicing and therefore absent in the mature cytoplasmic mRNA. The beginning and the end of the DNA region that is transcribted into RNA are indicated by CAP and STOP. The thick black lines represent the 5′ leader and the 3′ tail of the mRNA. Upstream of the CAP site is the TATA box, which is the binding site for RNA polymerase II and associated factors. The upstream sequence elements (USEs) can bind a variety of transcription factors (TFs). The drawing is not to scale!

The second class of DNA elements includes the binding sites for proteins that can interact with the RNA polymerase complex. Such *cis*-acting elements can function at variable distances from the TATA box. Even if their orientation is reversed they may still work. Often these elements are regulatory, i.e. they only enhance (or repress) transcription under specified cellular or environmental conditions. A classical example is the heat-shock element, which, when fused upstream of a TATA box/reporter gene, increases transcription only at high temperatures [76].

One of the best studied plant promoters is the 35S promoter. This very strong viral promoter produces the cauliflower mosaic virus (CaMV) 35S genomic RNA. In early experiments approximately 1000 bp of promoter DNA including a few basepairs beyond the transcription start site were fused to various reporter genes. Analysis in protoplasts, transformed calli and in transgenic plants demonstrated that the reporter genes were always expressed and at high levels, and were insensitive to various endogenous and environmental cues such as hormones, heat shock or light [43, 69, 72]. This so-called constitutive expression made the 35S promoter popular as a control for experiments analysing other, regulated promoters. Deletion analysis of the 35S promoter showed that the 350 bp adjacent to the TATA box were sufficient for high expression. The upstream 300 bp from about −350 to −50 (relative to the transcription start site and thus not including the TATA box) can be inverted and even placed 3′ of the reporter gene without loss of function [3]. A startling observation was made when the 35S promoter was deleted to −105 or −90: expression became organ-specific. No expression could be found in leaves or stems but in roots there were considerable levels of the CAT reporter gene mRNA and enzyme activity [79]. This finding naturally led to an important question. Is a constitutive promoter a simple promoter that contains one or more copies of a simple *cis*-

acting element that confers constitutive expression? Or is perhaps a constitutive promoter not simple at all but rather a complex array of various regulatory *cis*-acting elements, and is it the sum of all these specialized *cis*-elements that results in a constitutive, non-specialized promoter?

The latter model had proven correct for the SV40 promoter, a highly expressed constitutive animal viral promoter [71, 88]. Detailed studies on the 35S promoter have now been performed which demonstrate that subsegments of the 35S promoter confer widely varying patterns of gene expression upon the GUS reporter gene supporting the combinatorial model of promoter function (for review see [3]).

The *trans*-acting factors

The TATA box is the binding site for RNA polymerase II and its associated factors. The other *cis*-acting regulatory elements can bind a wide variety of DNA binding proteins. These proteins must interact with the RNA polymerase complex either directly, or via so-called bridging proteins which have no affinity for DNA themselves but are thought to have contact sites for both RNA polymerase and upstream DNA-binding proteins [54].

Some of the upstream binding proteins are probably general transcription factors, present in all or at least most cell types and active under most if not all conditions. Other factors may be more specialized. However, it should be kept in mind that the transcription rate and its regulation are very likely determined not just by the intrinsic properties of a transcription factor and its cognate binding site but rather by a complicated interplay of multiple factors and multiple binding sites (e.g. [26]). One factor may have different affinities to multiple sites and it may bind cooperatively. A factor may compete with another factor for a single or overlapping binding sites resulting in changed interactions with the RNA polymerase complex. Post-translational modification of transcription factors may influence all the above.

The above concepts derive mainly from experiments with bacteria, yeast and animals. However, also our knowledge of plant transcription factors is increasing rapidly. Here I review some of the information on plant transcription factors. A list of well-characterized factors is given in Table 1.

