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REVIEW ARTICLE

JIPs and RIPs: The Regulation of Plant Gene Expression by Jasmonates in Response to Environmental Cues and Pathogens

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INTRODUCTION

After its first description as a fragrant constituent of the essential oil of *Jasminum grandiflorum* (Demole et al., 1962), (−)-jasmonic acid methyl ester (methyl *cis*-2-(2-penten-1-yl)-3-oxocyclopentenyl acetate, also known as methyl jasmonate [MeJA]), and related compounds were found to be widespread in the plant kingdom (Meyer et al., 1984). In addition, jasmonates occur in fungi and algae (for references, see Sembdner and Parthier, 1993). Interestingly, MeJA is also a component of female-attracting pheromones in certain moths (Baker et al., 1991).

(−)-Jasmonic acid [(−)-JA] and its derivatives are cyclopentanone compounds that are synthesized from linolenic acid by a sequence of as yet poorly characterized reactions (Vick and Zimmerman, 1984). The natural biosynthetic product of this pathway in most plant species appears to be (+)-7-*iso*-JA, which is rapidly converted into (−)-JA by isomerization. (−)-JA has been shown to undergo various modifications, giving rise to numerous metabolites with differing structures and biological activities. Major physiologically active compounds are (−)-JA, MeJA, and the amino acid conjugates of (−)-JA with isoleucine, valine, or leucine (for review, see Sembdner and Parthier, 1993).

(−)-JA and MeJA (which will be collectively referred to throughout this review as jasmonates) affect plant growth and development in a pleiotropic manner (for reviews, see Parthier, 1990, 1991; Staswick, 1992; Sembdner and Parthier, 1993). Recently, jasmonates have been proposed to be stress-related compounds (Farmer and Ryan, 1990; Parthier, 1990, 1991; Enyedi et al., 1992; Farmer et al., 1992; Gundlach et al., 1992; Müller et al., 1993). MeJA, in particular, is a signal molecule that is released in plants in response to various stresses, such as wounding or pathogen attack (Creelman et al., 1992), treatment of plant tissues or plant cell cultures with fungal elicitors (Dittrich et al., 1992; Gundlach et al., 1992; Müller et al., 1993), or subjection of tissues to osmotica or desiccation (Parthier et al., 1992). Jasmonates are thought to be transported both locally and systemically within wounded plants (Anderson, 1985; Farmer and Ryan, 1990; Enyedi et al., 1992; Farmer et al., 1992; Hildmann et al., 1992). Because MeJA is a volatile compound, it is also able to traverse the atmosphere and thus can reach neighboring plants, in which characteristic defense reactions may be induced (Farmer and Ryan, 1990; Franceschi and Grimes, 1991).

All of the different plant responses to jasmonates, whether applied externally or released internally, appear to be correlated with alterations in gene expression. At least three major jasmonate effects have been reported to be exerted at the gene expression level: (1) the induction of novel abundant polypeptides, designated jasmonate-induced proteins (JIPs) (Parthier et al., 1987), (2) the selective repression of synthesis of several polypeptides that are present before jasmonate or stress treatment (Weidhase et al., 1987a; Reinbothe et al., 1993a, 1993b, 1993c), and (3) the temporally delayed general down-regulation of protein biosynthesis occurring in long-term MeJA-treated or long-term stressed leaf tissues (Reinbothe et al., 1994).

Figure 1 illustrates the first two effects in the polypeptide patterns of MeJA-treated leaf tissues in plants as diverse as barley, *Nicotiana plumbaginifolia,* and Arabidopsis. Except for a few evolutionarily conserved polypeptides (see below), most JIPs appear to be expressed in a genus- and species-specific manner (Figure 1; see also Herrmann et al., 1989). Their induction patterns differ markedly in both temporal and spatial aspects in different plant species (Müller-Uri et al., 1988; Herrmann et al., 1989; Farmer and Ryan, 1990; Staswick, 1990; Sánchez-Serrano et al., 1991; Gundlach et al., 1992; Reinbothe et al., 1992b, 1992c). In addition, qualitative and quantitative differences exist in the extent to which MeJA represses the synthesis of preexisting proteins (Figure 1). These results

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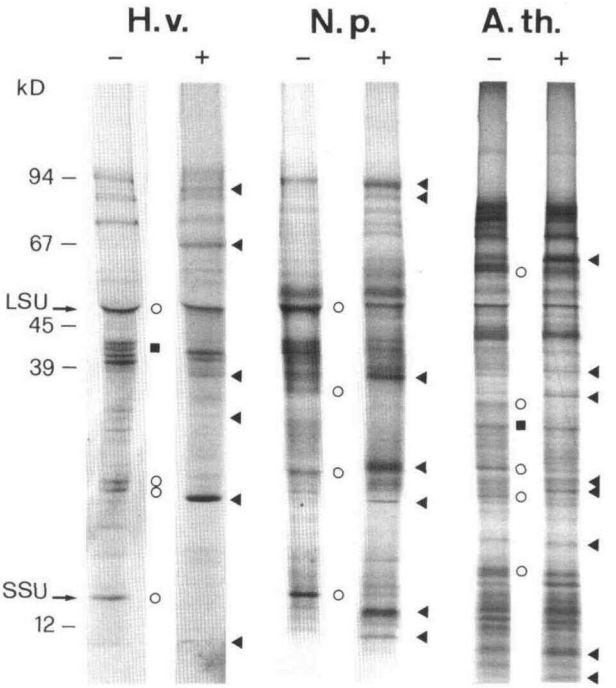

Figure 1. Methyl Jasmonate–Induced Alterations in Polypeptide Patterns.

Excised leaf tissues of barley (H.v.), *Nicotiana plumbaginifolia* (N.p.), and Arabidopsis (A.th.) were treated with water (–) or 45 μM MeJA (+) for 24, 72, and 16 hr, respectively. Proteins were then labeled with ^{35}S-methionine for 2 hr, separated in an 11 to 20% polyacrylamide gel, and detected by autoradiography. Arrowheads mark JIPs, and circles indicate polypeptides whose synthesis was reduced in MeJA-treated leaf tissues compared to water-treated leaf tissues. LSU and SSU designate the large and small Rubisco subunits, respectively. The positions of molecular mass standards are indicated by horizontal bars.

imply that plants differ in uptake and/or metabolization of MeJA and/or in their sensitivity (i.e., competence to respond) to this compound.

