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Update on Signal Transduction

Early Genes and Auxin Action[1]

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The plant hormone IAA (or auxin) is central to the control of plant growth and development. Processes governed by auxin in concert with other plant growth regulators include development of vascular tissues, formation of lateral and adventitious roots, control of apical dominance, and tropic responses (Went and Thimann, 1937). At the level of cellular physiology, auxin profoundly affects turgor, elongation, division, and cell differentiation, the major driving and shaping forces in morphogenesis and oncogenesis. The molecular mechanisms of auxin action are still unknown, although it is now well established that auxin modulates membrane function and gene expression (for review, see Napier and Venis, 1995). These biochemical changes, in turn, most likely affect fundamental aspects of plant morphology and physiology. However, a causal relationship between auxin-mediated alterations in gene expression or membrane function and a particular growth process has not yet been demonstrated. Despite its critical role in plant development and the immense volume of studies on the diverse auxin effects, understanding of the molecular mechanisms of auxin action remains one of the major challenges in plant biology.

The signal transduction cascades leading from auxin perception to altered gene expression or membrane function hold the key in our attempts to elucidate the primary mechanism(s) of auxin action. An array of experimental strategies has been mounted to investigate auxin signaling pathways. The combination of biochemical, molecular, and genetic approaches will allow for significant new insights into how the hormone works in molecular terms (Fig. 1). One strategy employs genetics and reverse genetics to construct transgenic plants with perturbations in auxin homeostasis and to screen for mutants with defects in auxin-related physiology. Transgenic plants expressing altered hormone levels have already resolved some longstanding questions in plant physiology. Mutant plants defective in auxin responses will rejuvenate and stimulate research by identifying novel genes involved in hormone perception, signal transduction, and physiological responses (for review, see Hobbie and Estelle, 1994; Klee and Romano, 1994). The first significant result (to our knowledge) of this approach was the cloning of the *AXR1* gene, which encodes a protein related to the ubiquitin-activating enzyme E1 (Leyser et al., 1993). Although AXR1 is probably not a functional E1 homolog, it is nonetheless an exquisite example of the potential of molecular genetics to connect the unexpected. The biochemical strategy is based on the identification of auxin receptors and subsequent isolation of interacting components. The search for auxin receptors has led to the discovery of a number of soluble and membrane-bound proteins that bind auxin with moderate but physiologically relevant affinity. Their functional role in auxin signaling is still unclear and is a major target of current research (for review, see Jones, 1994; Napier and Venis, 1995). Auxin-regulated genes provide yet another source of molecular tools to dissect auxin action. The hormone modulates gene expression in a wide variety of plant tissues and cell types over a broad period of time (for review, see Guilfoyle, 1986; Theologis, 1986). However, early genes selectively induced as a primary response to auxin and prior to the initiation of cell growth are likely candidates to play a pivotal role in mediating growth-stimulating effects of the hormone. This review focuses on recent advances in our knowledge on early auxin-inducible gene expression and possible functions of the polypeptides encoded.

THE POWER OF EARLY GENES

Early genes or primary-response genes—the latter term is based on a mechanistic definition—have played a decisive role in understanding how growth factors and tumor promoters act in animal cells (for review, see Herschman, 1991). The induction of primary-response genes is independent of de novo protein synthesis. This implies that the components required for their transcriptional activation are preexisting and that the extracellular stimulus is transduced to the nucleus via posttranslational processes. Consequently, the number of intervening steps is limited and the time elapsing between ligand perception and gene activation is short, usually in the range of minutes to a few hours. Thus, stimulus-responsive *cis*-elements of early genes provide a platform to explore signal transduction pathways in reverse. This concept is viable and has been successfully employed to trace back the short and direct path by which interferon signals are transmitted from the

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Abbreviations: AuxRE, auxin-responsive element; CHX, cycloheximide; *dgt*, diagetropica; GST, glutathione *S*-transferase; *ocs*, octopine synthase; SAUR, small auxin up RNA.

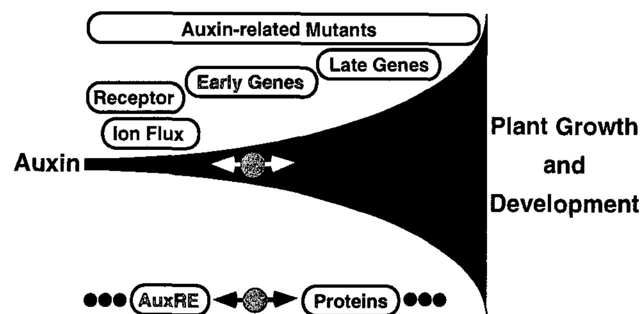

Figure 1. Molecular approaches for the dissection of auxin signal transduction cascades.

plasma membrane to the nuclear genome (Darnell et al., 1994).

