The promoter for the phaseolin (phas) bean seed protein gene adopts an inactive chromatin structure in leaves of transgenic tobacco. This repressive architecture, which confers stringent spatial regulation, is disrupted upon transcriptional activation during embryogenesis in a process that requires the presence of both a transcription factor (PvALF) and abscisic acid (ABA). Toward determining the need for de novo synthesis of proteins other than PvALF in transcriptional activation we explored the effect of several eukaryotic protein synthesis inhibitors. Surprisingly, cycloheximide (CHX), emetine, and verrucarin A were able to induce transcription from the phas promoter in tobacco and bean leaf tissue in the absence of either PvALF or ABA. This induction was decreased by the replication inhibitors hydroxyurea and aphidicolin but not by genistein or mimosine. Since protein phosphatases and kinases are essential components of the ABA signal transduction pathway, it is conceivable that CHX is also capable of inducing phosphorylation of proteins usually involved in ABA-mediated activation. Interestingly, okadaic acid, an inhibitor of serine/threonine phosphatase, also strongly activated transcription from the phas promoter. In contrast, the protein synthesis inhibitors anisomycin and puromycin did not activate transcription from the phas promoter, nor did the tyrosine phosphatase inhibitors phenylarsine oxide and sodium orthovanadate. These discrete but different results on transcriptional activation may reflect specific modes of action of the inhibitors, or they may reflect differential interactions of the inhibitors or of downstream events resulting from inhibitor activity with presently unknown components of the transcriptional activation system.

The necessity of de novo protein synthesis for gene activation can be investigated through the use of inhibitors. For example, the fact that the induction of most genes involved with indole acetic acid metabolism is insensitive to the protein synthesis inhibitor cycloheximide (CHX) suggests that newly synthesized protein is not necessary for activation of these genes (1). CHX has also been reported to enhance and prolong the accumulation of mRNA of expressing genes in a process termed superinduction (2) and to induce de novo transcription from nonexpressing genes as documented for PS-IAA4/5 (3), GAmyb (4), HVA22 (5), CBF1 (6), ATL2 (7), and mlip15 (8). Superinduction or de novo induction of genes by protein synthesis inhibitors is thought to occur through several distinct processes. CHX can enhance mRNA stability by preventing the synthesis of labile mRNA-degrading enzymes (9); alternatively, some protein synthesis inhibitors cause RNAs to be trapped on polysomes, thus shielding them from cytoplasmic ribonucleases (10, 11). For autorepressive genes, the inhibited translation of their protein product can lead to superinduction through an inability to shut off transcription (12). CHX may lead to transcriptional activation via the loss of labile negative regulators. CHX can induce the uncoupling of DNA replication and chromatin assembly (because of the continued DNA replication and the absence of histone synthesis during CHX treatment), preventing the formation of a repressive chromatin structure (13); alternatively, CHX may induce uncoupling of DNA replication and gene specific repressors, thereby releasing chromatin constraint on transcription. CHX can lead to direct transcription activation by eliciting chromatin-associated signals such as H3 phosphorylation (14) or by biochemical modifications that lead to the activation of positive (or the deactivation of negative) transcription factors.

Phaseolin, the major seed protein of bean, Phaseolus vulgaris, is encoded by a small gene family whose expression is tightly regulated both temporally and spatially (15). Expression of the β-phaseolin gene (phas) is totally inactive during vegetative phases of plant development (16). This is achieved by a repressive chromatin architecture (17) that is remodeled concomitant with gene activation in the developing seed, resulting in disruption of histone-mediated DNA wrapping and permitting abundant factor binding to the phas promoter (18). Activation of the phas promoter is a two-step process: chromatin modification mediated by the transcription factor PvALF, followed by abscisic acid (ABA)-mediated transcriptional activation (19).

