Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 739–743, February 1987
Botany

Hormonal regulation of protein synthesis associated with salt tolerance in plant cells

(abscisic acid/cultured plant cells/NaCl adaptation/26-kDa protein)

NARENDRA K. SINGH, P. CHRISTOPHER LAROSA, AVTAR K. HANDA, PAUL M. HASEGAWA, AND RAY A. BRESSAN*

Horticulture Department, Purdue University, West Lafayette, IN 47907

Communicated by John D. Axtell, September 23, 1986 (received for review July 14, 1986)

ABSTRACT **Cultured tobacco cells (*Nicotiana tabacum* L. cv. Wisconsin 38) synthesize a predominant 26-kDa protein upon exposure to abscisic acid (ABA). ABA also accelerates the rate of adaptation of unadapted cells to NaCl stress. The ABA-induced 26-kDa protein is immunologically cross-reactive to, and produces a similar pattern of peptides after partial proteolysis as, the major 26-kDa protein associated with NaCl adaptation. Both have pI values of >8.2. The synthesis of the ABA-induced 26-kDa protein is transient unless the cells are simultaneously exposed to NaCl stress. There is an association between increased intracellular accumulation of ABA during cell growth and commencement of synthesis of the 26-kDa protein. ABA induces the synthesis of an immunologically cross-reactive 26-kDa protein in cultured cells of several plant species. In tobacco plants, synthesis of the 26-kDa protein could be detected in several tissues but the highest level of expression was seen in outer stem tissue. In root tissues, exogenous ABA greatly stimulated the synthesis of 26-kDa protein as compared to outer stem tissue and leaf. We suggest that ABA is involved in the normal induction of the synthesis of 26-kDa protein and that the presence of NaCl is necessary for the protein to accumulate.**

Abscisic acid (ABA) has been implicated as having an important role in many plant physiological processes (1–3). However, molecular events mediated by ABA are poorly understood. ABA generally exerts an inhibitory effect on the metabolism of nucleic acids and proteins. Yet, it has been shown to induce the synthesis of specific mRNAs and proteins, notably in barley aleurone layers (4–7) and in other plants during seed germination and embryogenesis (8–14), desiccation and other stresses (15, 16), and dormancy (17). Barley aleurone cells have been used extensively to study ABA inhibition of giberellic acid-induced synthesis of α-amylase, where ABA appears to affect the level of transcription and transcript stability and processing (4, 5, 18–20). In other tissues, ABA appears to act at the level of translation (14, 17) or transcription (15, 16).

We have demonstrated that tobacco cells undergo phenotypic and physiological changes during exposure to and subsequent adaptation to NaCl and accumulate several proteins (21). In addition, we have shown that ABA accelerates the rate of adaptation to NaCl (22). In this report, we present evidence of the involvement of ABA in the synthesis of a 26-kDa protein which is associated with NaCl adaptation. We also show that the ABA-induced 26-kDa proteins from cultured cells of several plant species are immunologically related and that there is tissue-specific expression of the 26-kDa protein in differentiated tissues of tobacco plants.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

MATERIALS AND METHODS

Cell Culture. Suspension cultures of unadapted (S-0) cells and cells adapted to NaCl (25 g/liter) (S-25) of tobacco (*Nicotiana tabacum* L. cv. Wisconsin 38) were maintained as described (23). Also used were cell suspensions of millet (*Panicum miliaceum* L. cv. Abbar), soybean (*Glycine max* L. cv. Wayne), cotton (*Gossypium hirsutum* L. cv. Tamcot-SP37), carrot (*Daucus carota* cv. Danvers), potato (*Solanum tuberosum* L. cv. Superior), and tomato (*Lycopersicon esculentum* L. cv. VFNT-Cherry).

***In Vivo* Labeling.** Newly synthesized proteins were labeled in cells by adding $H_2^{35}SO_4$ to the cultures for 24 hr as described (21). Plant tissues were labeled with 100 μCi of $H_2^{35}SO_4$ (1 Ci = 37 GBq) by incubating discs of leaf and cut sections of root, pith, and stem outside of the pith (outer stem tissue) from greenhouse-grown tobacco plants in 2 ml of 20 mM potassium phosphate buffer (pH 7.0) containing 5 mM of each amino acid except cysteine and methionine. After labeling for 12 hr on a gyratory shaker at 25°C with constant room light, the tissues were washed in phosphate buffer, filtered, suspended in precooled (−20°C) acetone, and homogenized. All homogenized tissues were filtered and washed with cold acetone, air-dried, and stored desiccated. Filter-sterilized ABA was added to incubation flasks to a concentration of 10 μM at the time of inoculation.

