Proc. Natl. Acad. Sci. USA
Vol. 86, pp. 3169–3173, May 1989
Botany

Single-strand-preferring nuclease activity in wheat leaves is increased in senescence and is negatively photoregulated

(*Triticum aestivum*/photocontrol/activity gels/sodium dodecyl sulfate/polyacrylamide gel electrophoresis/cereals)

A. BLANK* AND THOMAS A. MCKEON

U.S. Department of Agriculture, Agricultural Research Service, Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710

Communicated by P. K. Stumpf, February 2, 1989 (received for review October 21, 1988)

ABSTRACT **Single-strand-preferring nucleases (EC 3.1.30.1) selectively cleave internucleotide bonds in single-stranded regions of predominantly duplex DNA and DNA·RNA hybrids and extensively degrade denatured DNA and RNA. The functions of single-strand-preferring nuclease in plants are unknown. We have monitored this nuclease activity in flag leaves of wheat (*Triticum aestivum* L. cv. Chinese Spring) undergoing natural senescence and in primary leaves of wheat seedlings undergoing dark-induced senescence. In flag leaves, nuclease activity remained at basal levels during the first 2 weeks after anthesis, while chlorophyll content increased to a maximum. Nuclease activity then rose in concert with a decline in chlorophyll, reaching a 16-fold elevation at 5 weeks post-anthesis, when 53% of the chlorophyll had been lost. When 8-day-old wheat seedlings were induced to senesce by placing them in darkness, nuclease activity rose without apparent lag, reaching a 13-fold elevation in 7 days, when 61% of the chlorophyll had been lost. The increase in nuclease activity was reversible upon reexposure of seedlings to light, a decline beginning without apparent lag. Reversibility was complete for plants that had been held in darkness for 5 days, with activity returning to the control level in 2 days. These senescence-related changes in nuclease activity, measured in conventional assays, were consistent with concomitant analysis by activity staining of sodium dodecyl sulfate/polyacrylamide gels. We conclude that an increase in single-strand-preferring nuclease activity is closely associated with wheat leaf senescence and that nuclease activity is subject to negative photoregulation.**

Endonucleases with a strong preference for polynucleotide substrates lacking ordered structure (EC 3.1.30.1) are well known in plants and in fungi (for reviews see refs. 1–4). These enzymes, such as S1 nuclease, discriminate between double- and single-stranded polynucleotides and have thus become an important reagent for identification and selective hydrolysis of single-stranded regions of DNA and DNA·RNA hybrids. More is known, in fact, about research applications of these single-strand-preferring (SSP) nucleases than is known about their physiological functions. The ability to selectively cleave single-stranded DNA is well suited for biological processes that require precise and limited restructuring of predominantly duplex DNA. Indeed, a role for SSP nuclease in recombination and DNA repair in fungi is indicated by the isolation of nuclease-deficient mutants that are recombination-defective and ultraviolet-sensitive (2). Selective cleavage of single-stranded regions of tRNA is also known to occur *in vitro*. On the other hand, the capacity of SSP nuclease to extensively degrade single-stranded polynucleotides (e.g., mRNA) is compatible with other, quite different biological roles. Despite its catalytic versatility, no specific functions have been documented for the nuclease in plants. This situation is especially interesting because plant tissues have been found to contain few DNases, whereas a multiplicity of DNases has been found in prokaryotic and animal cells (5, 6). Moreover, only one other enzyme that hydrolyzes DNA—i.e., phosphodiesterase I (EC 3.1.4.1 or EC 3.1.15.1)—has been reported to occur widely in plants, and its action is exonucleolytic (3, 4). Hence, it is possible that plants use the multifaceted SSP nuclease for some of the functions served by the DNases of other organisms. Here we present evidence that the nuclease is involved in both natural and dark-induced senescence of wheat leaves and that its activity is subject to negative photoregulation. Our findings are consistent with the suggestion that SSP nuclease participates in the extensive turnover of DNA and RNA that occurs during leaf senescence.

EXPERIMENTAL PROCEDURES

Plants. Wheat (*Triticum aestivum* L. cv. Chinese Spring) seedlings were grown in vermiculite in 7.5-cm pots at 25°C in a growth chamber under continuous light (3×10^{-4} $mol \cdot m^{-2} \cdot sec^{-1}$, cool white fluorescent and incandescent). Plants were given a modified Johnson's solution (7) [2 mM $(NH_4)H_2PO_4$/6 mM KNO_3/4 mM $Ca(NO_3)_2$/1 mM $MgSO_4$/20 μM FeNaEDTA/50 μM KCl/25 μM H_3BO_3/5 μM $MnSO_4$/2 μM $ZnCl_2$/0.5 μM $CuSO_4$/0.1 μM $(NH_4)_6Mo_7O_{24}$] on alternate days beginning on the fifth day and water on all other days. To induce senescence, 8-day-old seedlings (average length of primary leaves, about 21 cm) were placed in continuous darkness at 25°C. After various intervals in darkness, seedlings were returned to light. Primary leaves were sampled as described below.