The aim of this review is to summarize our current knowledge about changes in plant gene expression caused by jasmonates. We demonstrate that jasmonates influence gene expression at multiple levels, such as gene activation and transcription, post-transcriptional RNA processing and transcript stability, translation, and post-translational steps including protein modifications and protein degradation. As secondary messengers that are released in plants upon pathogen attack, jasmonates seem to set in motion an entire defense-related gene expression program that, as an early component, includes the synthesis of an array of diverse plant defense proteins and, as a later component, includes the general depression of plant protein biosynthesis. Both components appear to be causally related, because one of the recently identified JIPs, JIP60, is a ribosome-inactivating protein that irreversibly inactivates plant protein synthesis (Reinbothe et al., 1994) and initiates cell death. The putative role of JIP60 in the hypersensitive response and in stress reactions will be discussed.

PLANT DEFENSE AND PHOTOSYNTHETIC GENES ARE INVERSELY REGULATED BY JASMONATES

Several proteins whose synthesis is either induced or repressed by jasmonate treatment have been identified. Table 1 demonstrates that most JIPs have activities and/or expression patterns that suggest a function in plant defense and stress reactions, whereas proteins whose synthesis is reduced or even shut down by MeJA, at least in barley, are involved in photosynthetic carbon assimilation. Genes whose expression is apparently unaffected by jasmonates in barley leaf tissues (Table 1) seem to encode proteins with housekeeping functions, such as actin, tubulin, and plastid aminoacyl-tRNA synthetases (Reinbothe et al., 1993a, 1993b).

At least five groups of jasmonate-induced plant defense proteins can be distinguished: proteinase inhibitors, thionins, proline-rich proteins, enzymes involved in phenylpropanoid metabolism, and ribosome-inactivating proteins.

Proteinase inhibitors are ubiquitous proteins that inhibit the digestive proteinases of insects and microorganisms (Green and Ryan, 1972). By virtue of their activities, proteinase inhibitors have been proposed to protect plants against herbivory by decreasing the digestibility and nutritional quality of leaf proteins (Ryan, 1990).

Thionins occur in various plant species (Garcia-Olmedo et al., 1989; Bohlmann and Apel, 1991). Some of them have been shown to exhibit toxicity against several plant pathogens in vitro (Bohlmann et al., 1988; Molina et al., 1993). The leaf thionin genes of barley, which were found to be MeJA responsive (Andresen et al., 1992), comprise a family of closely related genes (Bohlmann and Apel, 1987; Bunge et al., 1992). Their encoded products accumulate both in the cell wall and in the vacuole (Reimann-Philipp et al., 1989).

Proline-rich proteins, and especially hydroxyproline-rich proteins, have been proposed to be involved in cell wall strengthening (Cassab and Varner, 1988). One of the proline-rich proteins has recently been shown to undergo a very rapid elicitor-induced oxidative isodityrosine cross-linking that is thought to modify the cell wall to prevent the spread of fungi beyond the infection site (Bradley et al., 1992). In growing zones of soybean seedlings, low water potential (i.e., water deficit) correlates with an increase in the endogenous content of jasmonates (Creelman et al., 1992) and has been shown to cause accumulation of mRNAs encoding proline-rich proteins (Creelman and Mullet, 1991a).

Phenylalanine ammonia-lyase, 4-coumarate CoA ligase, and chalcone synthase are enzymes involved in phenylpropanoid metabolism (for reviews, see Hahlbrock and Scheel, 1989; Dixon and Lamb, 1990). These proteins have recently been demonstrated to be induced by endogenous jasmonates that

Table 1. Jasmonic Acid/Methyl Jasmonate–Responsive Genes and Their Expression under Diverse Stress Conditions[a]

Gene Product	Species	Other Inducers/ Repressors	References
INDUCED (JIPs)			
Plant defense and stress proteins			
Proteinase inhibitors	Potato, tomato, alfalfa	Sucrose, ABA, wounding	Peña-Cortés et al. (1989); Farmer and Ryan (1990); Johnson and Ryan (1990); Farmer et al. (1992); Hildmann et al. (1992)
Thionins	Barley	Pathogens, divalent metal ions	Bohlmann et al. (1988); Andresen et al. (1992)
Proline-rich proteins	Soybean	Water deficit, ABA	Creelman and Mullet (1991a, 1991b); Creelman et al. (1992)
Phenylalanine ammonia-lyase	Parsley	UV light, fungal elicitors	Kuhn et al. (1984); Dittrich et al. (1992); Gundlach et al. (1992)
4-Coumarate:CoA ligase	Parsley	UV light, fungal elicitors	Kuhn et al. (1984); Dittrich et al. (1992)
Chalcone synthase	Parsley, petunia	Sucrose, UV light, fungal elicitors	Kreuzaler et al. (1983); Chappell and Hahlbrock (1984); Kuhn et al. (1984); Ryder et al. (1984); Tsukaya et al. (1991); Dittrich et al. (1992)
Berberine bridge enzyme	*Eschscholtzia californica*	Fungal elicitors	Gundlach et al. (1992)
Ribosome-inactivating proteins	Barley	ABA, sugars, desiccation, wounding	Becker and Apel (1992, 1993); Reinbothe et al. (1994)
Late embryogenesis abundant proteins (group III)	Cotton, *Nicotiana plumbaginifolia,* barley,	ABA, sugars, desiccation	Galau et al. (1986); Reinbothe et al. (1992b, 1992c); C. Reinbothe, unpublished data
Cruciferin	*Brassica napus*	ABA, sugars	Wilen et al. (1990, 1991)
Napin	*B. napus*	ABA, sugars	Wilen et al. (1990, 1991)
Vegetative storage proteins	Soybean	Water deficit, wounding, nitrogen supply	Mason and Mullet (1990); Staswick (1990); Mason et al. (1992)
Proteins involved in signal transduction of stress responses and in pathogen defense			
Lipoxygenase	Soybean	Water deficit, wounding	Bell and Mullet (1991); Tranbarger et al. (1991)
Systemin	Potato, tomato	Wounding	Pearce et al. (1991); McGurl et al. (1992)
REPRESSED			
Chloroplast proteins			
Nuclear-encoded			
SSU	Barley	ABA, sugars, desiccation	Reinbothe et al. (1992c, 1993b)
LHCP	Barley	ABA	Reinbothe et al. (1993b)
Plastid-encoded			
LSU	Barley	ABA, sugars, desiccation	Reinbothe et al. (1992c, 1993c)
68- and 66-kD proteins of photosystem I	Barley	ABA	Reinbothe et al. (1993c)
CONSTITUTIVELY EXPRESSED			
LRS1	Barley	ABA	Reinbothe et al. (1993b)
D1 protein of photosystem II	Barley	ABA	Reinbothe et al. (1993c)
Actin	Barley	ND	Reinbothe et al. (1993a)
Tubulin	Barley, soybean	Water deficit	Creelman and Mullet (1991a, 1991b); Reinbothe et al. (1993a)
HSC70	Barley	Heat shock	Müller-Uri et al. (1988)
Patatin	Potato	ABA	Peña-Cortés et al. (1992)