Immunoprecipitation and Electrophoresis. Radiolabeled cells or tissues were homogenized in extraction buffer [20 mM potassium phosphate buffer (pH 7.0) containing 0.5 M NaCl, 0.1% $NaDodSO_4$, 0.5% Triton X-100, and 1 mM phenylmethylsulfonyl fluoride] and placed in boiling water for 2 min. A clear supernatant was obtained by centrifugation at 12,000 × *g* for 5 min. ^{35}S incorporation into protein was measured after trichloroacetic acid precipitation as described (21). Twenty microliters of partially purified (IgG fraction) rabbit antiserum against 26-kDa protein from S-25 cells was used to immunoprecipitate ^{35}S-labeled protein (equal cpm) in 500 μl of clear supernatant by incubation at 4°C overnight. Antibody–antigen complex was precipitated by addition of protein A attached to *Staphylococcus aureus* wall as described by Kessler (24). The immunoprecipitated protein complex was dissociated in extraction buffer containing 65 mM Tris·HCl (pH 6.8), 2% $NaDodSO_4$, 5% (vol/vol) glycerol, 5% 2-mercaptoethanol, 2 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride by incubation in boiling water followed by centrifugation at 12,000 × *g* for 2 min. Conditions of single and two-dimensional electrophoresis, partial proteolysis, staining, and fluorography of gels were as described (21).

Measurement of ABA. ABA was extracted from cultured cells by homogenizing in 80% methanol containing acetic acid (2 ml/liter) and butylated hydroxytoluene (10 mg/liter), and

Abbreviation: ABA, abscisic acid.

*To whom reprint requests should be addressed.

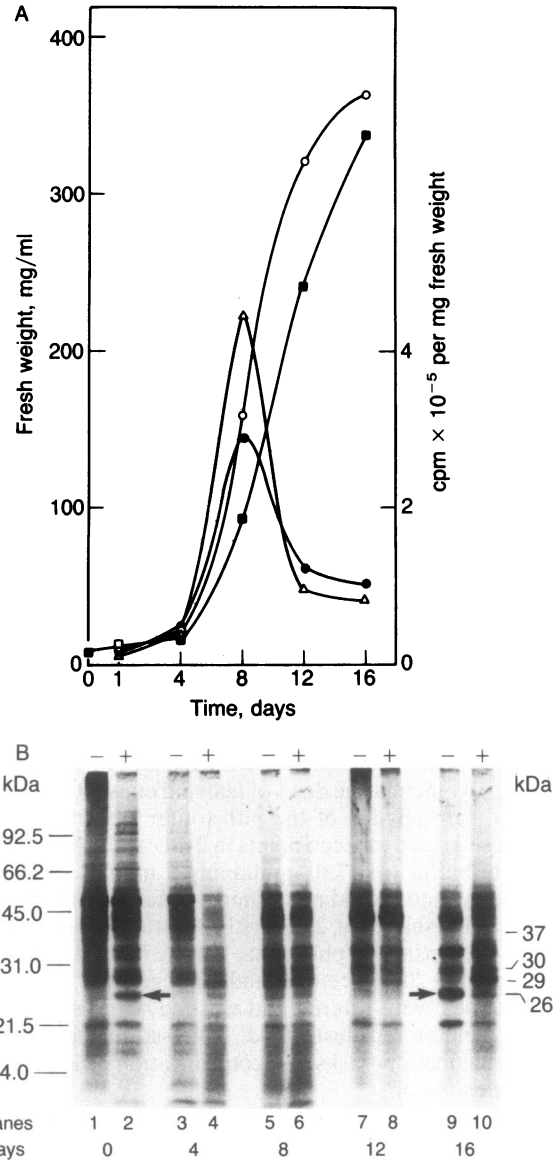

FIG. 1. (*A*) Effect of exogenous ABA on unadapted (S-0) cells in culture. Growth of cells in the presence (■) or absence (○) of ABA and ^{35}S incorporation in the presence (●) or absence (△) of ABA. (*B*) Fluorogram showing newly synthesized proteins from S-0 cells. Lanes corresponding to plus and minus ABA treatment are indicated at the top, while days after inoculation of each ± treatment is indicated at the bottom.