On the other hand, primary gene products likely play a regulatory role in late stimulus-induced cellular events, in particular when expressed as a specific response. The serum-elicited mitogenic response of quiescent cells was one of the first ligand-induced phenotypic responses studied in molecular terms (Herschman, 1991). Insofar as they have been identified, primary gene products induced in response to serum or growth factors serve mainly three functions: emergency rescue and stress adaptation, intercellular communication, and transcriptional regulation of secondary genes to establish and coordinate long-term biological consequences. Consistent with their limited and regulatory function, expression of primary gene products is often stringently regulated at multiple posttranscriptional levels to allow for rapid termination of the response and resetting of the inducible system (for review, see Hill and Treisman, 1995). Taken together, early genes establish a bridgehead and offer bidirectional molecular probes to explore upstream and downstream areas in the *terra incognita* of signal transduction pathways (Fig. 1).

EXPERIMENTAL SYSTEMS

Based on the physiological effects of auxin, several experimental systems have been established to isolate early-auxin-responsive genes. Auxin-mediated cell expansion is one of the fastest and phenotypically simplest hormonal responses known in plants, with a lag period of 10 to 25 min (for review, see Brummell amd Hall, 1987). Using differential cDNA screening strategies, etiolated legume seedlings have been the preferred tissues with which to search for early-auxin-inducible mRNAs that are induced prior to or concomitant with the onset of auxin-stimulated cell elongation (for review, see Key, 1989). The same technique has been applied to isolate early-auxin-responsive genes from proliferating cells. The initiation and execution of cell division is absolutely dependent on auxin; however, these processes are a long-term response in the range of several hours. To identify early genes in dividing cells, tobacco cell-suspension cultures have been established as well with conditions necessary to cultivate tobacco mesophyll protoplasts that are able to form calli at high frequency (for review, see Takahashi et al., 1995). Xylogenesis in cultured leaf mesophyll cells from *Zinnia elegans* is an excellent system in which to study auxin-dependent cell differentiation process. Isolated *Zinnia* mesophyll cells differentiate semisynchronously into tracheary elements within 3 d (Fukuda, 1992). A number of cDNAs have been isolated whose corresponding genes are expressed in close association with tracheary element formation (Ye and Varner, 1992); yet differential screening procedures to identify early-response genes have not been carried out.

PRIMARY GENES RESPONSIVE TO AUXIN

Using elongating tissues or dividing cells as the starting plant material, several classes of early-auxin-reponsive genes have been identified. These classes constitute multigene families (Table I). Cloning-by-homology approaches continue to identify new family members and shift the attention to *Arabidopsis thaliana*, which is an appropriate organism in which to study gene function by reverse genetics, and in which most progress is being made with respect to the isolation and molecular characterization of auxin-related mutants.

The first auxin-inducible genes to be identified, *Aux22* and *Aux28* from soybean (Ainley et al., 1988), are members of a large superfamily, hereafter collectively referred to as the *Aux/IAA* gene family. This class comprises related genes *GH1* from soybean (Guilfoyle et al., 1993), *PS-IAA4/5* and *PS-IAA6* from pea (Theologis et al., 1985), *ARG3* and *ARG4* from mung bean (Yamamoto et al., 1992), and at least 14 expressed genes from Arabidopsis, *IAA1* to *IAA14*, which include *AtAux2-11* (*IAA4*) and *AtAux2-27* (*IAA5*) (Conner et al., 1990; Abel et al., 1995b). In a specific response to auxin, steady-state mRNA levels of most *Aux/IAA* genes rapidly increase within 5 to 60 min. This response does not require de novo protein synthesis, which indicates direct gene activation. It is interesting that auxin-mediated expression of two late *IAA* genes from Arabidopsis, *IAA7* and *IAA8*, is dependent on protein synthesis and, therefore, may be considered as a secondary response to the hormone (Table I). *Aux/IAA* genes contain two to four introns at conserved positions and encode small hydrophilic polypeptides with molecular masses of 19 to 36 kD (Oeller et al., 1993). Although diverse (35–85% sequence identity), all deduced protein sequences contain four conserved domains and seven invariant amino acid residues in variable regions (Ainley et al., 1988; Abel et al., 1995b).

The most rewarding screen for early-auxin-inducible transcripts was carried out by Tom Guilfoyle's group. Members of four unrelated classes were identified in elongating soybean tissues: *GH1* (Guilfoyle et al., 1993), *GH3* (Hagen et al., 1991), *GH2/4* (Hagen et al., 1988), and *SAURs* (McClure et al., 1989). Since then, members of the *SAUR* gene family have been cloned from mung bean, pea, and Arabidopsis (Yamamoto et al., 1992; Guilfoyle et al., 1993; Gil et al., 1994). Expression of *SAUR* mRNAs is specifically induced by auxins within 2 to 5 min, and this response is insensitive to CHX (Table I). The five *SAUR* genes of soybean are closely clustered, do not contain introns, and give rise to small (0.5 kb) transcripts that encode highly similar polypeptides of 9 to 10 kD. The *GH3* gene is part of a small multigene family in soybean (Hagen et al., 1991), and re-