RNA polymerase and associated proteins

RNA polymerases will synthesize RNA when provided with a DNA template, Mg^{2+} ions and the four ribonucleoside triphosphates. Of the three RNA polymerases present in eukaryotes RNA polymerase II transcribes the nuclear protein-encoding genes. Plant RNA polymerase II has been isolated from a number of monocot and dicot species and displays similar subunit structure [32]. There are two large subunits with M_r 180–220 and M_r 140 and eight small subunits with M_r between 16 and 40. The largest subunit contains 35–40 tandem copies of the heptapeptide PTSPSYS at its carboxy-terminus. Similar repeats are also present in other eukaryotic RNA polymerases. Labelling with ^{32}P-phosphate indicated extensive phosphorylation probably at the threonine, serine and tyrosine residues of the heptapeptide repeat. Phosphorylation/dephosphorylation of an RNA polymerase may be important for interactions with histones or other transcription factors.

Of the accessory proteins TFIIA, B, D, E, F, known from HeLa cells, only TFIIA and TFIID have been characterized in plants. TFIIA appears to be very similar to its animal counterpart [11]. TFIID, which has some homology to bacterial sigma factor, binds to the TATA box and thus may be a key determinant of the transcription initiation site. Screening of an *Arabidopsis thaliana* cDNA library with heterologous probes revealed that *Arabidopsis* contains two distinct TFIID genes [25]. Whether these two genes code for functionally distinct proteins is an interesting speculation at present.

Table 1. Plant transcription factors.

Factor	Class	Target sequence	References
TFIIA	general	–	11
TFIID-1	general	TATA box	25
TFIID-2	general	TATA box	25
AT-1		AT-rich	16, 87
3AF-1	zinc-finger	AT-rich	49
no name		AT-rich	12
ASF-2		GATA	48
GA-1		GATA	19, 87
GC-1	Sp1-like?	GC-rich	87
GT-1		GTGG	29, 31, 87
GT-2		GTGG	18
Knotted-1	homeobox		96
Athb-1	HD-ZIP		80
Athb-2	HD-ZIP		80
HSF8	heat shock	GAAnnTTC	86
HSF24	heat shock	GAAnnTTC	86
HSF30	heat shock	GAAnnTTC	86
TGA1a + b	bZIP	TGACG	41, 42, 98
GBF		G/CACGTG	20, 87
OCSTF	bZIP	GACGTA	89a
TAF1	bZIP	ACGTG	70
O-2	bZIP		34, 55, 89
HBP-1 a + b	bZIP	GACGTG	91, 92
EmBP-1	bZIP	G/CACGTG	33
CPRF-1, 2, 3	bZIP	CACGTG	97
Deficiens	MADS		90
Agamous	MADS		14
TM3-TM8	MADS		78
AGL1-AG26	MADS		59
B1	HLH	CAGGTGC	28
Myb-like	HLH		38
Lc	HLH		56, 57
C1	HLH		27, 75
floricaula			15
viviparus-1			64

Abbreviations: HD-ZIP, homeodomain-leucine zipper; bZIP, basic domain-leucine zipper; MADS, MCM1-*Agamous-Deficiens*-SRF1 family. HLH, helix-loop-helix.

Specific DNA-binding proteins and (putative) transcription factors

The initial characterization of proteins binding to well-characterized *cis*-acting elements was made by gel retardation and footprinting assays [30]. In a gel retardation assay a labelled DNA fragment is incubated with a nuclear extract and then run on a non-denaturing gel. A protein-DNA complex will migrate slower compared to free DNA. The specificity of the interaction can be monitored by adding excess of unlabelled DNA to the binding reaction. DNA with a sequence related to the *cis*-acting element will compete for binding, unrelated DNA will not.

DNA footprinting techniques rely on the principle that proteins will protect their DNA-binding sequences from attack by nucleases or chemical agents. Footprinting can thus identify the protein-binding sites on a piece of DNA with high resolution. More recently, methods have been developed to clone the genes for DNA-binding proteins. As yet no plant transcription factor has been purified directly from nuclear extracts. However, methods have been devised to clone the genes for DNA-binding proteins.