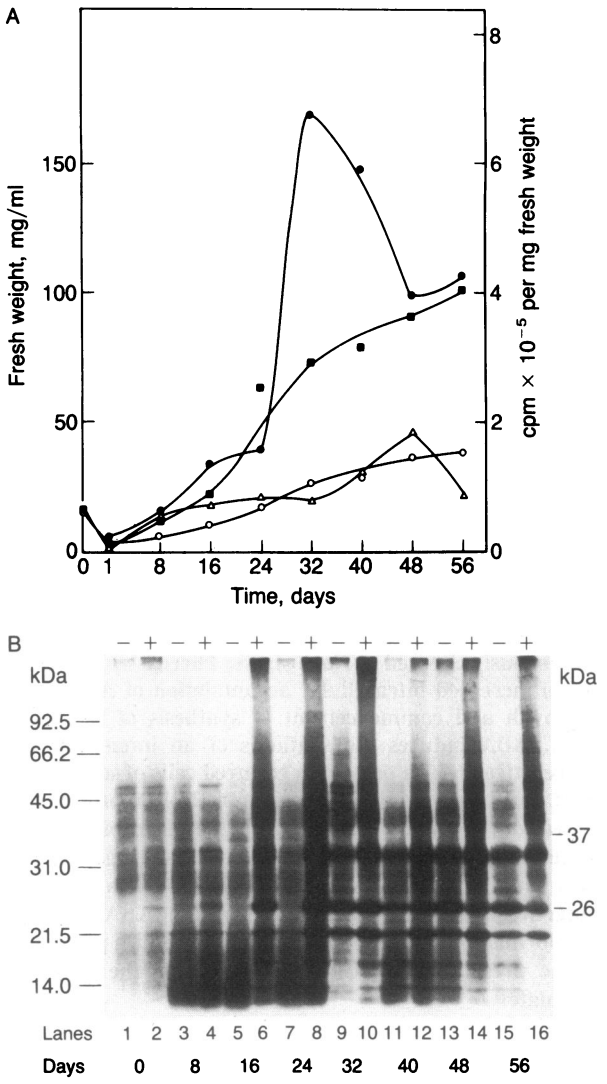

FIG. 2. (*A*) Effect of exogenous ABA on unadapted cells during adaptation to medium containing NaCl (16 g/liter). Growth of cells in the presence (■) and absence (○) of ABA and ^{35}S incorporation in the presence (●) and absence (△) of ABA. (*B*) Fluorogram showing newly synthesized proteins of S-0 cells in medium containing NaCl (16 g/liter). Lanes corresponding to plus and minus ABA treatment are indicated at top, while days after inoculation of each ± treatment is indicated at bottom.

recovery was monitored with [^{3}H]ABA (25). The homogenate was filtered and the methanol was removed by rotoevaporation. The pH was adjusted to 2.5–3.0 with 1 M HCl and the extract was partitioned against redistilled hexane on a column of diatomaceous earth (26). ABA was eluted with redistilled ethyl acetate and concentrated by rotoevaporation. The samples were further purified by TLC (27) and were methylated with diazomethane in ethyl acetate. The amounts of ABA methyl ester in derivatized samples were measured by GLC on a 1-m OV-11 packed column with a ^{63}Ni electron capture detector, based on detection of known amounts of ABA. The identity of putative ABA methyl ester peaks resolved by GLC was confirmed by comparing the retention time with the authentic ABA methyl ester, chromatographic behavior with [^{3}H]ABA (purified to constant specific radioactivity), UV light-induced racemerization of putative and authentic ABA to a 1:1 mixture of *cis*- and *trans*-ABA, and mass spectra of putative and authentic ABA methyl ester by electron impact mass spectrometry.

RESULTS

Effect of Exogenous ABA on Protein Synthesis in the Presence and Absence of NaCl. Exogenous ABA partially inhibited the growth and ^{35}S incorporation into protein of S-0 cells in the absence of NaCl stress (Fig. 1*A*). However, ABA increased the rate of adaptation of the S-0 cells in the presence of NaCl as much as 2- to 3-fold, as determined by increased fresh weight gain and increased ^{35}S incorporation into protein (Fig. 2*A*).