Toward evaluating the need for the synthesis of protein factors additional to PvALF to permit expression from the phas promoter, leaves of line 58.1A plants (tobacco transformed with –1470phas/uidA, see Ref. 19) and line PVAll-14 plants (a 58.1A plant retransformed with CaMV35S/PvAlf, see Ref. 19) and bean leaves were treated with protein synthesis inhibitors. To our surprise, we found that three potent eukaryotic protein synthesis inhibitors (CHX, verrucarin A, and emetine) were able to induce transcriptional expression from the phas promoter in these vegetative tissues without the presence of either PVAlF or ABA. We demonstrated that phas activation can also be induced by the protein phosphatase inhibitors okadaic acid...
and cantharidin. The stimulation of phas expression by protein synthesis inhibitors and by phoshatase inhibitors suggests that transcription from the phas promoter is regulated through a complex network of interactive signaling components and pathways.

**EXPERIMENTAL PROCEDURES**

**ABA Induction**—Seeds collected from either 58.1A plants or from line PvAlf-14 were surface-sterilized with 20% (v/v) household bleach and then rinsed three times in sterile water. Seedlings (10 days) or leaves from seedlings (30 days), selected on MS medium containing either 400 μg/ml kanamycin or 50 μg/ml hygromycin, were incubated in liquid basal MS medium with or without 100 μM ABA (cis/trans isomer; Sigma) for 24 h (6 h for the RNase protection experiment) in the dark with gentle shaking at room temperature.

**Fluorometric and Histochemical GUS Assays**—Histochemical analysis of GUS activity was according to Jefferson et al. (20). For fluorometric (MUG) assay, leaves or callus were homogenized in the GUS extraction buffer (50 mM NaH₂PO₄, pH 7.0, 10 mM EDTA, 0.1% Sarkosyl, 0.1% Triton X-100, 10 mM β-mercaptoethanol) and centrifuged for 5 min in a microcentrifuge. Then, 200 μl of extract were mixed with 200 μl of substrate solution (GUS extraction buffer + 4-methylumbelliferyl β-D-glucuronide: 4-MUG, Fluka) and incubated at 37 °C; 100-μl aliquots were removed at 0, 60, or 120 min and the reaction terminated by addition of 900 μl of NaNO₃. Fluorescence was measured on a fluorometer (VersaFluo™ fluorometer, Bio-Rad). Protein concentrations were determined using the colorimetric assay of Bradford (21). Specific GUS activity was calculated as pmol 4-MU h⁻¹ μg⁻¹ protein.

**RNase Protection Assay (RPA)**—Antisense constructs for generating riboprobes were prepared by subcloning segments containing the 3’ end of the uidA and the region encoding 18 S RNA into the vector pPCR-script amp sk (Stratagene, La Jolla, CA). Antisense uidA and 18 S rRNA riboprobes of 310 and 200 nucleotides, respectively, were synthesized by transcription in vitro, using T3 or T7 polymerase on a HindIII- or EcoRI-linearized plasmid.

RPA were performed in reactions containing 5–10 μg of total RNA using RPAII kit (Ambion, Austin, TX). The protected fragments were analyzed by electrophoresis on a 5% polyacrylamide, 8 M urea gel.

**Isolation of Histones**—Approximately 5 g of leaf tissue was powdered in a mortar and pestle under liquid nitrogen, then treated with nuclei isolation buffer NIB1 (0.5 M hexylene glycol, 20 mM KCl, 20 mM PIPES pH 6.5, 0.5 mM EDTA, 0.4% Triton X-100, 0.05 mM spermine, 0.125 mM spermidine, 7 mM 2-mercaptoethanol, 0.5 mMphenylmethylsulfonyl fluoride and 0.5% (v/v) aprotinin) in the presence of protein phosphatase inhibitors (10 mM NaF, 1 mM sodium orthovanadate). Nuclei were recovered by centrifugation at 1400 g for 10 min and washed twice in NIB2 (NIB1 without Triton X-100). All centrifugations were carried out at 4 °C. Nuclei were resuspended in 3 ml RSB (22) buffer (10 mM NaF, 1 mM sodium orthovanadate, 0.5% (v/v) aprotinin, 10 mM NaF, 1 mM sodium orthovanadate) and treated with nuclei isolation buffer NIB1 (0.5 M hexylene glycol, 20 mM KCl, 20 mM PIPES pH 6.5, 0.5 mM EDTA, 0.4% Triton X-100, 0.05 mM spermine, 0.125 mM spermidine, 7 mM 2-mercaptoethanol, 0.5 mM phenylmethylsulfonyl fluoride and 0.5% (v/v) aprotinin) in the presence of protein phosphatase inhibitors (10 mM NaF, 1 mM sodium orthovanadate). The samples were precipitated with trichloroacetic acid and then resuspended in TE buffer containing protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 0.5% (v/v) aprotinin, 10 mM NaF).