Table I. *Early auxin-responsive genes*

Gene	Plant Species	Response within[a]	Sensitivity to CHX	Other Inducers[b]	Reference[c]
		min			
Aux/IAA gene family					
Aux22	*Glycine*	15	n.d.[d]	n.d.	Ainley et al. (1988)
Aux28	*Glycine*	30	n.d.	n.d.	Ainley et al. (1988)
AtAux2-11 (*IAA4*)	Arabidopsis	30	n.d.	n.d.	Conner et al. (1990)
AtAux2-27 (*IAA5*)	Arabidopsis	90	n.d.	n.d.	Conner et al. (1990)
ARG3	*Vignia*	20	n.d.	CHX	Yamamoto et al. (1992)
ARG4	*Vignia*	20	n.d.	CHX	Yamamoto (1994)
GH1	*Glycine*	15	Insensitive	–	Guilfoyle et al. (1993)
PS-IAA4/5	*Pisum*	5	Insensitive	CHX	Oeller et al. (1993)
PS-IAA6	*Pisum*	8	Insensitive	CHX	Oeller et al. (1993)
IAA1–IAA6	Arabidopsis	5–25	Insensitive	CHX	Abel et al. (1995b)
IAA7, IAA8	Arabidopsis	60–120	Sensitive	–	Abel et al. (1995b)
IAA9–IAA14	Arabidopsis	15–60	Insensitive	CHX	Abel et al. (1995b)
SAUR gene family					
SAURs	*Glycine*	3–5	Insensitive	CHX	McClure et al. (1989)
ARG7	*Vignia*	5	n.d.	CHX	Yamamoto (1994)
SAUR-AC1	Arabidopsis	n.d.	Insensitive	CHX	Gil et al. (1994)
GH3 gene family					
GH3	*Glycine*	5	Insensitive	–	Hagen et al. (1991)
Genes encoding GST-like proteins[e]					
GH2/4 (*Gmhsp26-A*)	*Glycine*	15	Insensitive	CHX, Cd^{2+}	Hagen et al. (1988)
parA	*Nicotiana*	20	Insensitive	CHX, Cd^{2+}	Takahashi et al. (1995)
parB	*Nicotiana*	20	n.d.	n.d.	Takahashi and Nagata (1992a)
parC	*Nicotiana*	10	Insensitive	CHX	Takahashi and Nagata (1992b)
pCNT103	*Nicotiana*	15	Insensitive	CHX, SA	Van der Zaal et al. (1987)
pCNT107 (*parC*)	*Nicotiana*	15	Insensitive	ABA, SA	Van der Zaal et al. (1987)
pCNT114 (*parA*)	*Nicotiana*	30	Insensitive	Cu^{2+}, SA	Van der Zaal et al. (1987)
Genes encoding ACC synthase					
ACS4	Arabidopsis	25	Insensitive	CHX	Abel et al. (1995a)
CM-ACS2	*Cucurbita*	20	n.d.	n.d.	Nakagawa et al. (1991)
OS-ACS1	*Oryza*	n.d.	Insensitive	CHX, anaerobiosis	Zarembinski and Theologis (1993)
Miscellaneous genes					
ARG1	*Vignia*	20	n.d.		Yamamoto et al. (1992)
ARG2	*Vignia*	20	n.d.	Heat shock	Yamamoto (1994)
arcA	*Nicotiana*	60	n.d.	n.d.	Ishida et al. (1993)

[a] As detected by in vitro nuclear runoff transcription or by changes of steady-state mRNA levels. [b] A dash indicates that none of the agents tested other than active auxins are able to elicit a response. [c] The reference given does not necessarily coincide with the original publication. [d] n.d., Not determined. [e] For more genes encoding GST-like proteins, see Takahashi et al. (1995).

lated genes exist in Arabidopsis (Guilfoyle et al., 1993). *GH3*-like genes are composed of three exons that encode a protein of approximately 70 kD. Induction by auxin is rapid and qualifies as a primary response (Table I).

The soybean *GH2/4* gene is a member of a large and divergent superfamily encoding proteins with GST activity (Takahashi and Nagata, 1992a; Droog et al., 1993; Guilfoyle et al., 1993). Although *GH2/4*-like transcripts rapidly increase in abundance after auxin application, even in conditions of protein synthesis inhibition induction of these genes is not specific for the hormone. *GH2/4*-like genes are often induced by a wide variety of agents and environmental cues, including nonauxin analogs, other plant hormones, heavy metals, and various stress conditions (Table I).

Three novel, auxin-regulated transcripts have recently been isolated: *ARG1* and *ARG2* from elongating hypocotyls of mung bean and *arcA* from the tobacco BY-2 cell line. *ARG1* has high similarity to *fad3*, a gene encoding fatty acid desaturase in Arabidopsis (Yamamoto, 1994), and *arcA* is a member of the WD-40 repeat family (Ishida et al., 1993). Another class of primary auxin-response genes has been identified in studies to determine the expression profiles of multigene families encoding ACC synthase in various plant species. Those analyses revealed that certain *ACS* gene family members are induced by auxin in a primary fashion (Table I; for review, see Zarembinski and Theologis, 1994).