A 26-kDa protein was synthesized by S-0 cells in response to ABA on day 0 within 24 hr (Fig. 1*B*, lane 2). However, the synthesis of ABA-induced 26-kDa protein was greatly reduced by the 4th day after inoculation (Fig. 1*B*, lane 4). A low level of synthesis of 26-kDa protein began 1 hr after ABA treatment, and after 8 hr this synthesis increased to ≈8% of the total newly synthesized protein (data not shown). By the 16th day after inoculation, a 26-kDa protein was synthesized by S-0 cells in the absence of ABA (Fig. 1*B*, lane 9), and this synthesis was reduced in the ABA-treated cells (Fig. 1*B*, lane 10). However, the 26-kDa protein made by S-0 cells in the

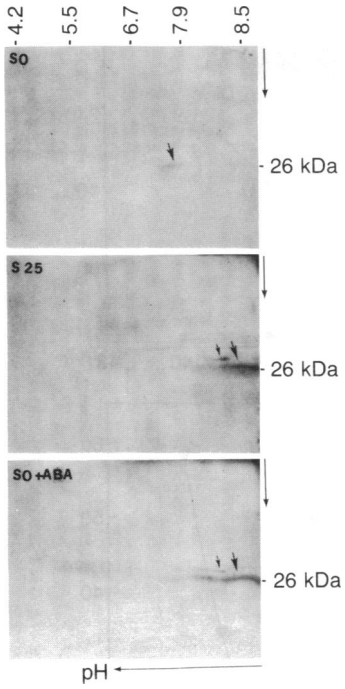

FIG. 3. Fluorogram of two-dimensional gel of immunoprecipitated ^{35}S-labeled 26-kDa protein from stationary phase (without any added ABA) S-0 cells on day 14 of culture growth, logarithmic phase S-25 cells on day 10 of culture growth and S-0 cells treated with ABA on day 2 of culture growth.

absence of ABA had a different pI than the 26-kDa protein made by S-25 cells or S-0 cells treated with ABA (Fig. 3). The cells treated with ABA produced two additional major polypeptides corresponding to 29 kDa and 30 kDa (Fig. 1*B*, lane 10) on day 16. Although the 26-kDa protein was synthesized in S-0 cells in the presence and in the absence of ABA treatment at different times, the protein was not accumulated in sufficient amount to be detected by Coomassie blue staining of the gel, even at the end of culture growth (data not shown).

Unadapted cells under NaCl stress (16 g of NaCl per liter) responded to exogenous ABA by rapidly synthesizing 26-kDa protein (Fig. 2*B*, lane 2), and this synthesis continued until the end of culture growth (Fig. 2*B*). When stressed in the absence of ABA, the cells began to synthesize 26-kDa protein only on day 32 (Fig. 2*B*, lane 9) and continued until day 56 and beyond. Under NaCl stress, 26-kDa protein accumulated in

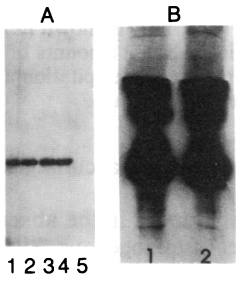

FIG. 4. (*A*) Fluorogram showing immunoprecipitated protein after ^{35}S labeling for 24 hr. ABA or NaCl was added to the culture along with ^{35}S. Lanes 1–4, immunoprecipitated protein from S-25 cells ^{35}S labeled at lag phase on day 7, S-25 cells at stationary phase on day 30, lag phase S-0 cells treated with ABA and ^{35}S-labeled on day 2, and stationary phase S-0 cells labeled on day 14, respectively. Lane 5, lag phase S-0 cells transferred to medium containing NaCl (10 g/liter) and labeled. Equal amounts of immunoprecipitated ^{35}S protein (4×10^3 cpm) were applied in each lane except in lane 5, where no immunoprecipitated ^{35}S was detected. (*B*) Peptide map after partial proteolysis by *S. aureus* V-8 protease of immunoprecipitated 26-kDa proteins from S-25 cells on day 7 (lane 1) and of S-0 cells treated with ABA on day 2 (lane 2). Equal amounts of immunoprecipitated ^{35}S protein were digested and applied to each lane.

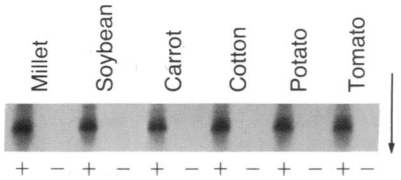

FIG. 5. Fluorogram of the ^{35}S-labeled and immunoprecipitated 26-kDa proteins from ABA-treated cultured plant cells of different species. ^{35}S-labeled protein (5×10^5 cpm) was immunoprecipitated from extracts of each species. Suspension cultured cells untreated and treated with ABA were used. Equal cpm of immunoprecipitated ^{35}S-labeled protein were applied in lanes marked (+), indicating ABA treatment. Equal volume of immunoprecipitated extract was applied to each lane, indicating no ABA treatment (−).

the cells and was easily detectable by Coomassie blue staining of proteins at the end of culture growth (data not shown). A coincidental inhibition of synthesis of 37-kDa protein with the commencement of synthesis of 26-kDa protein was observed in every experiment (Fig. 1*B*, lane 2; Fig. 2*B*, lanes 2 and 9).