Mature plants were started from seedlings grown for 4 weeks at 12°C with 8 hr of low-intensity light per day. Five such vernalized seedlings were placed in each 15-cm pot and kept in a greenhouse at 20–30°C. Plants were given water every third day and quarter-strength modified Johnson's solution (7) once weekly. As individual shoots reached anthesis, they were tagged and dated. Flag leaves were sampled as described below.

Sampling and Extraction. Eight-centimeter sections of primary leaves, cut 2 cm from the tip, were frozen in liquid nitrogen and stored at −70°C until used. Leaf sections (0.40 g) were ground in liquid nitrogen with a mortar and pestle, and the powder was carefully transferred to a chilled plastic tube. Ice-cold buffer (0.05 M Tris·HCl, pH 7.5/0.15 M NaCl/1 mM *N*-ethylmaleimide; 10 ml/g of leaf) was added and the suspensions were homogenized in an ice bath for *ca.* 15 sec by using a Brinkmann Polytron. Aliquots (1 ml) of homogenates were centrifuged for 8 min at top speed (*ca.* 14,000 × *g*) in an Eppendorf microcentrifuge at 6°C. The

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.

Abbreviation: SSP nuclease, single-strand-preferring nuclease.

*Present address: Department of Pathology, SM-30, University of Washington, Seattle, WA 98195.

supernatant solutions were frozen in small aliquots on dry ice and stored at −70°C.

Entire flag leaves were harvested at intervals after anthesis, leaf areas were determined, and the leaves were frozen at −70°C. Individual leaves (0.35–0.89 g) were ground in liquid nitrogen and extracted as described above with 4.5–11 ml of buffer per leaf. Specific area (cm^2/g) of leaves varied no more than 25% and showed no trend with age during the experimental period.

Assays. For chlorophyll determinations, aliquots of leaf homogenates were extracted in 80% acetone and centrifuged as described above. Chlorophyll content of the supernatants was determined according to Arnon (8). Nuclease activity was assayed by measuring the release of acid-soluble material from denatured calf thymus DNA. Assay mixtures (300 μl) containing 0.1% DNA, 0.1% bovine serum albumin, 0.1 M Tris·HCl (pH 7.5), and leaf extract (2.5–50 μl) were incubated for both 6 min and 66 min at 31°C. After addition of 1 ml of 3.4% perchloric acid, suspensions were held on ice for 10 min and then centrifuged for 5 min at *ca.* 14,000 × *g* in an Eppendorf microcentrifuge at 6°C. Absorbance of the supernatants was read at 260 nm, and the difference in A_{260} between 66 min and 6 min was determined; this difference was linear with added enzyme up to an A_{260} of 0.9. One unit is that amount of enzyme yielding an absorbance change of 1.0 per min per ml of incubation mixture.

Activity Gels. Nuclease was detected by activity staining in $NaDodSO_4$/12.5% polyacrylamide gels essentially as described (9). Gels were loaded with crude leaf extracts heated for 2 min at 100°C in buffer containing 2% $NaDodSO_4$, or 2% $NaDodSO_4$ and 10 mM dithiothreitol, as indicated in the text. Separation gels contained 0.3 mg of denatured calf thymus DNA or rRNA and 0.1 mg of bovine fibrinogen (10) per ml in the matrix and were run at room temperature. After electrophoresis, enzymes were renatured by gentle agitation of the gels for 45 min in 125 ml of 0.01 M Tris·HCl (pH 7.5) containing 20% (vol/vol) 2-propanol with three changes of solution, followed by shaking in buffer alone for 30 min with two changes of solution. Thereafter gels were incubated at room temperature in 0.1 M Tris·HCl (pH 7.5) for 15 hr to allow enzymatic degradation of embedded substrate and then were stained with toluidine blue.