[a] HSC70, heat shock cognate protein of M_r 70,000; JIPs, jasmonate-induced proteins; ABA, abscisic acid; LHCP, light-harvesting chlorophyll *a/b* binding protein; LRS1, plastid leucyl-tRNA synthetase; LSU, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit; SSU, ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit. ND, not determined.

are released in diverse plant species after treatment with fungal elicitors (Dittrich et al., 1992; Gundlach et al., 1992). The final products of the divergent branches of this pathway are toxins produced by plants to deter pathogenic microorganisms and herbivores.

In contrast to genes for plant defense (and stress) proteins, genes that encode proteins involved in photosynthetic carbon assimilation are negatively regulated by MeJA (cf. Table 1). In both the nucleocytoplasmic and plastidic compartments of barley leaf cells, MeJA affects various steps in the formation of chloroplast proteins such as the small and large subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the light-harvesting chlorophyll *a/b* (cab) binding proteins, and the 65- and 68-kD proteins of photosystem II (cf. Table 1). These effects ultimately lead to characteristic senescence symptoms within the plastid compartment, such as chlorophyll loss and Rubisco degradation (Weidhase et al., 1987b; Reinbothe et al., 1993b, 1993c). The amino acids released by the proteolytic degradation of chloroplast proteins are thought to be reutilized for cytosolic JIP formation (Reinbothe et al., 1993a). Chloroplast protein turnover thus appears to be required to ensure rapid JIP synthesis (Weidhase et al., 1987b; Reinbothe et al., 1993b, 1993c).

CONTROL OF GENE EXPRESSION BY JASMONATES

Positive Transcriptional Control of JIP Genes: *cis*-Elements and *trans*-Acting Factors

Numerous JIP genes have been identified. The promoter of *vspB*, which encodes a vegetative storage protein of soybean, contains a C-rich sequence and a G-box motif, separated by a stretch of 26 bp, that have recently been demonstrated to constitute a MeJA-responsive domain (Mason et al., 1993). C-rich motifs similar to that in *vspB* were found in the soybean *vspA* promoter and also upstream of other wound- and jasmonate-inducible genes, such as those encoding phenylalanine ammonia-lyase and chalcone synthase (for references, see Mason et al., 1993). The promoter of the proteinase inhibitor II gene of potato also contains both C-rich and G-box sequences (Kim et al., 1992).

Interestingly, the promoter elements of many genes regulated by abscisic acid (ABA) are similar to those found in JIP genes. They contain *cis*-regulatory regions that are closely related or even identical to the G-box motif (for reviews, see Reinbothe et al., 1992a; Williams et al., 1992). The G-box thus seems to be essential to confer both MeJA- and ABA-regulated transcriptional control. However, there is an increasing number of reports demonstrating distinctions between the responses to MeJA and ABA (Hildmann et al., 1992; Reinbothe et al., 1992c). This poses the question of how individual genes may be differentially regulated in response to either of these two compounds.

The G-box is known to be a general recognition and binding site for a family of transcription factors sharing the bZIP structure, a unique domain composed of basic amino acids and a leucine zipper (Williams et al., 1992). One might speculate that jasmonates and ABA might be able to physically interact differentially with these or other constitutively expressed transcription factors. Alternatively, jasmonates and ABA might induce the formation de novo of distinct transcription factors that, either directly or via their interaction with other factors, regulate transcription of either jasmonate- or ABA-responsive genes. An alternative possibility might be that MeJA and ABA increase the stability, and thus the amount, of different factors that normally limit transcription of either JIP or ABA-responsive genes (Hattori et al., 1992).

Negative Transcriptional Control: Photosynthetic Genes Encoded in the Nucleus

MeJA exerts a negative effect on the transcription of ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit *(rbcS)* and *cab* genes. Recent determinations of transcription rates in nuclear runoff assays using isolated nuclei from MeJA-treated leaf tissues (S. Reinbothe, unpublished results) demonstrate a decline in *rbcS* and *cab* transcription that is in contrast to the continuous transcription of JIP genes. By analogy to MeJA, ABA inhibits the expression of the *rbcS* and *cab* genes in barley (Reinbothe et al., 1992c), tomato (Bartholomew et al., 1991), and wheat (Williamson and Quatrano, 1988). These findings suggest the existence of common as well as distinct *cis*-regulatory regions within the promoters of MeJA-, light-, and ABA-responsive genes. Because the promoters of many *rbcS* and *cab* genes contain, at least in most dicot species, G-box–related sequences (for review, see Schindler and Cashmore, 1990), transcription factors that are involved in the activation of MeJA- and ABA-responsive genes might also act as repressors of light-regulated genes. On the other hand, MeJA has been demonstrated to eliminate the cell-specific expression of *vsp* genes in soybean (Huang et al., 1991), implying that this compound might also influence organ- and tissue-specificity in the expression of other genes.