Most successfully, radioactively labelled oligonucleotides comprising well-defined *cis*-acting elements have been used as probes to screen expression libraries. The resulting cDNAs in most cases have been shown to code for proteins with characteristics of animal and yeast transcription factors. From the accumulated data an interesting yet somewhat confusing picture is emerging. The probes used for the library screens corresponded to very diverse *cis*-acting regulatory elements. Yet many of the genes isolated so far appear to be structurally related (Table 1). Many fall into the class of the so-called bZip proteins, putative transcription factors that contain a leucine zipper dimerization motif and a basic DNA-binding domain. In particular, over the basic DNA binding domain, there is a high degree of similarity. This is unexpected since the factors were isolated using *cis*-acting elements from genes regulated by cues as different as light and abscisic acid. Close inspection of the *cis*-acting elements, however, reveals that all have a CACGTG palindromic core motif or closely related sequence. Although no exhaustive analysis has been made, in several cases it could indeed be shown that the putative transcription factors could bind to more sites than only their cognate *cis*-element. For instance, factor TAF-1 binds not only to the cognate *cis*-element in the ABA-regulated rice *rab16* gene, but also to G-box motifs found in various light regulated genes [70]. How to explain this? One possibility is that all these factors are general factors that are only indirectly involved in regulation of gene expression. Other as yet unidentified factors may interact with the general factors to bring about regulated gene expression. A second possibility is that the binding affinities *in vitro* do not reflect the *in vivo* reality. Gel retardation assays measure only binding affinities and are not necessarily a good indication of transcription rates, the biologically relevant parameter. It should be pointed out that only in a few cases has evidence been presented that these binding proteins can actually modulate transcription. TGA-1 stimulates transcription in HeLa cell and plant *in vitro* systems [42, 99] and TAF-1, when introduced into whole plants as a cDNA, can increase expression of a reporter gene carrying copies of the cognate *cis*-acting element [70].

A completely different approach has also led to the cloning of genes coding for (putative) transcription factors. Since the beginning of the century a considerable number of regulatory mutations have been described. In maize, mutants regulating anthocyanin biosynthesis or storage protein production have been well characterized. In *Antirrhinum majus*, pea and *Arabidopsis* so-called homeotic mutants are known that alter the identity of an organ. Great progress has been made recently in cloning the genes defined by these genetic defects. Virtually all of such genes seem to code for transcription factors. I take as an example the *opaque-2* mutation in maize. Maize homozygous for the *o–2* mutation has a reduced content of the 22 kDa zein storage proteins and a protein called b32, whereas the 19 kDa zeins are relatively unaffected. The lack of b32 and the 22 kDa zeins appears to result from a

lack of the corresponding mRNAs. The *o–2* mutation maps to the short arm of chromosome 7 and is unlinked to known 22 kDa zein genes. An *o–2* mutant allele caused by insertion of transposon Spm1 was cloned using the transposon as a probe [34, 55, 89]. The wild-type *o–2* gene could then be isolated from a wild-type maize library. Sequence analysis shows that the *o–2* gene encodes yet another bZIP transcription factor. Indeed, the *o–2* protein binds to *cis*-elements in the b-32 target gene and transient expression studies show that it can activate a reporter gene preceded by b-32 *cis*-acting elements [34, 55]. Therefore the *o–2* gene, genetically defined as a specific regulator of a specific subset of storage protein genes, belongs to a family of ubiquitous transcription factors. Homeotic genes from *Antirrhinum* and *Arabidopsis* defined by the *deficiens* and agamous floral mutations were cloned using similar strategies as for the maize *o–2* gene. The deduced proteins have sequence homology over the DNA-binding domain to yeast and human transcription factors. They are now collectively named MADS box proteins [14, 91]. The *def* and *agamous* proteins seem to be very precise regulators of steps in the pathway of floral development, although it is not known yet what their target genes are. On the other hand, it has been found in *Arabidopsis* and tomato that the MADS box genes are members of multigene families, some of which appear to be expressed ubiquitously [78, 59].

In summary, a growing number of transcription factors are being characterized. Many are structurally related. Detailed knowledge about their *in vitro* binding specificity is accumulating. The challenge is now to understand how these factors bring about the very diverse and very precise regulation of target genes.

Table 1 gives an overview of cloned or at least well-characterized (putative) plant transcription factors. Clearly many of these factors are structurally related and bind to very similar DNA sequences.