ABA-Induced 26-kDa Protein Is Not Salt Shock-Induced but Appears to be the Same as 26-kDa Protein Made by Salt-Adapted Cells. Newly synthesized 26-kDa protein was detected in S-25 cells both in lag phase on day 7 and in stationary phase on day 30. It also was detected in S-0 cells treated with ABA early in growth on day 2 and in S-0 cells late in growth on day 14 (Fig. 4*A*, lanes 1–4, respectively). S-0 cells during early growth phase did not synthesize 26-kDa protein, even upon exposure to NaCl at 10 g/liter for 24 hr (Fig. 4*A*, lane 5) without ABA treatment.

Immunoprecipitated 26-kDa protein from early logarithmic phase S-25 cells and early logarithmic phase S-0 cells treated with ABA exhibited an identical peptide pattern after *S. aureus* V-8 proteolysis (Fig. 4*B*). This pattern was similar to that produced from proteolysis of 26-kDa protein from late growth phase S-0 cells (21). However, the pI of immunoprecipitated 26-kDa protein from late growth phase S-0 cells was 7.8, while the pI values of 26-kDa protein from ABA-treated early logarithmic phase S-0 cells and S-25 cells were >8.2 (Fig. 3). In both ABA-treated S-0 cells and S-25 cells, a slightly larger protein with a lower pI coimmunoprecipitated (marked with smaller arrows in Fig. 3) with the 26-kDa protein.

Tobacco 26-kDa Protein Is Immunologically Related to Protein from Different Plant Species. A 26-kDa protein could be immunoprecipitated from extracts of cultured cells of several plant species when treated with ABA (Fig. 5). The immunoprecipitated 26-kDa protein was highest in tomato cells and was considerably lower in potato, carrot, cotton, millet, and soybean cells. No detectable 26-kDa protein was present in cells not treated with ABA. Increased levels of immunologically related 26-kDa protein were observed after adaptation of cultured cells of different species (e.g., tomato, soybean, field bindweed) to NaCl at 10 g/liter or higher (unpublished result).

Expression of 26-kDa Protein in Differentiated Plant Tissues. Much more immunoprecipitable 26-kDa protein was synthesized in outer stem than in leaf or root of tobacco (Fig. 6). Exogenous ABA appeared to stimulate the synthesis of 26-kDa protein more in root than in outer stem or leaf. Immunoprecipitated 26-kDa protein could be detected in pith with or without ABA treatment only after very long exposure of x-ray film.

Association of 26-kDa Protein Synthesis with Endogenous ABA Level. The maximum level of ABA measured in both S-0 and S-25 cells was 0.75–0.8 nmol per g fresh weight (Fig. 7). The period of maximum ABA accumulation by S-0 cells was on day 10 (early growth phase), while in S-25 cells the

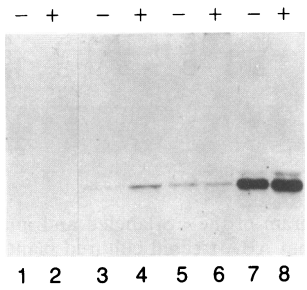

FIG. 6. Immunoprecipitation of 26-kDa protein from tobacco plant tissue treated with or without ABA. Equal cpm (2×10^5) of ^{35}S-labeled protein were used for immunoprecipitation and total immunoprecipitated ^{35}S-labeled protein was added to each lane. Plant tissues used were stem pith (lanes 1 and 2), root (lanes 3 and 4), leaf (lanes 5 and 6), and outer stem tissue (lanes 7 and 8). (+) and (−) ABA treatments are indicated.

maximum ABA level was attained in the lag phase between 4 and 5 days after inoculation. The synthesis of 26-kDa protein in S-0 cells began during late logarithmic phase between days 12 and 16, while in S-25 cells this synthesis was greatly increased during the lag phase on day 5 (21). These results indicate a coincidental increase of ABA content in the cells with the commencement of synthesis of 26-kDa protein in both NaCl-adapted and unadapted cells.