Post-Transcriptional Transcript Modification: Alternative Processing Inactivates Translation of the Plastid *rbcL* Transcript

Little is known about the effect of jasmonates on post-transcriptional RNA modifications, such as endonucleolytic cleavage of primary transcripts, exonucleolytic removal of nucleotides, capping, and polyadenylation. Just one transcript has been shown to be modified at its 5′ end in MeJA-treated leaf tissues of barley, the plastid *rbcL* transcript encoding the Rubisco large subunit, shown in Figure 2. Three different *rbcL* transcripts have been identified, beginning at positions −316,

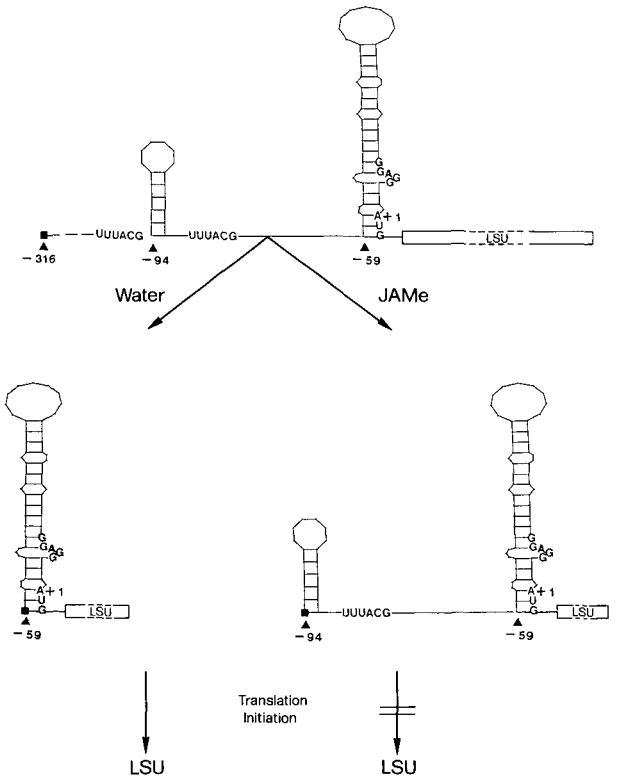

Figure 2. Proposed Secondary Structure and Putative Processing of the Barley Plastid *rbcL* Transcript.

The barley plastid *rbcL* transcript is formed as a large primary transcript whose 5′ end maps at position −316. The Shine-Dalgarno sequence (5′-GGAGG-3′) is indicated. The Rubisco large subunit (LSU) coding region is boxed. Two putative stem–loop structures within the 5′ untranslated leader of the primary *rbcL* transcript are indicated (top). A hexameric sequence, 5′-UUUACG-3′, found upstream and downstream of the more 5′ stem–loop structure and hypothesized to have a function in differential processing of the primary transcript is indicated. Processing downstream of the more 5′ stem–loop structure would give rise to *rbcL* transcripts beginning at position −59 (bottom left). Both the primary and −59 transcripts occur in freshly harvested leaves and are maintained in excised leaf tissues treated with water. By contrast, processing upstream of the more 5′ stem–loop structure in jasmonate-treated leaf tissues would generate −94 transcripts (bottom right). The two differentially processed −59 and −94 *rbcL* transcripts differ remarkably in their capability to initiate translation of LSU in vivo, in organello, and in vitro. Modified after Reinbothe et al. (1993c), with permission of The EMBO Journal, Oxford University Press.

−94, and −59 (Reinbothe et al., 1993c). Whereas the largest *rbcL* transcript is probably generated by transcription initiation, the two shorter transcripts are likely to arise from alternative processing of the primary transcript (Reinbothe et al., 1993c). −94 *rbcL* transcripts specifically appear upon MeJA treatment and displace −59 *rbcL* transcripts present in untreated leaf tissues.

Differential 5′ end formation of the primary transcript may be caused by a MeJA-induced change in the target site specificity of a putative endoribonuclease that recognizes a common hexameric sequence motif, UUUACG, localized both upstream and downstream of a predicted stem–loop structure in the 5′ untranslated region of the −316 *rbcL* transcript (Figure 2). If endonucleolytic cleavage upstream of the predicted stem–loop structure in the *rbcL* primary transcript occurred in MeJA-treated leaf tissues, −94 *rbcL* transcripts would be formed. By contrast, endonucleolytic cleavage plus exonucleolytic removal of nucleotides downstream of this putative RNA secondary structure could generate −59 *rbcL* transcripts in water-treated tissues (Figure 2).

The resulting two divergently processed transcripts differ remarkably in their ability to initiate translation of the large subunit in vitro, in organello, and in vivo (Reinbothe et al., 1993c) (Figure 2). Whereas −94 *rbcL* transcripts are impaired in the formation of functional 30S initiation complexes, *rbcL* transcripts ending at position −59 can be translated efficiently (Reinbothe et al., 1993c). As a result of a striking complementarity of the extra 35 bases in the −94 transcript to the extreme 3′ terminal end of both the 16S and 18S rRNAs, correct translation initiation is inhibited in both prokaryotic and eukaryotic systems (Reinbothe et al., 1993c). Depending on the ratio of ribosomes and −94 *rbcL* transcripts, either the translation of the Rubisco large subunit would be inhibited, or plastid protein synthesis would be generally inactivated. In barley plastids, an excess of ribosomes seems to ensure that plastid-encoded transcripts such as *psbA*, which encodes the D1 protein of photosystem II, can be translated during that stage of the jasmonate response, however (Reinbothe et al., 1993c).

Data base searching indicates that nucleotide sequences similar to the 35-base motif present in the barley *rbcL* gene can also be found in plastid-encoded *rbcL* and *atpB* genes of other plant species. Provided that MeJA operates via a mechanism similar to that postulated here for *rbcL*, the expression of the β-subunit of the chloroplast CF_0-ATPase would be down-regulated. This effect would cause a decrease in ATP synthesis and thus could, after depletion of other intracellular energy sources, nonspecifically influence all other metabolic functions in MeJA-treated leaf tissues.