CONTROL OF PRIMARY GENE EXPRESSION

Auxin enhances the abundance of a selective and conserved set of mRNAs in various plant species (Table I). The individual responses are rapid and in many instances hormone specific and sensitive to physiologically relevant auxin concentrations (10^{-8} to 10^{-6} M). Intricate control of expression is a hallmark of genes encoding regulatory polypeptides. Regulation of gene expression at different

levels has been most thoroughly examined for members of the *SAUR* and *Aux/IAA* families, and those studies suggest an important function in auxin action of the proteins encoded.

Transcriptional Activation

Early-auxin-reponsive genes are rapidly activated at the transcriptional level (Hagen and Guilfoyle, 1985; McClure et al., 1989; Koshiba et al., 1995). Protein synthesis inhibitors have traditionally been used to unmask regulatory mechanisms of early gene activation (Hill and Treisman, 1995). Induction of most early-auxin-responsive genes is independent of de novo protein synthesis, suggesting that the hormone signal is transmitted to the nucleus via preexisting components. It is interesting that protein synthesis inhibitors such as CHX are the only other known inducers of *SAUR* and *Aux/IAA* genes (Table I). Gene activation by inhibition of protein synthesis is a common characteristic of various mammalian oncogenes. In many early-inducible systems, including *SAUR* and *Aux/IAA* genes, the concomitant presence of CHX and the inducer results in superinduction of the mRNAs (Herschman, 1991). Koshiba et al. (1995) studied the effect of protein synthesis inhibition on *PS-IAA4/5* and *PS-IAA6* expression in more detail. Their experiments indicate a dual effect of the inhibitor CHX: activation of transcription and stabilization of the inducible mRNAs. A popular interpretation of the first effect is that CHX prevents synthesis or activation of a short-lived transcriptional repressor whose subsequent physical or functional loss results in gene activation (Koshiba et al., 1995). Such eukaryotic transcriptional repressors have been described (Herschbach and Johnson, 1993). The second effect has been explained by disruption of translation-coupled mRNA degradation or by depleting the pool of a labile enzyme that degrades specific transcripts (Koshiba et al., 1995).

The simplest view of how auxin activates transcription is the following: the small and amphiphilic IAA molecule enters the cell by an active transport mechanism and rapidly diffuses into the nucleus, where it is sensed by a protein that either directly or indirectly interacts with an *AuxRE* (Ballas et al., 1993; Koshiba et al., 1995). Alternatively, auxin is perceived by a receptor located in the plasma membrane (Jones, 1994). This interaction activates an intracellular signal transduction pathway, e.g. a protein kinase cascade, or may generate second messengers, such as Ca^{2+} or inositol triphosphate, which activate transcription factors involved in auxin-regulated gene expression (Koshiba et al., 1995). Finally, it has been suggested that the differential length of the lag periods of primary gene induction reflects differences in the biochemical properties of the components of the signaling apparatus responsible for their transcriptional activation and processing of their transcripts (Koshiba et al., 1995).

Posttranscriptional Regulation

Expression of *SAUR* and *Aux/IAA* genes is tightly regulated at the posttranscriptional level. The mRNA half-lives for members of both classes have been determined and auxin does not significantly alter their stability. The turnover rates of *SAUR* mRNAs (40–50 min; Franco et al., 1990) and of *PS-IAA4/5* and *PS-IAA6* transcripts (60–75 min; Koshiba et al., 1995) are comparable to those of many unstable mRNAs of mammalian early-response genes. A high mRNA turnover has already been indicated in many of the original reports, where control incubations of tissues in the absence of exogenous auxin caused a pronounced decrease in transcript abundance. Similarly, transient accumulation of *IAA3* and *IAA6* transcripts in response to auxin is consistent with a short mRNA half-life (Abel et al., 1995b). In addition to mRNA instability, *Aux/IAA* gene expression is attenuated at the level of protein availability. The PS-IAA4 and PS-IAA6 proteins have extremely low abundance in vivo, representing no more than 0.0001% of the total protein in pea epicotyl tissue (Oeller and Theologis, 1995). The low abundance of both polypeptides has been attributed to their short metabolic half-lives (6–8 min), which are among the shortest known for eukaryotic proteins and are characteristic of regulatory proteins (Abel et al., 1994). Furthermore, a study of the induction kinetics of PS-IAA4 and PS-IAA6 indicates that expression of both proteins is likely under translational control. Although their coding mRNAs are highly abundant, both proteins are only transiently synthesized (Oeller and Theologis, 1995).