In S-0 cells that were treated with ABA and simultaneously exposed to NaCl (10 g/liter) (Fig. 8*A*), the endogenous ABA level increased very rapidly (within 6 hr) to 30 nmol per g fresh weight and then dropped to 4 nmol per g fresh weight on day 2. The immediate increase in the ABA content was caused at least in part by a rapid uptake of ABA, which was twice as high in the presence of NaCl (data not shown). A gradual increase in the level of ABA after day 3, to 24 nmol per g fresh weight on day 14 (Fig. 8*A*), was also the result of uptake of ABA from the medium (data not shown). ABA-treated cells under NaCl stress began synthesis of 26-kDa protein immediately after inoculation and the synthesis continued until day 40 (Fig. 8*A Inset*). Under these conditions, 26-kDa protein began to accumulate near the onset of cell growth.

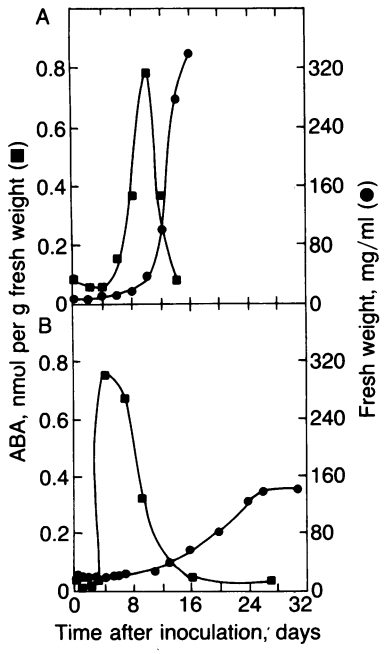

FIG. 7. Endogenous levels of ABA during growth of S-0 cells in medium without NaCl (*A*) and of S-25 cells in medium with NaCl (25 g/liter) (*B*).

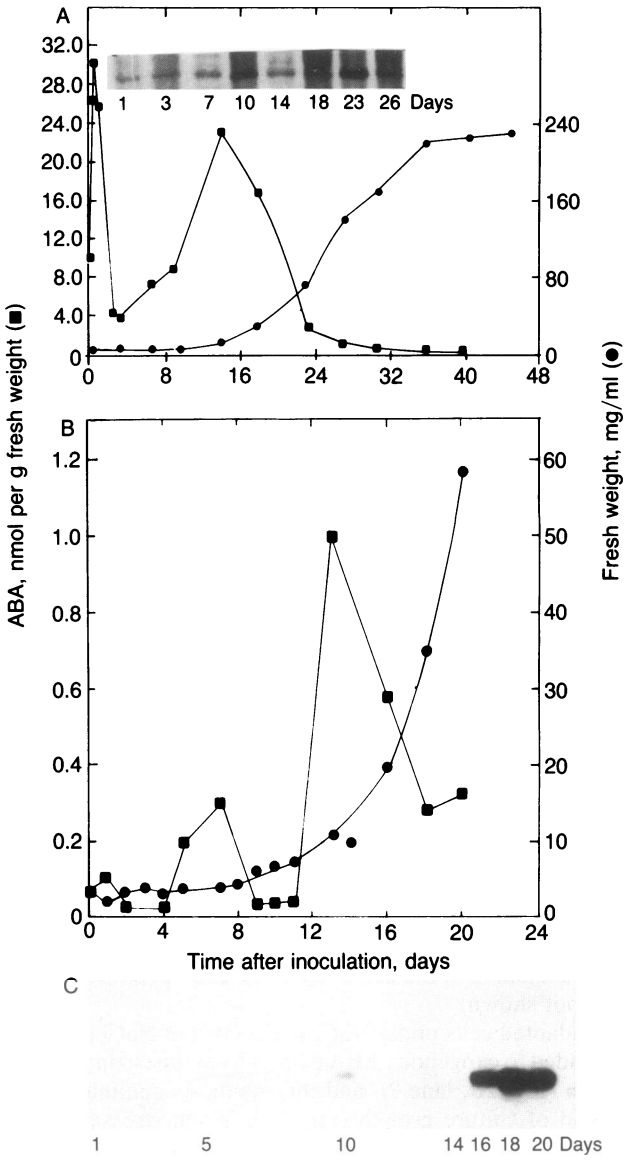

FIG. 8. Endogenous levels of ABA during adaptation of S-0 cells to medium containing NaCl (10 g/liter) in the presence (*A*) and absence (*B*) of ABA. The synthesis of immunoprecipitable 26-kDa protein in cells treated with ABA is shown in *A* (*Inset*) and in cells not treated with ABA in *C*. Equal amounts of total labeled protein (10^5 cpm) were used for immunoprecipitation and total immunoprecipitated ^{35}S was applied to each lane.