Interestingly, the prosystemin gene, which encodes the precursor of systemin, an 18–amino acid peptide that is involved in systemic signaling of wound responses in potato and tomato (Pearce et al., 1991; Farmer and Ryan, 1992; McGurl et al., 1992), also contains a sequence with remarkable homology to the 35-base motif found in the barley *rbcL* gene (McGurl and Ryan, 1992). However, this sequence is located within the second intron of the tomato prosystemin gene (McGurl and Ryan, 1992) and thus might be inactive in terms of translation of the prosystemin mRNA. If MeJA were to cause a defect in splicing of the prosystemin primary transcript, this RNA might be a very powerful specific or general inhibitor of translation in wounded or pathogen-infected tomato and potato leaves.

Destabilization of *rbcS* and *cab* Transcripts

When barley leaf tissues are exposed to MeJA for periods longer than 24 hr, *rbcS* and *cab* transcript levels start to decline (Reinbothe et al., 1993b). Based on the comparison of in vitro transcription rates in nuclear runoff assays (S. Reinbothe, unpublished results) and transcript levels by RNA gel blot hybridizations (Reinbothe et al., 1993b), differential changes in message abundances suggest an additional negative effect exerted by MeJA on the stabilities of *rbcS* and *cab* but not JIP transcripts.

ABA has previously been shown to influence the accumulation of transcripts such as *rbcS*, *cab*, and *Em* in wheat (Quatrano et al., 1983; Williamson and Quatrano, 1988). Although the levels of *rbcS* and *cab* transcripts declined in embryos treated with ABA, the levels of the *Em* transcript, which encodes a group II late embryogenesis abundant (LEA) protein (see Dure et al., 1989; Reinbothe et al., 1992a, for a compilation of LEA proteins and their genes), remained stable (Quatrano et al., 1983). Williamson and Quatrano (1988) proposed that the *Em* mRNA might be preferentially stabilized by endogenous ABA in wheat embryos as compared to other ABA-regulated transcripts. Inclusion of the 5′ untranslated region of *Em* led to a 10-fold increase in β-glucuronidase activity in the absence of ABA and to a 20-fold increase in the presence of ABA, suggesting that the 5′ region of *Em* may stabilize the reporter transcript (or lead to its preferential translation) in rice protoplasts (Marcotte et al., 1989; Hetherington and Quatrano, 1991).

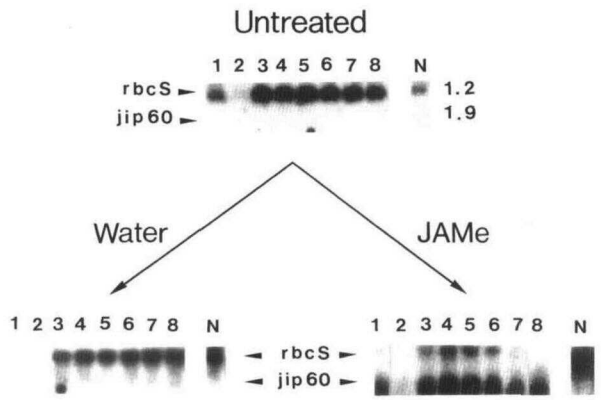

Figure 3. Association of *rbcS* and *JIP60* Transcripts with Polysomes and Nonpolysomal Ribonucleoprotein Material in Barley.

Polysomes were isolated by Mg^{2+} precipitation from an equal number of barley leaf segments that had been either exposed to water or MeJA for 8 hr or left untreated. Ribonucleoprotein material not recovered by this procedure (i.e., containing nonpolysomal RNA) was ethanol precipitated from leaf homogenates. Polysomal RNAs were fractionated in discontinuous sucrose step gradients, and individual fractions, numbered 1 through 8, were harvested. Polysomal (1 to 8) and nonpolysomal (N) RNAs prepared by phenol extraction and ethanol precipitation were separated in denaturing agarose gels, and the filter-bound RNAs were hybridized with *rbcS* and *JIP60* cDNA probes as described previously (Reinbothe et al., 1993b, 1994). The sizes of the detected transcripts are given in kilobases.

Translational Control: Ribosomes Discriminate against *rbcS* and *cab* mRNAs in Favor of JIP mRNAs in the Early Stage of the Jasmonate Response

One of the most remarkable effects of MeJA in barley is to repress the synthesis of most preexisting ("control") proteins (Weidhase et al., 1987a). Although the population of translatable control mRNAs, including *rbcS* and *cab*, is conserved in MeJA-treated barley leaf tissues for at least 24 hr, most of these mRNAs are no longer translated into protein in vivo (Müller-Uri et al., 1988; Reinbothe et al., 1993a, 1993b). By contrast, leaf tissues exposed to water instead of MeJA continue to translate control mRNAs (Müller-Uri et al., 1988; Reinbothe et al., 1993a, 1993b). The observed repression of protein synthesis appears to be highly specific in vivo, because polysomes isolated from short-term MeJA-treated leaf tissues efficiently translate JIP mRNAs as well as certain other mRNAs, such as those encoding tubulin, actin (Reinbothe et al., 1993a), and the nuclear-encoded plastid leucyl-tRNA synthetase (Reinbothe et al., 1993b). This result implies that polysomes of MeJA-exposed leaf tissues discriminate specifically against control mRNAs in favor of JIP and constitutively translated mRNAs.

Figure 3 shows that control transcripts, such as *rbcS*, are confined to small polysomes and to the nonpolysomal fraction in MeJA-treated barley leaf tissues, in contrast to JIP transcripts, which are present in all of the different polysomal fractions. In freshly harvested barley leaves and leaf tissues that have been exposed to water, however, the *rbcS* transcripts are associated with large polysomes (Figure 3). This finding suggests that translation initiation at these transcripts is repressed in MeJA-treated leaf tissues compared to water-treated leaves (Reinbothe et al., 1993a, 1993b). Similarly, most non-heat shock mRNAs are displaced from polysomes in favor of heat shock mRNAs in numerous plant species under heat shock and other stress conditions (Nover et al., 1989, 1990; Apuya and Zimmerman, 1992).