Developmental Control

Expression during plant development has been studied in some detail for *GH3*, *SAUR* genes, and members of the *Aux/IAA* gene family (Gee et al., 1991; Hagen et al., 1991; Wyatt et al., 1993; Wong et al., 1996). These studies reveal spatio-temporal patterns of expression that largely correlate with cells, tissues, and developmental processes known to be affected by auxin. Expression of primary auxin genes is confined to a few cell and tissue types at specific developmental stages. Those expression patterns are not restricted to elongating cells but include dividing cells or cells with the potential to divide as well as highly specialized cell types. This suggests multiple functions of early auxin genes during plant development. A comparative study of *GH3* and *SAUR* gene expression in soybean demonstrates both unique and common patterns of tissue-specific gene activation. Gee et al. (1991) inferred from these observations the existence of multiple auxin receptors and/or auxin signal transduction pathways. This assumption is corroborated by the study of Wong et al. (1996), which indicates discrete and yet common spatio-temporal expression patterns of *PS-IAA4/5* and *PS-IAA6*, members of the *Aux/IAA* gene family (Fig. 2). We anticipate, therefore, a complexity of partially overlapping expression patterns of the various members of *Aux/IAA* multigene families.

Defective Expression in Auxin-Related Mutants

In an effort to elucidate mechanisms of auxin signal transduction, a number of mutants have been isolated with physiologically well-characterized defects in auxin re-

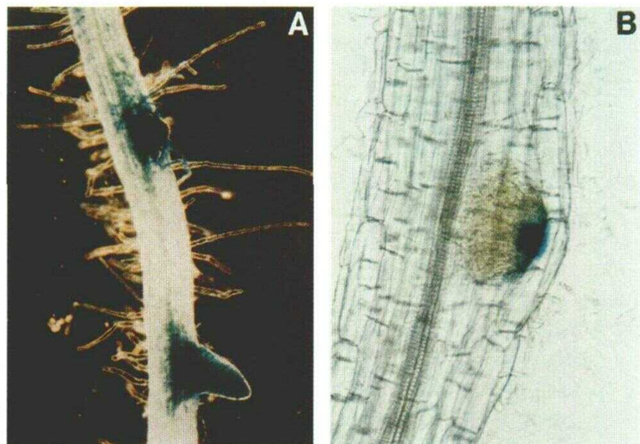

Figure 2. Overlapping expression pattern of *PS-IAA4/5-GUS* (A) and *PS-IAA6-GUS* (B) during lateral root development of transgenic tobacco seedlings.

sponses. Early-auxin-inducible genes provide probes suitable to extend those analyses at the molecular level. The various genetic lesions may discriminate between auxin-responsive gene families or family members and thereby illuminate the nature of the signal transduction pathways involved. The expression of *SAUR-AC1*, all *IAA* genes, and *ACS4* is similarly inhibited in Arabidopsis auxin-resistant mutants *axr1*, *axr2*, and to a less-severe extent in *aux1* (Gil et al., 1994; Abel et al., 1995a, 1995b). These mutants have a pleiotropic but auxin-related phenotype with severe defects in apical dominance and gravitropism (Hobbie and Estelle, 1994). The *axr1* phenotype has been explained by a reduced sensitivity to auxin (Hobbie and Estelle, 1994), which is supported by dramatically reduced levels of the early-auxin-responsive mRNAs tested, although auxin inducibility is maintained (Abel et al., 1995a, 1995b). As implicated by the *AXR1* gene product, the possibility exists that ubiquitin-dependent proteolysis plays an important role in rapid auxin responses. For instance, AXR1 may control the level but not the auxin sensitivity of the short-lived repressor postulated to regulate *PS-IAA4/5* gene expression (Koshiba et al., 1995). A coordinate inhibition of several classes of primary auxin-response genes supports the previous notion that either mutation acts far upstream in an auxin-response pathway and probably affects a general component in hormone signaling (Hobbie and Estelle, 1994).

A similar analysis has been performed in the *dgt* mutant of tomato, which is auxin insensitive and defective in some responses to the hormone, such as auxin-induced ethylene production. Partial tomato cDNA sequences related to *Aux/IAA*, *SAUR*, and *GH2/4*-like transcripts were cloned and used to characterize their expression. It is interesting that auxin-inducible expression of the *SAUR* and *Aux/IAA* tomato homolog but not of the *GH2/4*-like gene is significantly inhibited in the *dgt* mutant (Mito and Bennett, 1995). Likewise, expression of all auxin-inducible *ACS* genes of tomato is also impaired in the *dgt* background (K. Kawakita and A. Theologis, unpublished results). This suggests the function of a common component in auxin signal transduction pathways for various auxin-specific primary genes. A different, probably stress-related pathway regulates expression of *GH2/4*-like genes (Fig. 3).