Cis-acting elements for post-transcriptional regulation?

The majority of the *cis*-acting elements have been found in the 5′ upstream regions of plant genes. In most cases it has been proven, or at least assumed, that these elements are involved in the modulation of transcription rates. However, it must also be pointed out that in most cases a search was made exclusively for such upstream transcriptional elements.

Possible *cis*-acting elements downstream of the TATA box, modulating either transcription or post-transcriptional processes are often not considered in experimental designs and could easily be overlooked. *Cis*-acting elements in 'unusual' places have been described in several genes. The first intron of the maize ADH gene is required for high transcript levels, a phenomenon that is not clearly defined as purely transcriptional [58]. The ABA-responsive *Em* gene from wheat has an upstream regulatory element that mediates ABA responsiveness. Then there is a second element encompassing the 5′ non-translated leader that enhances reporter gene expression 10-fold [37, 60]. It is easy to imagine that this second element does not function at the DNA level, but rather influences stability or translation of the mRNA. In the pea gene coding for ferredoxin the only light-regulatory elements encountered are in the protein coding region. Again, although effects on transcription cannot be ruled out, a role in mRNA stability may be more likely [21, 95].

Research focused on mRNA stability determinants has only just begun. Detailed information on the *cis*-acting RNA sequences and the proteins that interact with them should become available in the near future.

The formation of 3′ ends of mRNAs appears to be different between plant and mammalian genes. The conserved hexanucleotide AATAAA found in most mammalian genes 10–30 basepairs before the 3′ end is absent in many plant genes. The requirement for such a site may be less stringent. No sequences downstream of the polyadenylation site appear to be necessary, but further upstream elements have been found [65, 83]. An

interesting problem is posed by the termination and polyadenylation of the CaMV 35S RNA [84]. This RNA is transcribed from the circular CaMV DNA genome as a terminally redundant RNA, i.e. transcription goes all around the circle, passes the transcription start site and stops some 200 nucleotides beyond. These last 200 bp are sufficient for correct termination of reporter gene constructs and the question is why transcription does not terminate during the first passage over the termination site. With a number of constructs having increasing length of DNA between transcription start and termination sites it could be shown that a minimal distance between the two is required for proper termination.

Translational regulation can be important too

An example of very well documented translational regulation of nuclear gene expression is provided by the *Amaranthus rbcS* genes [5, 6]. The light-responsive expression of the *rbcS* genes is one of the paradigms of transcriptional regulation in plants. Studies by Klessig and coworkers make it clear that there exists a second tier of regulation. With *Amaranthus* seedlings these authors could show that after transfer from light to dark, mRNA levels for both *rbcS* and the chloroplast-encoded *rbcL* subunits remain unchanged for at least 6 h. On the other hand, incorporation of ^{35}S-methionine in the encoded proteins ceases completely within 2 h. Subsequent experiments demonstrated that the mRNA remains bound to polysomes, implicating regulation at the level of translation elongation. Based on the animal literature a possible involvement of elongation factor EF-2 can be surmised [82]. In contrast, when seedlings were transferred from dark to light, recruitment of *rbcS* mRNA into polysomes was observed, indicating regulation at the translation initiation step. Thus one of the workhorses for transcription studies is also extremely useful for research on translation.

In *Volvox* cultures synchronized by a light-dark cycle, the major events in the juvenile-to-adult-transition are light-dependent. The effect of light is not exerted at the transcriptional but at the translational level [44].

The promoter for the CaMV 35S RNA has been studied by several groups in great depth and with exciting results (see before). The translation of the proteins encoded by this mRNA is at least as interesting. Translation of the genome-size RNA is thought to give rise to at least five proteins. The existence of such polycistronic mRNAs in eukaryotes has been in doubt for many years and only relatively recently was it shown unambiguously for poliovirus RNA that downstream open reading frames can be translated through internal initiation [77]. A number of dicistronic reporter gene constructs were prepared and transient expression in host protoplasts measured. The conclusion from these experiments was that always only the first open reading frame in a dicistronic construct is translated. Expression of downstream cistrons, however, could be observed when the viral ORF VI gene was co-transfected. Effects on splicing, nuclear-cytoplasmic transport or mRNA stability could be ruled out. Thus the ORF VI gene product acts as a trans-activator to stimulate translation from downstream open reading frames in polycistronic mRNAs [7].