When S-0 cells were grown in the absence of ABA under NaCl stress, the level of endogenous ABA peaked on day 6 at 0.35 nmol per g fresh weight and on day 14 at 1 nmol per g fresh weight (Fig. 8*B*). Immunoprecipitated 26-kDa protein appeared as very faint bands from day 5 to day 14 and as greatly intensified bands from day 16 to day 40 (data shown until day 20 in Fig. 8*C*). These results demonstrate that, under NaCl stress, ABA accumulation did not occur immediately, and that the synthesis of 26-kDa protein followed an increase in the ABA content of the cells.

DISCUSSION

Synthesis of 26-kDa protein was induced within 8 hr by exogenous ABA in unadapted cells, but the synthesis did not continue beyond 4 days after treatment unless the cells were simultaneously exposed to NaCl, where the synthesis of

26-kDa protein also was rapidly induced by ABA and continued until the end of cell growth. In addition, the ABA-treated cells synthesized new 29-kDa and 30-kDa proteins on the 16th day. There was always an inhibition of synthesis of 37-kDa protein with the beginning of synthesis of 26-kDa protein. Hammerton and Ho (28) have shown that, in barley aleurone layer, a 37-kDa protease is inhibited by ABA.

The 26-kDa protein synthesized by NaCl-adapted cells and the one induced by ABA are similar based on immunological crossreactivity, production of identical peptide products upon proteolysis, and similar pI values. The 26-kDa protein synthesized by S-25 cells and by S-0 cells treated with ABA differed from the 26-kDa protein made by untreated S-0 cells by the period of the growth cycle during which they were made and by their pI values. This might be explained by the presence of a family of genes for 26-kDa protein or by posttranscriptional processing of message and/or post-translational modification.

Since anti-26-kDa protein raised against tobacco protein can immunoprecipitate 26-kDa protein from cells of unrelated plant species, this protein may be highly conserved. Lin and Ho (6) have reported that an ABA-induced 26-kDa protein in barley aleurone cells is not immunologically crossreactive with tobacco 26-kDa protein. This may not be surprising in view of the fact that the expression of the tobacco 26-kDa protein is tissue specific and appears to be induced by ABA mainly in root. When cells of species other than tobacco were adapted to grow in NaCl, they also began to accumulate 26-kDa protein (unpublished data). This suggests that the protein may be conserved to perform a function related to NaCl adaptation. Furthermore, the highest level of synthesis of the protein occurs in outer stem tissue of tobacco, which contains the water- and solute-transporting vascular tissues.

A close association between an increase in the endogenous ABA content, the synthesis of 26-kDa protein, and the subsequent adaptation of S-0 cells to NaCl, which involves considerable osmotic adjustment (29), suggests that these processes may be interdependent. Although the maximum level of endogenous ABA in adapted and unadapted cells was similar, S-0 cells accumulated ABA later in the growth cycle. This may have been too late (because of a lack of receptivity) to induce the synthesis of the 26-kDa protein made by adapted cells. Also, localization of ABA in S-0 cells may make the endogenous ABA ineffective. Synthesis of 26-kDa protein during or after adaptation to salt always followed an increase in endogenous ABA level. Although this does not prove that endogenous ABA levels regulate the synthesis of this protein, the correlation between the ABA content of the cells and the induction of 26-kDa protein synthesis provides a strong argument favoring this hypothesis.

The mRNA from S-25 cells and S-0 cells treated with ABA produced a precursor of 26-kDa protein when *in vitro* translated even in the presence of high (80 mM) NaCl concentration (30, 31). The translation of mRNA from S-0 cells was greatly inhibited under high NaCl conditions and a precursor of 26-kDa protein was not made. It may be that induced synthesis or posttranscriptional processing of mRNA for 26-kDa precursor protein is regulated by ABA and the accumulation of 26-kDa protein is modulated by the effect of NaCl on its relative translation. Given the complexity of events required for adaptation to NaCl, it is unlikely that there exists only one mode of action of ABA-mediated regulation of adaptation to NaCl. However, the ABA-regulated synthesis of 26-kDa protein appears to be one important component of the overall process of adaptation to NaCl.

We thank J. Clithero and G. McClatchey for excellent technical assistance and M. Goodwin for mass spectrometry. Original cell cultures of carrot, cotton, and millet were obtained from J. Widholm (University of Illinois), P. Heinstein (Purdue University), and N. C. Carpita (Purdue University), respectively. This is paper no. 10,827 (Purdue University Agricultural Experiment Station). This research was supported by U.S. Department of Energy Grant DE13109.