AUXIN-RESPONSIVE *CIS*-ELEMENTS

Comparisons of promoter sequences of early auxin-inducible genes have identified various conserved regions that have been proposed as candidates for *AuxREs* (for review, see Guilfoyle et al., 1993; Napier and Venis, 1995). Here attention will be given to *cis*-elements that have been experimentally identified in tests for auxin inducibility, since only those elements provide the specific tools to probe auxin signaling in reverse. The first element shown to be functionally active is the 164-bp auxin-responsive region of the *PS-IAA4/5* gene (Ballas et al., 1993). Further loss-of-function analysis by linker-scanning mutagenesis divided this region into two auxin-responsive domains, domain A (48 bp) and domain B (44 bp), which, in gain-of-function tests, could confer auxin inducibility to minimal promoters (Ballas et al., 1995). Domain A acts like an auxin-dependent switch, whereas domain B has the features of an enhancer element. Both domains function cooperatively to stimulate transcription and, as implicated by in vivo competition experiments, interact specifically with low-abundance, positive transcription factors (Ballas et al., 1995). Domain A contains the sequence TGTCCCAT, which is well conserved in the promoters of *Aux/IAA*, *SAUR*, and auxin-inducible *ACS* genes (Oeller et al., 1993; Abel et al., 1995a).

A similar functional analysis of the *GH3* promoter identified three independently acting *AuxREs* that con-

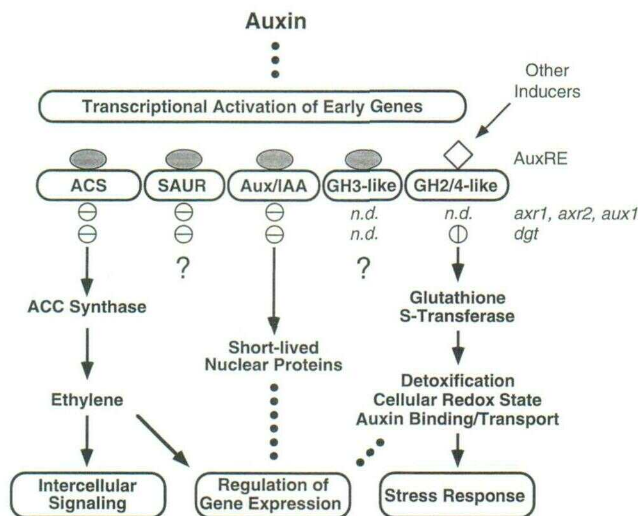

Figure 3. A model of early-auxin-regulated gene expression and gene function. Expression of early auxin genes in several auxin-insensitive mutants of Arabidopsis (*axr1*, *axr2*, and *aux1*) and *Lycopersicon* (*dgt*) is either unaffected (open valve), inhibited to varying degrees (closed valve), or not determined (n.d.). A common set of functionally identified *AuxREs* used by at least three major classes of auxin-specific early genes is indicated by a shaded ellipsoid. The *ocs*-like element of GH2/4-like genes is indicated by an open diamond.

tribute incrementally to the overall auxin inducibility of *GH3* (Liu et al., 1994). The major response to auxin is mediated by a 76-bp promoter region that contains two independent *AuxREs*, domain D1 (25 bp) and domain D4 (32 bp). Both domains contain the core sequence TGTCTC-(nnn)AATAAG. A more detailed analysis indicates that the minimal *AuxREs* of both domains are composed of two cooperating elements (Ulmasov et al., 1995). One element, TGTCTC, confers auxin inducibility, and an upstream variable element confers constitutive expression. The variable element is proposed to function as a coupling factor that may confer tissue specificity (Ulmasov et al., 1995). It is interesting that a motif similar to the D1 core sequence is also present in domain A of *PS-IAA4/5*, and the TGTCTC motif is contained in the 30-bp NDE region of *SAUR* genes. The NDE element of the *SAUR 15A* promoter is necessary and sufficient for auxin inducibility and contains the conserved sequence TGTCTC(nnnnnn)GGTCCCAT (Li et al., 1994). The downstream motif of this sequence is strikingly similar to the core sequence of domain A in the *PS-IAA4/5* promoter. A structural and possibly functional relationship between motifs TGTCTC and TGTCCCAT has been pointed out, but this remains to be proven by analyses to determine minimal promoter elements conferring auxin inducibility (Li et al., 1994; Liu et al., 1994; Ulmasov et al., 1995). Nonetheless, these functional studies strongly indicate that three classes of auxin-specific primary genes utilize a common set of *cis*-elements in a combined manner that specifies a certain modular organization of auxin-responsive promoter regions. Although not yet tested, auxin-regulated *ACS* genes may constitute a fourth class that exploits the same set of elements (Abel et al., 1995a). Modular construction is a hallmark of promoters and allows for autonomous as well as cooperative regulation of transcription in a context-dependent manner specified by the stimulus, tissue type, and developmental stage.