Inhibitor experiments with cycloheximide further show that the repression of translation initiation of control mRNAs in MeJA-treated barley leaves is only partial, because the repressed *rbcS* and *cab* transcripts, as well as other mRNAs, can be shifted toward larger polysomes (Reinbothe et al., 1993a, 1993b). Because these control mRNAs remain translatable in MeJA-treated barley leaf tissue (Reinbothe et al., 1993a, 1993b), their specific inactivation (e.g., by modifications at their 5′ ends) is unlikely. Alternatively, the 5′ leader sequences of control and JIP mRNAs might differ structurally, causing control mRNAs to be sequestered (and thus inactivated) in specialized subcellular structures such as messenger ribonucleoprotein particles (Larson and Sells, 1987; Scherrer, 1990).

A similar mechanism has previously been demonstrated for most tomato and carrot non-heat shock mRNAs, which become attached to the intermediate filament network of the cytoskeleton and are preserved in so-called heat shock granules under heat shock conditions (Nover et al., 1989; Apuya and Zimmerman, 1992). A different possibility is that JIP mRNAs might contain translational enhancers within their 5′ untranslated leaders that are recognized by the protein biosynthetic machinery. However, in vitro translation experiments demonstrate that JIP mRNAs do not have higher rates of translation initiation than the average control mRNAs (Reinbothe et al., 1993a).

If JIP mRNAs do exhibit some unique features in primary sequence or secondary structure that favor their translation in vivo (but not in vitro), this information must be decoded by factors interacting with such putative sequences or structures. Modifications of distinct ribosomal proteins or of proteins interacting with them, for example by phosphorylation or dephosphorylation, have been reported to occur under diverse stress conditions (Scharf and Nover, 1982; Nover and Scharf, 1984; Bailey-Serres and Freeling, 1990; Crosby and Vayda, 1991; Apuya and Zimmerman, 1992) and might also be required to ensure the selectivity of JIP protein synthesis.

General Depression of Translation: JIP60 Inactivates Protein Synthesis in Long-Term MeJA-Treated Leaf Tissues

Barley leaf tissues exposed to MeJA for periods longer than 48 hr start to down-regulate their protein biosynthetic machinery. Recent analyses of ribonucleoprotein material in sucrose step gradients demonstrate a successive decay into their ribosomal subunits of cytoplasmic polysomes in long-term MeJA-treated leaf tissues (Reinbothe et al., 1994). This finding is consistent with a general depression of translation initiation caused by MeJA. In contrast, water-treated leaf tissues maintain their polysomes (Reinbothe et al., 1994) and continue to synthesize control proteins for at least 72 hr (Müller-Uri et al., 1988). These differences raise the possibility that MeJA may repress the synthesis of proteins that are required for translation initiation, such as initiation factors, or may cause the synthesis of proteins that induce polysome decay in vivo.

Interestingly, one of the recently identified barley JIPs, JIP60 (Becker and Apel, 1992), shares amino acid sequence homology with plant ribosome-inactivating proteins (RIPs) (Stirpe and Barbieri, 1986; Katzin et al., 1991) and certain bacterial toxins, such as Shiga and Shiga-like toxins (Calderwood et al., 1987). The N-terminal half of JIP60 is related to both type I and type II RIPs, such as the *Mirabilis jalapa* antiviral protein (Habuka et al., 1989), saporin-6 from *Saponaria officinalis* (Benatti et al., 1989), and ricin from *Ricinus communis* (Lamb et al., 1985) (see Reinbothe et al., 1994, for comparison). Whereas type I RIPs are basic proteins that consist of a single polypeptide chain of 25 to 32 kD (for references, see Bass et al., 1992), type II RIPs contain, in addition to the N-terminal RIP domain, a second domain that confers lectinlike properties to these proteins (Olsnes and Pihl, 1973, 1982). Both types of RIP are exceptionally potent inhibitors of eukaryotic protein synthesis (Coleman and Roberts, 1982; Stirpe et al., 1988). All RIPs characterized thus far repress protein synthesis at the level of translation elongation by catalyzing the cleavage of a specific adenine nucleoside residue in the 28S rRNA (Endo et al., 1988; Stirpe et al., 1988). This irreversible modification impairs binding of elongation factor II, thereby inhibiting the progression of the ribosome along the translated mRNA (Brigotti et al., 1989).

In contrast to previously characterized RIPs, JIP60 appears to exhibit an unusual mode of action and thus may be termed a type III RIP. JIP60 cleaves polysomes from plant, microbial, and animal origin into their ribosomal subunits (Reinbothe et al., 1994). Particularly interesting is the observation that JIP60 is able to cleave barley polysomes only from long-term MeJA-treated leaf tissues or from leaf tissues that have been exposed to stressors that cause high level jasmonate accumulation, such as harsh wounding, osmotic stress, or desiccation (Reinbothe et al., 1994). Polysomes isolated from non-jasmonate-treated or unstressed barley leaf tissues do not serve as substrates for cleavage by JIP60, suggesting a specific MeJA-induced marking of ribosomes for this destiny. It is as yet unknown when and how this specific marking of ribosomes is initiated. Nevertheless, it is tempting to speculate about modifications similar to those that have been suggested to be involved in ensuring the discrimination of JIP versus control mRNAs in the very early stage of the MeJA response (see above), i.e., phosphorylation/dephosphorylation of distinct ribosomal proteins or proteins interacting with ribosomes.

JIP60, A PUTATIVE PLANT DEFENSE PROTEIN INVOLVED IN LOCAL PATHOGEN RESISTANCE

The coordinate interplay between JIP60 and marked ribosomes ultimately leads to cell death in long-term MeJA-treated and long-term stressed leaf tissues. This effect seems to share some similarity to the hypersensitive response, a phenomenon that is observed when plants are challenged by viral, bacterial, or fungal pathogens. Around the sites of pathogen infection, which are often wound sites, specific lesions indicative of localized cell death appear. This cell suicide is thought to prevent the spread of pathogens beyond the necrotic lesion (for reviews, see Collinge and Slusarenko, 1987; Lamb et al., 1989; Madamanchi and Kuc, 1991).