**Electrophoresis and Western Blotting**—Proteins were analyzed by SDS-15% polyacrylamide gels. The proteins were visualized by Coomassie Brilliant Blue staining or transferred to nitrocellulose membranes and analyzed by electrophoresis on a 5% polyacrylamide, 8 M urea gel.

**RESULTS**

**Phas Expression Can Be Induced in Leaf Tissue by CHX**—To examine whether de novo protein synthesis is required for PALF-dependent induction of expression from the phas promoter in the presence of ABA, leaves from either 58.1A (tobacco transformed with $-1470{phas}/uidA$, see Ref. 19) or PvAlf-14 plants (line 58.1A additionally transformed with CaMV35S/PvAlf) were subjected to CHX treatment in the presence or absence of ABA. Half of the CHX-treated leaves was used for MUG assays, the other half was used for RPA experiments. MUG analysis showed that CHX was very effective in preventing de novo protein synthesis, since no GUS accumulation was detected in PvAlf-14 seedling leaves treated with both ABA and CHX (Fig. 1A). Despite the complete inhibition of de novo protein synthesis, phas mRNA was still synthesized (Fig. 1B, compare lanes 5 and 6), suggesting that de novo protein synthesis is not required for phas activation. The synthesis of uidA mRNA appeared to be slightly reduced in the presence of CHX. However, this difference may be due to the variation of PALF levels among plants (19). The similar levels of 18 S rRNA seen in each lane of the RPA assay show that comparable amounts of RNA were loaded.

Although the above experiments show that the phas promoter can be activated in the absence of de novo protein synthesis, it is likely that this expression is not through ABA- or PALF-mediated processes. As shown in Fig. 1B, treatment of PvAlf-14 leaves with CHX in the absence of ABA or treatment of 58.1A plants with CHX in the presence or absence of ABA can also induce phas mRNA accumulation (Fig. 1B, lanes 2 and 4). This reveals that the CHX treatment can bypass the absolute requirement for PALF and ABA to activate phas in transgenic tobacco (19). The samples were cultured in CHX containing MS liquid medium for 6 h in these and subsequent CHX
experiments reported in this paper because this period gave maximal accumulation of mRNA from 58.1A leaves (Fig. 1C).

A similar induction of expression from the phas promoter by CHX to that seen in transgenic tobacco was obtained using leaf tissue of its native species, P. vulgaris (Fig. 1D). This shows conclusively that the induction of expression seen in tobacco is not due to the use of a heterologous system.

**Phas Activation by CHX Is Concentration-dependent**—To investigate whether phas activation by CHX results from the inhibition of protein synthesis, we tested phas induction under different concentrations of CHX. As shown in Fig. 2A, lanes 2 and 3, the phas promoter is not activated at low concentrations (0.1 and 1 μM) of CHX that do not greatly reduce protein synthesis (Fig. 2B). However, a low level of transcription (detected as uidA mRNA; Fig. 2A, lane 4) occurred after 5 h of 10 μM CHX treatment, when protein synthesis was reduced to 25% compared with the control. At 100 μM, CHX was 98% effective in inhibiting protein (GUS) synthesis (Fig. 2B), and transcription from the phas promoter was strongly induced (Fig. 1A).

**Differential Effects of Replication Inhibitors on CHX-induced phas Expression**—It has been hypothesized that treatment of cells with an efficient inhibitor of protein synthesis, such as CHX, would be expected to uncouple DNA synthesis and chromatin assembly and that this may have widespread consequences in relieving chromatin-mediated general transcriptional repression (13). To determine whether uncoupling of DNA replication and chromatin assembly is responsible for protein inhibitor-inducible phas expression, we tested the effect of DNA replication inhibitors on CHX-induced phas expression. As shown in Fig. 3A, pretreatment with 10 mM hydroxyurea (HU), a replication inhibitor that inhibits ribonucleotide reductase, for 30 min before adding CHX substantially reduced the degree of induction by CHX. Pretreatment of leaves with 10 mM HU for 24 h before adding CHX further reduced the degree of induction of uidA expression by CHX (Fig. 3A, lanes 4 and 5 compared with lanes 1 and 3). This reduction was not due to HU toxicity, since HU concentrations of up to 100 mM did not reduce transcription from the CaMV35S promoter (Fig. 3B).