The study by Ulmasov et al. (1994) indicates that *GH2/4*, a representative of the fifth major class of early-auxin-inducible genes, is activated by auxin via an *ocs*-like element. This element belongs to a family of related sequences that regulate transcription of several viral and agrobacterial genes. *ocs*-like elements contain two strictly spaced binding sites for plant basic Leu zipper proteins (core sequence ACGT), and occupation of both sites is required for function (Ellis et al., 1993). The *GH2/4* promoter was the first plant gene identified that contains a functional *ocs*-like element, TG**ATGTA**AGAGATT**ACGTA**A, and similar sequence elements were found by database searches in the related gene *parA* and those encoding *CNT103*-like mRNAs (Ellis et al., 1993). Consistent with the induction profile of *GH2/4*-like genes, the *ocs*-like element is activated not only by auxin but also by other plant growth substances, inactive auxin analogs, and stress-inducing agents. Therefore, it has been argued that *ocs*-like elements are the target of a common stress-related signal transduction pathway that is different from auxin-specific signaling cascades (Ulmasov et al., 1994). In fact this assumption is strongly supported by the apparent absence of functional *ocs*-like elements in the promoters of auxin-specific primary-response genes, and by the unaffected expression of a *GH2/4*-like gene in the *dgt* mutant of tomato (Mito and Bennett, 1995).

FUNCTION OF EARLY GENE PRODUCTS

With the exception of ACC synthase, the biological role and precise biochemical function of primary-auxin-responsive gene products are unknown. First functional clues have emerged for proteins encoded by *Aux/IAA* and *GH2/4*-like genes, mainly inferred from similarities of their deduced primary and predicted secondary structures with proteins of known function. Thus far, such similarities have not been noticed for the SAUR and GH3 polypeptides. However, differential activation of *SAUR* genes during tropic responses and the high degree of instability of their transcripts suggest an important role of SAUR polypeptides in cell elongation growth (Guilfoyle et al., 1993).

ACC Synthase

ACC synthase is the key regulatory enzyme of ethylene biosynthesis. Ethylene serves as a signaling molecule to initiate and coordinate profound physiological adaptations during plant development. Consequently, its biosynthesis is stringently regulated. The rate-limiting reaction is the conversion of *S*-adenosylmethionine into ACC, the immediate ethylene precursor. The committed step is catalyzed by ACC synthase, which is subject to transcriptional and posttranscriptional control. ACC synthase is a short-lived cytosolic enzyme and is encoded by a multigene family in all higher plant species studied. Each gene is differentially regulated in a tissue-specific manner by a distinct subset of agents and environmental conditions. Auxin is a known inducer of ethylene production and regulates specific *ACS* genes. Ethylene itself regulates gene expression at the transcriptional and posttranscriptional level (for review, see Theologis, 1993; Zarembinski and Theologis, 1994). The relative physiological roles of auxin and ethylene were studied in transgenic plants in which the effects of both hormones are uncoupled. Those studies indicate that auxin overproduction alone is responsible for increased apical dominance and leaf epinasty, but that ethylene affects plant stature by reducing internode length (for review, see Klee and Romano, 1994). Primary auxin-responsive *ACS* genes offer suitable probes for the study of molecular aspects of the intimate interrelationship of auxin and ethylene action.

GST

Members of the large and divergent *GH2/4*-like gene family were the first auxin-responsive genes identified to encode polypeptides with an enzymatic activity. GSTs are a ubiquitous family of enzymes that catalyze the nucleophilic addition of the thiol group of reduced glutathione to the electrophilic moiety of a wide variety of hydrophobic chemicals (for review, see Rushmore and Pickett, 1993). Based primarily on studies with bacterial and animal GSTs, several controversial hypotheses on the putative function of related plant enzymes have been proposed. The best-studied function associated with GSTs are detoxification of xenobiotics and of endogenous toxic compounds. A related

function is protection against the adverse effects of oxidative reactions, such as lipid peroxidation of membranes during mechanical trauma and pathogen infection. Auxin affects a large number of metabolic and cell physiological processes that lead to the formation of oxidizing by-products. It is interesting that *ARG1*, an early auxin gene, appears to encode a fatty acid desaturase that is thought to change membrane properties by oxidizing unsaturated fatty acids (Yamamoto, 1994). Thus far, conjugation of herbicides is the only described function of plant GSTs (Timmerman, 1989). Members of GST gene families are induced by various electrophilic chemicals, stress conditions, and heavy metals that directly provoke oxidative damage. Therefore, it has been proposed that high concentrations of auxin, in particular 2,4-D, are perceived by plants as xenobiotics (Droog et al., 1993; Ulmasov et al., 1994). However, there is no direct evidence as yet that auxins and their chemical derivatives bind to the catalytic site of GSTs and undergo glutathione conjugation (Bilang and Strum, 1995). In addition to the catalytic site, animal GSTs possess a noncatalytic site that is thought to be involved in intracellular transport of a wide range of hydrophobic and amphipathic molecules. Based on the enzymatic characterization of a GST from *Hyoscyamus muticus*, which was identified by photolabeling with 5-azido-IAA, Bilang and Sturm (1995) proposed that IAA binds to such a noncatalytic site. This site, as proposed in analogy to cytoplasmic retinoic acid binding proteins, may control intracellular concentrations and gradients of auxin or may regulate GST enzyme activity, which then modulates the cellular redox potential (Bilang and Sturm, 1995). It is interesting that in a similar scenario, noncompetitive binding of salicylic acid to catalase inhibits enzyme activity and increases hydrogen peroxide concentrations, which finally leads to modulation of specific gene expression associated with systemic acquired resistance (Chen et al., 1993). It has been proposed that auxin may affect gene expression via reactive oxygen species as second messengers by regulating GST gene expression and/or GST enzyme activity (Jones, 1994). Nuclear localization of the parA polypeptide, and similarity of *GH2/4*-like gene products to the stringent starvation protein of *Escherichia coli*, which forms an equimolar complex with the holoenzyme of RNA polymerase, are yet other pieces of cirumstantial evidence that suggest a role of GST-like proteins in gene regulation (Takahashi et al., 1995). Clearly, the various hypotheses on GST function and their twisting relationship to auxin action warrant critical investigation.