Figure 4 shows a working model describing the putative involvement of JIP60 in local pathogen resistance. We propose that MeJA released in situ upon pathogen attack or wounding might cause the induction of RIPs with activities similar to that reported here for the barley RIP, JIP60. Simultaneously, wounding, operating via MeJA, might elicit a marking of ribosomes, targeting them for degradation by this enzyme. The latter effect, however, would require a critical threshold concentration of

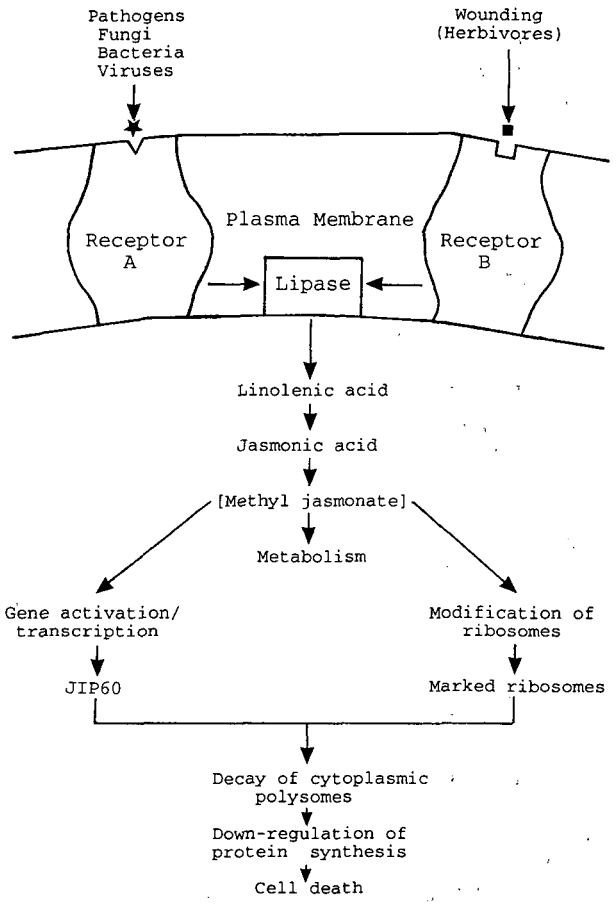

Figure 4. Working Model for JIP60's Involvement in the Local Resistance Response to Pathogen Infection and Wounding.

See text for details. The upper part was redrawn and modified after Farmer and Ryan (1992).

MeJA that could be reached only after a certain period of continuous production of this compound from linolenic acid, reflecting the irreversible damage to the plasma membrane of wounded or pathogen-attacked cells. Thus, the coordinate interplay between JIP60 and marked ribosomes could take place only in the immediate vicinity of wounded or pathogen-attacked, structurally damaged cells.

Although distant plant organs and tissues could import MeJA from its site of continuous production, they would not be necessarily destined to down-regulate their protein biosynthetic machinery because they would be able to rapidly metabolize this compound (Sembdner et al., 1990; Gundlach et al., 1992; Müller et al., 1993). By lowering the effective MeJA concentration, intact plant tissues could thus protect themselves efficiently against the wasteful degradation of their ribosomes. However, the actual jasmonate concentration would likely still be sufficiently high to induce the expression of an array of diverse plant defense proteins (see above). In concert with other defense-related compounds, such as secondary metabolites, these proteins might help to confer systemic resistance against subsequent pathogen attack.

In addition to its role in the general down-regulation of plant protein biosynthesis during pathogenesis, we propose that JIP60 might have a direct defense function, acting as a lectin similar to ricin (summarized in Katzin et al., 1991). Ricin is an extraordinarily toxic molecule. Its lectin domain binds specifically to carbohydrate moieties of eukaryotic cell surfaces. In turn, the entire RIP molecule enters the cell via endocytosis, and, once it is in the cytoplasm, the RIP domain subsequently attacks ribosomes of the target cell (e.g., pathogens), leading to inactivation of protein biosynthesis. By analogy to ricin, JIP60, by virtue of its putative lectin domain, might enter the cells of pathogens and could then inactivate protein synthesis. However, the proposed operation of this mechanism would require that JIP60 be released from the cytosol of pathogen-infected, structurally damaged plant cells. Alternatively, JIP60 might be secreted to the cell wall or the vacuole to accomplish its defense function. This proposal implies that JIP60 may be differentially targeted to either the cytosol or the wall of the plant cell. In fact, the JIP60 homolog in Arabidopsis, traced in protein gel blots with antibodies directed against the barley protein (kindly provided by J. Lehmann, Halle/Saale, Germany), accumulates first as the unspliced 67-kD protein and then as the spliced polypeptide lacking the signal peptide in MeJA-treated leaf tissues (S. Reinbothe, unpublished results). Thus, this polypeptide may be transported into the endoplasmic reticulum of plant cells treated with MeJA or challenged by pathogens.

In barley, JIP60 is synthesized and primarily localized in the cytosol (Hause et al., as cited in Parthier, 1991). However, JIP60 may also be secreted, because its predicted N-terminal amino acid sequence (Becker and Apel, 1992) contains a tripartite structure reminiscent of signal peptides of proteins that enter the secretory pathway (for review, see Chrispeels, 1991). Whether and when JIP60 can enter the secretory pathway is as yet undetermined. It might be that fungal or bacterial toxins, of which some are known to affect intracellular protein trafficking in plants (summarized in Bednarek and Raikhel, 1992), could induce secretion of JIP60. Alternatively, the intracellularly produced MeJA could cause JIP60 to enter the secretory pathway. Once directed into the endoplasmic reticulum, JIP60 would no longer be able to act intracellularly on marked ribosomes. However, one might speculate that membrane-bound JIP60 may be able to reenter the cytosol of the plant cell when leaf tissues are exposed to prolonged pathogen attack. The ability to cross membranes in order to reach the cytosol is quite common among toxins of diverse origin (e.g., Wiedlocha et al., 1992). It remains to be investigated whether JIP60 primarily operates intracellularly in the general down-regulation of protein synthesis, extracellularly to deter pathogens, or even both, and whether its localization changes in plants after pathogen attack and in response to stress.