We also found that CHX-mediated activation of phas is higher in young leaves where there is more DNA replication than that in older leaves (Fig. 3C, compare lanes 2 and 4). However, aphidicolin, another DNA replication inhibitor that inhibits DNA polymerase α, had only a small inhibitory effect on CHX-induced phas expression (Fig. 3A, compare lanes 7 and 8 with lane 1). Contrary to the histone depletion hypothesis, mimosine and genistein, compounds shown to inhibit DNA replication in animal (24, 25) and plant (rice, data not shown) cells, were found to superinduce phas expression in the presence of CHX (Fig. 3D).

**phas Can Be Strongly Induced by Some, but Not All, Protein Synthesis Inhibitors**—To further evaluate the mechanism of CHX-induced phas activation, we examined the effect of alternative protein synthesis inhibitors (verrucarin A, emetine, anisomycin, and puromycin) on activation in leaf tissues of transgenic tobacco plants. Like CHX, verrucarin A and emetine strongly induced phas expression (Fig. 4, A and B). However, puromycin and anisomycin did not induce phas activation when used at concentrations that are effective for inhibition of protein synthesis and activation of gene expression in animal cell lines (Fig. 4, B and C). Overexposure of the gel revealed uidA mRNA expression at levels barely above background for two different concentrations of puromycin and anisomycin (Fig. 4C). The trace levels of uidA mRNA in the presence of 100 μg/ml puromycin and 10 μg/ml of anisomycin could be attributed to poor inhibition of protein synthesis at these concentrations, as indicated by MUG assays (Fig. 4D). However, 10-fold higher concentrations of these inhibitors (1 mg/ml puromycin or 100 μg/ml anisomycin) are very effective in inhibiting protein synthesis (Fig. 4D), yet no uidA mRNA was detected in 58.1A leaves. This suggests that inhibition of protein synthesis alone will not lead to transcriptional activation from the phas promoter.
Induction of phaseolin Expression in Leaf Tissues in Response to Phosphatase Inhibitors—In addition to inhibiting protein synthesis, CHX has been shown to induce histone H3 phosphorylation (14). Because of the role of chromatin in silencing expression from the phaseolin promoter, we decided to explore the use of okadaic acid, a serine/threonine phosphatase inhibitor that has been shown to induce H3 phosphorylation and early-response gene expression in mammalian cells (14). Okadaic acid was able to induce transcriptional expression from the phaseolin promoter in leaf tissue of both 58.1A and PvAlf-14 plants (Fig. 5A, lanes 2 and 4). The induction of phaseolin expression from leaves of PvAlf-14 was about twice as strong as that from leaves of 58.1A. A similar serine/threonine phosphatase inhibitor, cantharidin, can also induce phaseolin expression (data not shown).

We then tested whether CHX- or okadaic acid-induced phaseolin activation is related to bulk H3 phosphorylation, as has been shown for early-response genes (14). Western blotting with antibodies to pH3 showed that there is no discernible difference between the amount of pH3 in transgenic tobacco leaves in the presence or absence of CHX treatment (Fig. 5B, compare lanes 3 and 4), suggesting that bulk H3 phosphorylation is not responsible for phaseolin activation.

Tyrosine phosphatase inhibitors, phenylarsine oxide and sodium orthovanadate, a specific inhibitor for dual specificity phosphatase and tyrosine phosphatase, were not effective in inducing uidA mRNA expression from the phaseolin promoter. Slightly higher than background levels of uidA mRNA were detected only after overexposure of the gel for more than 2 days using a PhosphorImager (Fig. 5C).