Short-Lived Nuclear Proteins

Three features of Aux/IAA polypeptides suggest a function as regulators of gene expression in a secondary phase of auxin signal transduction. First, Aux/IAA proteins are nuclear localized and contain conserved targeting signals that are essential for nuclear transport (Abel et al., 1994; Abel and Theologis, 1995). Second, expression of *PS-IAA4/5* and *PS-IAA6* is stringently regulated at multiple levels, a hallmark of genes encoding regulatory proteins. This is best illustrated by the short metabolic half-lives of PS-IAA4 and PS-IAA6 (Abel et al., 1994). Thus, the physical presence and functional competence of Aux/IAA proteins in the nuclear compartment is limited and may reflect a switch-like function in auxin-regulated growth processes. Third, we have proposed that an anticipated nuclear function of Aux/IAA polypeptides is linked to five invariant hydrophobic amino acids that are predicted to form an amphipathic helix. This helix contributes to a putative $\beta\alpha\alpha$-fold that is reminiscent of the β-ribbon DNA recognition domain of prokaryotic repressor polypeptides, such as Arc of *Salmonella* phage P22 and MetJ of *E. coli* (Abel et al., 1994). If experimentally proven, Aux/IAA proteins would represent the first eukaryotic members of this class of transcription factors. In comparison to the prokaryotic $\beta\alpha\alpha$-fold, DNA binding ability, as well as homo- and heterodimerization of Aux/IAA polypeptides, have been proposed (Abel et al., 1994, 1995b). In fact, we have recently demonstrated both homo- and heterotypic interactions of Aux/IAA proteins from pea and Arabidopsis using a yeast two-hybrid system (J. Kim and A. Theologis, unpublished data). Furthermore, the plant $\beta\alpha\alpha$ domain selects DNA binding sites in vitro (S. Abel and A. Theologis, unpublished data). In view of the highly differential mode of *Aux/IAA* gene expression with respect to timing, hormone concentration, and spatially restricted expression patterns (Wyatt et al., 1993; Abel et al., 1995b; Koshiba et al., 1995; Wong et al., 1996), the prospect arises that cell-specific restriction of *Aux/IAA* gene expression is a developmental process that plays a fundamental role in specifying a combined code of spatio-temporal co-expression of the various family members. This code, in turn, could determine a spectrum of heterotypic interactions of Aux/IAA proteins and thereby the permissible repertoire of biological responses to auxin for a given cell or tissue type.

CONCLUSIONS AND PROSPECTS

The paradigm of early genes induced by IAA is a viable concept from which to elucidate molecular mechanisms of auxin action. As primary genes identified in animal systems, early-auxin-inducible gene products appear to play important roles in emergency rescue and stress adaptation, intercellular communication, and regulation of late gene expression (Fig. 3). However, a causal role of any auxin-responsive gene in cellular physiology and regulation of secondary-phase gene expression remains to be established, and we wish we could have titled this review "Early Genes in Auxin Action." The construction of mutant plants, preferably knock-outs defective in the expression of those genes, is imperative. The stage has been set with the isolation of homologous mRNAs and parental genes from *A. thaliana*, a plant species of choice for reverse genetics. Much will be learned from the *Aux/IAA* gene family. Identification of the regulatory targets of Aux/IAA proteins bears the potential to link mechanisms of auxin perception and stimulus transduction to auxin-induced biochemical and physiological processes responsible for plant cell growth, such as cell cycle control, cell turgor regulation, secretion, or cell-wall biosynthesis. The elucidation of the function of the SAUR and GH3-like polypeptides is also a task for the

future. Auxin-responsive *cis*-elements have been identified for members of all major classes of early auxin genes. Proteins that bind specifically to those functionally characterized elements have not yet been isolated and tested for function in signal transmission, the next step in an effort to trace auxin-signaling cascades. More importantly, auxin-specific expression of early genes, mediated by characterized *AuxRE*s, provides a long-sought specific molecular phenotype to design genetic screens for mutants primarily defective in auxin signal transduction. Such reverse genetic screens aim at the processing machinery upstream of early gene activation. This approach will greatly aid in bridging the gap between auxin perception and primary gene activation, a principal goal of the early gene concept.

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