1. Addicott, F. T. & Carns, H. R. (1983) *Abscisic Acid*, ed. Addicott, F. T. (Praeger, New York).
2. Rhodes, D. (1986) in *The Biochemistry of Plants*, ed. Davies, D. D. (Academic, New York), Vol. 9, in press.
3. Walton, D. C. (1980) *Annu. Rev. Plant Physiol.* **31,** 453–489.
4. Higgins, T. J. V., Jacobson, J. V. & Zwar, J. A. (1982) *Plant Mol. Biol.* **1,** 191–215.
5. Jacobsen, J. V. & Beach, L. R. (1985) *Nature (London)* **316,** 275–277.
6. Lin, L. & Ho, D. T. H. (1986) *Plant Physiol.* **82,** 289–297.
7. Mozer, T. J. (1980) *Cell* **20,** 479–485.
8. Bray, E. A. & Beachy, R. N. (1985) *Plant Physiol.* **79,** 746–750.
9. Choinski, J. S., Jr., Trelease, R. N. & Doman, D. C. (1981) *Planta* **152,** 428–435.
10. Crouch, M. L. & Sussex, I. M. (1981) *Planta* **153,** 64–74.
11. Dommes, J. & Northcote, D. H. (1985) *Planta* **165,** 513–521.
12. Dure, L., III, Greenway, S. C. & Galau, G. A. (1981) *Biochemistry* **20,** 4162–4168.
13. Quatrano, R. S., Ballo, B. L., Williamson, J. D., Hamblin, M. T. & Mansfield, M. (1983) in *Plant Molecular Biology—UCLA Symposium on Molecular and Cellular Biology*, ed. Goldburg, R. B. (Liss, New York), Vol. 12, pp. 343–353.
14. Rodriguez, D., Nicolas, J. J., Aldasoro, J. J., Hernandez-Nistal, J., Babiano, M. J. & Matilla, A. (1985) *Planta* **164,** 517–523.
15. Heikkila, J. J., Papp, J. E. T., Schultz, G. A. & Bewley, J. D. (1984) *Plant Physiol.* **76,** 270–274.
16. Jacobsen, J. V., Hanson, A. D. & Chandler, P. C. (1986) *Plant Physiol.* **80,** 350–359.
17. Smart, C. C. & Trewavas, A. J. (1984) *Plant Cell Environ.* **7,** 121–132.
18. Ho, D. T. H. & Varner, J. E. (1976) *Plant Physiol.* **57,** 175–178.
19. Muthukrishnan, S., Chandra, G. R. & Allaugh, G. P. (1983) *Plant Mol. Biol.* **2,** 249–258.
20. Zwar, J. A. & Hooley, R. (1986) *Plant Physiol.* **80,** 459–463.
21. Singh, N. K., Handa, A. K., Hasegawa, P. M. & Bressan, R. A. (1985) *Plant Physiol.* **79,** 126–137.
22. LaRosa, P. C., Handa, A. K., Hasegawa, P. M. & Bressan, R. A. (1985) *Plant Physiol.* **79,** 138–142.
23. Hasegawa, P. M., Bressan, R. A. & Handa, A. K. (1980) *Plant Cell Physiol.* **21,** 1347–1355.
24. Kessler, S. W. (1981) *Methods Enzymol.* **73,** 442–459.
25. Pierce, M. & Raschke, K. (1980) *Planta* **148,** 174–182.
26. Dumbroff, E. B., Walker, M. A. & Dumbroff, P. A. (1983) *J. Chromatogr.* **256,** 439–446.
27. Zeevaart, J. A. D. (1977) *Plant Physiol.* **59,** 788–791.
28. Hammerton, R. W. & Ho, D. T. H. (1986) *Plant Physiol.* **80,** 692–697.
29. Binzel, M. L., Hasegawa, P. M., Handa, A. K. & Bressan, R. A. (1985) *Plant Physiol.* **79,** 118–125.
30. Bressan, R. A., Singh, N. K., Handa, A. K., Kononowicz, A. & Hasegawa, P. M. (1985) in *Plant Genetics—UCLA Symposium on Molecular and Cellular Biology*, ed. Freeling, M. (Liss, New York), Vol. 35, pp. 755–769.
31. Singh, N. K., Bressan, R. A., Hasegawa, P. M. & Handa, A. K. (1985) in *Abstracts First International Congress of Plant Molecular Biology*, ed. Galau, G. A. (Univ. of Georgia Press, Athens, GA), p. 138.