CHX and Okadaic Acid Can Induce the Expression of Various Seed-specific Genes—Given the remarkable stimulation of expression from the phaseolin promoter, we were interested in exploring the ability of CHX and okadaic acid to induce expression of other seed storage protein genes. As shown in Fig. 6, RPA analysis clearly demonstrated that expression of uidA from the normally seed specific DC3 (26), HaG3 (27), and Arcelin (28) promoters was induced in leaves of tobacco transformed with these constructs by treatment with CHX or okadaic acid.
expression from several seed storage protein genes. A, the effect of CHX on the expression of DC3, HaG3, and Arcelin genes. B, okadaic acid can activate the expression of uidA from DC3, HaG3, and Arcelin promoters in transgenic tobacco.

Daic acid, albeit at different efficiencies: DC3 was strongly induced but HaG3 and Arcelin were induced only weakly.

**DISCUSSION**

We have shown previously that the phas promoter adopts an inactive chromatin structure in leaf tissue of transgenic tobacco and that the repressive chromatin structure is disrupted upon transcriptional activation during embryogenesis, processes that require both PvALF and ABA (17, 18). The presence of PvALF results in remodeling of the chromatin architecture over the phas promoter, allowing ABA-stimulated transcriptional activation (19). Here we show that several eukaryotic protein synthesis inhibitors can induce transcription from the phas promoter in tobacco and bean leaf tissue in the absence of either PvALF or ABA. The expression seen in leaves from bean, the plant from which phas was isolated (29), confirms that the induction observed in the case of tobacco does not result from the use of a heterologous system. We also demonstrated that CHX-induced phas activation is associated with inhibition of protein synthesis, although this is not the only requirement. Since phosphatase inhibitors can also induce phas activation, it is possible that protein synthesis inhibitor-induced expression from the phas promoter is mediated through a general signal transduction pathway involving phosphorylation/dephosphorylation events. The stimulation of transcription from the Arcelin, De3, and HaG3 seed-specific promoters by CHX and okadaic acid (Fig. 6) indicates the generality of transcriptional activation by these compounds.

**CHX-induced phas Expression and Uncoupling of DNA Replication and Chromatin Assembly**—CHX-induced mRNA stability or CHX-inhibited autorepression can only contribute to superinduction of genes after their transcription has been activated. Since phas is not expressed in leaf tissues of transgenic tobacco plants, as shown both by nuclei run-on and by RPA experiments (17, 19), accumulation of its mRNA in the presence of CHX cannot result from mRNA stabilization or release from autorepression. Further support that mRNA stability is not involved is provided by the decreased level of uidA mRNA seen in leaves after 6 h of exposure to CHX (Fig. 1C). Additional evidence against the involvement of mRNA stability is provided by the fact that verrucarin A also induces phas expression (Fig. 4A), even though its mode of action is to dissociate mRNAs from ribosomes, thereby exposing them to cytoplasmic ribonucleases.

An attractive explanation for protein synthesis inhibitor-inducible phas expression is the uncoupling of DNA replication and chromatin assembly. In the presence of protein synthesis inhibitors, the synthesis of both histones and/or non-nucleosomal repressors will be affected. If DNA replication occurs during CHX treatment, histone- or gene-specific repressors may not be present in sufficient quantity to cover the newly replicated phas promoter. In such cases, phas will be expressed, since the transcription complex is still functional (13). This scenario suggests that CHX-induced transcription should be reduced upon addition of DNA replication inhibitors. Pretreatment of leaf tissue with the DNA replication inhibitor HU did indeed reduce induction by CHX and greater reduction was observed if more HU was added or if the pretreatment was longer (Fig. 3A). This reduction is not due to HU toxicity, since higher levels of HU did not affect transcription from the constitutive CaMV35S promoter (Fig. 3B). Furthermore, CHX-mediated induction was much greater for young leaves (in which replication is active) than for mature leaves (Fig. 3C), where little replication occurs. A slight reduction in CHX-mediated activation was also observed in the presence of aphidicolin (Fig. 3A).

In contrast to the above results, incubation of PvAlf-14 leaves with mimosine and genistein, two other inhibitors of replication, led to enhancement of CHX-mediated induction of expression from the phas promoter (Fig. 3D). Although the different outcomes of experiments using replication inhibitors are puzzling, it is feasible that mimosine and genistein have other functions in addition to the inhibition of replication.