SUMMARY AND PERSPECTIVE

As outlined in this review and summarized in Figure 5, jasmonates influence plant gene expression in a pleiotropic manner. Whether released endogenously upon pathogen attack or stress treatment or applied externally, jasmonates induce numerous plant defense and stress proteins and simultaneously lower or even shut down the expression of photosynthetic and various other genes. JIP genes are rapidly transcriptionally activated, and the resulting JIP mRNAs are preferentially translated compared to most preexisting mRNAs, including *rbcS* and *cab*. The molecular basis for such efficient ribosomal discrimination against control mRNAs in favor of JIP mRNAs is as yet unknown. Modifications of distinct ribosomal proteins or of factors that interact with them, or specifically induced translational activators, might be involved in deciphering the structural information that is likely to be contained in the 5′ regions of JIP transcripts. In this early stage of the jasmonate response, *rbcS* and *cab* transcripts are shifted into the nonpolysomal fraction and are preserved in the cell, potentially to allow recovery of the cell from MeJA or stress treatment or pathogen attack. At a later stage, transcription of the *rbcS* and *cab* genes is reduced and finally turned off, and the levels of the *rbcS* and *cab* transcripts decline.

Within the plastid compartment, early changes in transcript functionality, as discussed for *rbcL*, appear to be superimposed on delayed MeJA effects on plastid transcription and RNA stability. Chloroplast proteins of cytoplasmic and plastid origin, such as the small and large subunits of Rubisco, respectively, are degraded, and their amino acids are likely to be used for cytoplasmic JIP formation.

Collectively, the various mechanisms discussed in this review seem to ensure that plant cells, by reprogramming their gene expression, are able to deter pathogens and to respond to stress. Synthesis of plant defense proteins seems to be an early event in plants challenged by pathogens. A temporally delayed response is the general down-regulation of protein biosynthesis. This mechanism may initiate localized cell death, thus preventing the spread of bacteria, viruses, and fungi beyond the infection site, a defense strategy that closely resembles the hypersensitive response. In the case of the jasmonate response, the underlying molecular mechanism appears to require the induction of JIP60 and the specific marking of the plant ribosome. The coordinate interplay between JIP60 and its intracellular target, marked ribosomes, is thus a rather late event and probably proceeds much slower than the rapid changes occurring during the hypersensitive response. Nevertheless, one might speculate that pathogens may be able to elicit the immediate marking of the plant ribosome. Consistent with this idea, pathogen-encoded enzymes have been shown to be involved in the signal transduction chain by which plants activate local and systemic resistance responses against pathogens (Daniels et al., 1984; Dow et al., 1987). It remains to be investigated how plant ribosomes are marked for cleavage by JIP60 and whether pathogens may promote this effect.

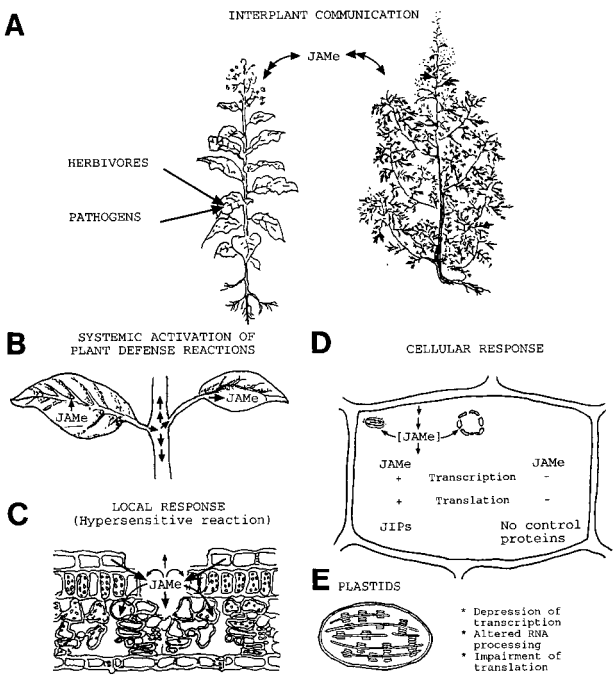

Figure 5. Hypothetical Hierarchy of Methyl Jasmonate Action in Plants.

(A) MeJA (JAMe) is distributed widely in plants, but plants differ in the extent to which they produce MeJA under non-stress conditions. Plants containing low endogenous jasmonate levels, such as tobacco (left), respond to pathogen attack or wounding by inducing MeJA synthesis. This compound is volatile and is presumably released to the atmosphere. Plant species producing constitutively high levels of MeJA, such as sagebrush (right), would also transmit this compound to their environment. In both cases, the airborne MeJA could induce defense responses in neighboring plants.

(B) MeJA (JAMe) produced upon pathogen attack or wounding can also be transported to distant plant organs, causing systemic activation of plant defense genes.

(C) Plant tissues challenged with incompatible pathogens often respond with the hypersensitive reaction. MeJA (JAMe) produced at sites of pathogen infection could set in motion a cascade of cellular responses that lead to localized cell death.

(D) and **(E)** At the molecular level, MeJA (JAMe) is synthesized from linolenic acid released from the plasma membrane of wounded or pathogen-attacked, structurally damaged cells. MeJA causes changes in gene expression in both the nucleocytoplasmic **(D)** and plastid **(E)** compartments. In the nucleocytoplasmic compartment **(D)**, MeJA induces the synthesis of JIPs and simultaneously depresses the formation of several other polypeptides. Similar alterations in gene expression are caused by other stressors, such as osmotica or desiccation, that cause plasma membrane damage and subsequent MeJA production.

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