The efficiency of translation of ORF VII, the first gene in the 35S mRNA, is severely impaired by sequences in the 600 nt leader sequence preceding ORF VII. Within these 600 nt, mutational analysis identified a mosaic of inhibitory and stimulatory elements. None of the mutations influenced steady state mRNA levels to any great extent and thus, the effects again, must be at the level of translation [23]. Translational enhancer sequences have been described for the 5′ leaders of several plant viruses [24, 40].

In all branches of molecular biology the interest in the mechanisms of translation declined dramatically in the 1980s. This may be due to the fact that most often regulation of gene expression is at the level of transcription. Translational regulation of the GCN4 in yeast and of the ferritin gene in animal cells are two of the rare genes in which gene-specific translational regulation has been demonstrated and studied in great detail [67, 13]. And in these two genes the traditional translation

initiation and elongation factors do not seem to be centrally involved. Yet, translation initiation factors are likely to play crucial roles in cellular responses. For instance, the gene for translation initiation factor eIF-4E, the cap-binding protein, has recently been shown to act as an oncogene when overexpressed in mammalian cells [52]. Translation initiation factor eIF-4A, a putative RNA helicase, is thought to unwind secondary RNA structure in the 5′ leader of mRNAs to enable the scanning ribosome to reach the initiator AUG. Injection of purified eIF-4A into *Xenopus* oocytes can activate dormant mRNAs [2].

Plant translation initiation factors have been fairly well-characterized from wheat germ extracts [39, 51]. They are very much like the factors in rabbit reticulocytes. This is not surprising since wheat germ and rabbit reticulocyte cell-free extracts are both standard systems for *in vitro* translation of mRNAs and the differences between the two systems are minor. We have recently isolated genes for plant eIF-4A and found a multigene family of highly divergent genes [73]. This is in contrast to yeast and mouse where duplicate genes code for identical or highly similar proteins, respectively. The divergence of the plant eIF-4A genes suggests that they may have dissimilar functions, for example they could perhaps translate various mRNAs with different efficiencies.

Signal transduction

Molecular-biological experiments have provided ample evidence that internal and external signals can modulate the expression of specific genes. A major question remaining pertains to the intermediary steps. In the case of light: how does light succeed in turning transcription on or off? The first step is relatively well defined, at least for red/far-red reversible reactions. The light is perceived by the photoreceptor phytochrome. A large body of data documents how red light can change the physical conformation of the $M_r = 120000$ chromoprotein [94]. Far-red light can reverse this conformational change. But, what comes afterwards? The idea that the activated photoreceptor could bind directly to *cis*-acting regulatory elements – as is the case in glucocorticoid-induced gene expression in mammalian systems – has been abandoned. Thus, there must be intermediary steps to transduce the signal from the activated photoreceptor to the transcriptional apparatus. Research into the nature of these intermediaries has so far mostly followed along the lines set out for non-plant systems. Evidence implicating protein kinases, Ca^{2+} and calmodulin, G-proteins, phosphoinositides has been obtained in various systems. A good example is again the phytochrome mediated response. The approach usually taken is to find compounds that can interfere with the signal transduction chain with the goal of eliciting the response in the absence of the natural stimulus. Clearly, a multicellular plant is less suitable as an experimental system and single cell systems have been sought that retain phytochrome responsiveness. Wheat protoplasts respond to red light treatment by increasing in volume and this red-light-induced swelling is far-red-reversible. The red-induced swelling requires Ca^{2+}, and importantly, swelling can occur in the dark when the protoplasts are incubated in the presence of Ca^{2+} and Ca^{2+} ionophores. The results are interpreted to mean that phytochrome induces the opening of Ca^{2+} channels in the plasma membrane. Subsequent experiments with phorbol esters and GTP/GDP analogues indicate the involvement of a membrane-bound GTP-binding protein [89]. Evidently, it will be interesting to compare these results with results obtained in other phytochrome-mediated systems.