**CHX-inducible phas Expression Is Not Entirely Due to the Loss of Labile Negative Regulators**—It has been well documented that many protein synthesis inhibitors, such as CHX, superinduce mRNA synthesis by de novo transcriptional activation (2, 30–33). A widely accepted interpretation of this effect is that a labile transcription repressor is degraded following protein synthesis inhibition, resulting in transcriptional activation. The strong correlation seen here between protein synthesis inhibition and phas promoter activation supports this interpretation: cycloheximide (100 μg/ml), verrucarin A, and emetine are effective protein synthesis inhibitors and all induce expression from the phas promoter. In contrast, puromycin (100 μg/ml) and anisomycin (10 μg/ml) are not effective inducers of phas activation and are relatively weak inhibitors of protein synthesis in plants (puromycin at 100 μg/ml was only 45% effective in inhibiting GUS protein synthesis, and anisomycin at 10 μg/ml was 64% effective). However, at higher concentrations, puromycin (1 mg/ml) and anisomycin (100 μg/ml) are very effective in inhibiting protein synthesis (Fig. 4D), but transcription was not activated in their presence (Fig. 4C). While these results appear to detract from the possibility that a labile repressor is involved in regulating expression from the phas promoter, or that uncoupling of DNA replication and chromatin assembly is the cause of phas induction, they may reflect the inhibition of specific components of the activation processes. For example, the individual protein synthesis inhibitors may be differentially effective with regard to specific components or those inhibitors that do induce expression may have side effects that lead to transcriptional activation.

**CHX-inducible phas Expression Is Not Due to Global H3 Phosphorylation**—Another possible explanation for CHX-inducible expression from the phas promoter is that CHX can actively elicit chromatin-associated signals that lead to transcription activation. CHX has been shown to superinduce the c-jun gene in human cells (14), which was later shown to be correlated with bulk histone H3 phosphorylation (34). In this case, activation of gene expression was not related to the inhibition of protein synthesis, and a subinhibitory concentration of protein synthesis inhibitor was able to superinduce c-jun transcription (35). Although the phosphatase inhibitors okadaic acid and cantharidin can induce phas activation (Fig. 5), immunoblotting of bulk histone with anti-pH3 antibodies showed that CHX treatment does not increase the level of bulk histone
We favor the active pathway as being the predominant explanation for our results, because the passive events require significant depletion of histones; this should be especially apparent for cells that are actively replicating. However, while our experiments with the replication inhibitor hydroxyurea (Fig. 3A) and to a lesser extent, aphidicolin, do show a decrease in protein synthesis inhibitor-induced activation, the effect is not striking and does not appear to be sufficient to account for the rapid induction of expression from the phas promoter upon exposure of leaves to protein synthesis inhibitors (Fig. 1C). In contrast, post-translational modifications, such as phosphorylation of pre-existing substrates, appear to provide a feasible basis for the rapid induction of phas expression.

In the putative active pathway, we postulate that a key protein phosphatase exists. The depletion of this phosphatase by protein synthesis inhibitors (Fig. 7, step F) and the inhibition of its activity by the inhibitors okadaic acid and cantharidin (Fig. 7, step C1) result in the induction of expression from the phas promoter. Either or both depletion and inhibition of the phosphatase (Fig. 7, steps C1–C3 or step F) could lead to the modification of the activity of transcription factors, directly or indirectly through the activation of additional phosphatase(s) or kinase(s). An alternative or additional set of events resulting from the depletion or inactivation of the postulated protein phosphatase could lead to chromatin potentiation as shown in Fig. 7, steps D and E. Potentiation can be achieved by targeted alterations in the chromatin environment encompassing specific genes, both directly, by phosphorylation of nucleosomal and chromatin proteins, or indirectly by allowing the recruitment of histone acetyltransferases to specific phosphoepitopes on transcription factors (Fig. 7, step E).

Our experiments provide clues regarding the nature of the putative protein phosphatase. Because okadaic acid and cantharidin can induce phas expression while sodium orthovanadate cannot suggest that this protein phosphatase is a serine/threonine protein phosphatase (PP1, PP2A, or PP2B) that normally negatively regulates expression from the phas promoter. Interestingly, okadaic acid has been shown to inhibit most gibberellic acid-inducible events and partly inhibits the induction of a HVA gene by ABA (37). Kinase and phosphatase inhibitors have been shown to affect gene expression in response to ABA (38–40). For example, the DNA binding activity of the G-box-binding bZIP factor GBF1 can be stimulated in vivo (41), and it is known that phosphorylation of bZIP factors in response to ABA (38–40). Kinase and phosphatase inhibitors have been shown to affect gene expression in response to ABA (38–40). For example, the DNA binding activity of the G-box-binding bZIP factor GBF1 can be stimulated in vivo (41), and it is known that phosphorylation of bZIP factors

ph3 level. This suggests that CHX-induced phas expression is not through global H3 phosphorylation, but certainly does not eliminate the possibility that localized H3 phosphorylation of phas chromatin is involved.

A Possible Explanation for Inhibitor-induced phas Transcriptional Activation—It is likely that the inhibition of protein phosphatase by protein synthesis inhibitors or of its synthesis by protein synthesis inhibitors has several biochemical consequences on signal transduction mechanisms. These could include phosphorylation or dephosphorylation events that lead to the activation of positive, or the deactivation of negative, transcription regulators (36). Indeed, our experiments suggest that the inhibitors can act through a passive activation pathway (Fig. 7, steps A or B) or an active pathway (Fig. 7, steps C–F).

FIG. 7. Possible modes of action of protein synthesis, DNA replication, and protein phosphatase inhibitors in phas promoter activation. Expression from the phas promoter typically involves potentiation (chromatin remodeling instigated by PvALF, dark green arrow) and ABA-mediated activation (light green arrow) steps. Two pathways for inhibitor-induced activation are portrayed. In the Passive Pathway, inhibition of translation (A) by protein synthesis inhibitors (cycloheximide, emetine, and verrucarin A) leads to a decrease in histone synthesis and failure to establish an inactive nucleosomal architecture over the phas promoter after DNA replication, yielding a potentiated state that is accessible to the activators (black arrow). B, inhibition of DNA replication (by hydroxyurea) eliminates the need for new histones; nucleosome architecture over the promoter remains unperturbed and the inactive state is maintained (magenta arrows). In the Active Pathway, protein phosphatase inhibitors (okadaic acid and cantharidin) inhibit the activity of a postulated protein phosphatase (C1). The absence of dephosphorylation (C2) causes an accumulation of the phosphorylated (active) state of a putative transcription factor (orange ball) that activates transcription (C3, red arrows). Alternatively, protein phosphatase inhibitors may prevent dephosphorylation of histones, leading to hyperphosphorylated nucleosomes (D) that can potentiate the promoter by recruiting nucleosome remodeling activity, such as histone acetyltransferase (blue arrows). E, if the unknown factor (orange ball) is a protein kinase, rather than a transcription factor, its activated state may hyperphosphorylate histones and hence result in nucleosome remodeling (purple arrows), as in D. F, it is also possible that the protein synthesis inhibitors cycloheximide, emetine, and verrucarin A function by preventing the synthesis of a labile protein phosphatase (black arrow), leading to hyperphosphorylation and activation by pathways C, D, or E.
must be a component of the ABA signal transduction pathway that leads to transcriptional activation of the phaseolin gene (19).

The results obtained here are dramatic with regard to our understanding of the regulation of expression from the phaseolin promoter. First, they show that the normally tight repression of expression from the phaseolin promoter in vegetative tissue can be overcome rapidly by exposure to the potent inhibitor of protein synthesis, CHX. Second, while tyrosine phosphatase inhibitors had no discernible effect, inhibition of by okadaic acid activated transcription from the phaseolin promoter, strongly implicating the presence of a serine/threonine phosphatase as a key regulator. Third, our findings extend previous studies that have demonstrated the ability of CHX to induce or superinduce transcription (2) by showing that other protein synthesis inhibitors can overcome rapidly by exposure to the potent inhibitor of protein synthesis, CHX. Second, while tyrosine phosphatase inhibitors or of downstream products of the inhibitors or of downstream products may reflect differential interactions of the inhibitors or of downstream products resulting from inhibitor activity with components of the signal transduction and transcriptional activation systems that remain to be identified.

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