Signal transduction during development: the events upstream and downstream

The signal pathway leading to the activation of light-regulated genes is likely to involve more than just a linear amplification of the signal. Some light-regulated genes are turned on faster or at lower fluence rates than others; some are turned off by light. Also the light pathway must interact with other pathways that determine cell specific-

ity or hormone responsiveness. To understand how signals cross-react and network to induce highly specific patterns of gene expressions is a challenge for the future.

In the case of light regulation we know very well at least what is at the end of the signal transduction chain. The *rbcS* gene is transcribed, the transcript is translated, the protein transported into the chloroplast and combined with the chloroplastic *rbcL* gene product to form the Rubisco enzyme. Finally, there is a wealth of data about the enzymatic activities of the protein. Thus the steps after transcription initiation are known in detail.

What about developmental pathways? In some cases we know, or think we know, the signals and we may have some ideas about how they modulate gene expression. Nodule formation on leguminous roots can be initiated by an oligosaccharide secreted by the infecting *Rhizobium* [53; T. Bisseling's paper elsewhere in this volume]. An early signal in flower development is florigen, which is not so well-characterized but appears to be produced by leaves and transported to the vegetative shoot apex where it is thought to initiate the floral transition [4]. At the end of the signal transduction chain is the flower, a complex structure, very distinct from the vegetative organs. Between florigen and flower must be many steps of which we know only two: the homeotic genes and the flower-specific genes.

Mutations in homeotic genes drastically alter the identity of organs. Thus in the *Antirrhinum majus deficiens* mutant petals are changed into sepals and carpels form instead of stamens [14]. Best known are the homeotic mutants that alter flower development. However, homeotic mutations in vegetative organs have also been described [62]. The *deficiens* gene and several other floral homeotic genes have been cloned and sequenced and been shown to have strong homology to known transcription factors, in particular over the DNA-binding domains [14]. Homeotic genes that determine organ identity are well known from *Drosophila* and many of them also code for transcription factors. In flies, genes have been described that act even earlier and specify the body plan [68]. In the context of this review it is interesting that one of these very early genes codes not for a transcription factor but for a putative RNA helicase and thus may act at the post-transcriptional level [35, 50]. Very early developmental mutations have recently also been described in *Arabidopsis*, and their characterization at the molecular level is eagerly awaited [63].

It is attractive to think of homeotic genes as central switches, reacting to positional, developmental and environmental cues, and determining a cascade of subsequent events, finally leading to organ formation.

The signal transduction pathways that turn the central switches (i.e. lead to expression of homeotic genes) are not known in detail. The chemical nature of florigen remains elusive despite intensive efforts. On the other hand, what is the result of the expression of the homeotic genes? Since most appear to code for transcription factors it is reasonable to assume that they will activate target genes downstream in the pathway. Genes that are expressed only in petals, in stamens, in the tapetum layer of the stamen etcetera have been isolated and their spatial and temporal expression determined in great detail [45]. The question to be answered in the near future is how the *cis*-acting elements of these target genes interact with the homeotic-type transcription factors.

Conclusion

Ten years ago a small number of plant genes had been cloned and sequenced. Today not only have more genes been sequenced, we have also learned a great deal about how they are expressed. Small *cis*-acting elements have been delineated, mostly in the upstream sequences, that can confer correct regulation upon reporter genes. More recently genes have been isolated coding for proteins that bind to these *cis*-acting elements. A major object of research in the near future will be to understand how the often ubiquitous transcription factors cooperate with one another, with as yet undiscovered factors, and with the *cis*-acting

elements, to bring about the finely tuned regulation of individual genes. In summary, the molecular cloning of plant genes has allowed for an unprecedented level of detail in the characterization of the gene products. We are beginning to understand how genes are regulated.

Acknowledgements

Numerous colleagues contributed to this review by making (p)reprints available. Drs Urs Feller, Andrew Fleming, Susan Flores and Gunther Neuhaus were very helpful by critically reading the manuscript. I am extremely grateful to Ms L. Häusermann, M. Zeder and R. Hintermann for their expert secretarial assistance and for their patience.

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