RESULTS

Nuclease Activity in $NaDodSO_4$/Polyacrylamide Gels. An essential aspect of our study was the use of $NaDodSO_4$ activity gels to monitor nuclease activity in extracts of senescing leaves. In initial experiments, we characterized the nuclease from senescent and nonsenescent wheat leaves in these gels. Figs. 1 and 2 show that nuclease activity in leaf extracts is electrophoretically heterogeneous and has properties like those described for SSP nuclease purified from wheat seedlings (11), young wheat leaves (12), and other sources (1–4, 13). SSP nuclease is readily identified in our gels by its characteristic substrate preference—i.e., similar activity toward denatured DNA (Fig. 1*A*) and rRNA (Fig. 1*B*) and greatly reduced activity toward native DNA (Fig. 1*C*). Notably, SSP nuclease bands in the different leaf extracts in Fig. 1 comprise the only activity in gels cast with DNA. Also, the banding patterns observed in DNA-containing gels are preserved in detail in RNA-containing gels. A second set of bands in Fig. 1*B*, active only on RNA, is due to RNase WL_B (14). Consistent with published data (11), nuclease activity in gels is diminished but not abolished by EDTA (Fig. 1*D*) and by $MgCl_2$ (Fig. 1*E*). A broad pH optimum ranging from *ca.* pH 7.2 to pH 7.6, like that previously reported for the wheat leaf enzyme (12), was found in RNA-cast gels for the activity from both senescent and nonsenescent primary leaves. This pH dependence was established by incubating gel sections (not shown) in 0.1 M Tris·HCl/NaCl buffers of varying pH and constant ionic strength ($\mu = 0.15$). As shown in Fig. 2, nuclease activity bands in reduced and denatured samples display molecular weights ranging from 32,000 to 38,000. The most intense band in nonsenescent flag leaves is at M_r 35,000, while the two most intense bands in senescent leaves form a doublet at M_r 38,000. Renaturation of reduced and denatured nuclease is enhanced by Zn^{2+} (Fig. 2*B* vs. Fig. 2*A*), consistent with the finding that Zn^{2+} stabilizes the wheat seedling enzyme at low pH (11) and that SSP nuclease from several sources appears to be a zinc metalloprotein (1, 2).

We have relied on activity gels to correlate activity measured in solution assays with activity of defined polypeptides, but we have not relied on them for quantitation, for two reasons. First, different isozymes may not renature with equal efficiency, even if they have equal catalytic activity. Were isozyme distribution to change during senescence, quantitation of overall activity in gels could be misleading. A related reason is increased proteolysis in senescent tissue (16); such proteolysis may generate nicked polypeptides that retain secondary structure and catalytic activity in solution but renature poorly if at all in gels.

Nuclease Activity in Dark-Induced Senescence of Primary Leaves. When placed in darkness, intact wheat seedlings undergo a series of changes that provides a model of naturally occurring leaf senescence (17). These changes encompass the cardinal biochemical events of senescence, including loss of chlorophyll and photosynthetic capacity and loss of soluble protein. Light reverses the changes if restored sufficiently early. To determine whether SSP nuclease is involved in this

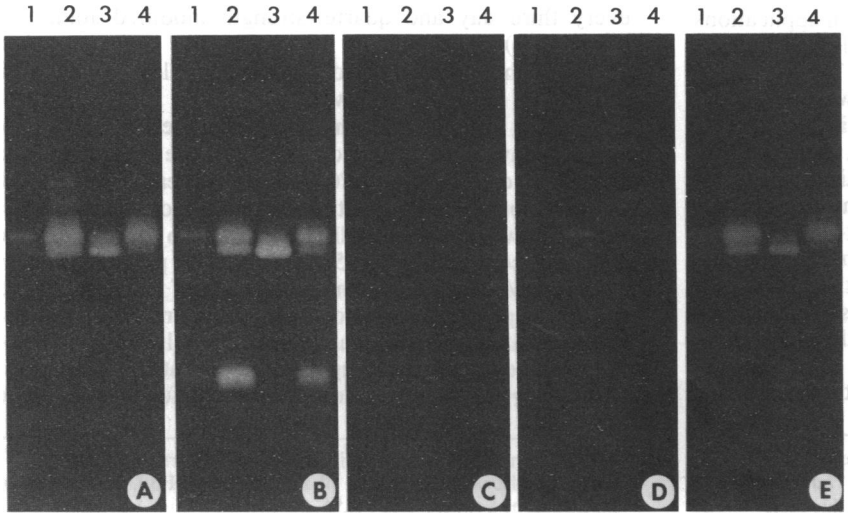

FIG. 1. Characterization of SSP endonuclease in $NaDodSO_4$ activity gels. Leaf extracts were heated in buffer containing 2% $NaDodSO_4$ and were subjected to polyacrylamide gel electrophoresis and activity staining as described in *Experimental Procedures*. (*A–C*) Gels contained denatured DNA (*A*), rRNA (*B*), or native DNA (*C*) in the matrix and were incubated in 0.1 M Tris·HCl (pH 7.5) for enzymatic degradation of embedded substrate. (*D* and *E*) Gels contained denatured DNA and were incubated in 0.1 M Tris·HCl (pH 7.5) containing either 1 mM EDTA (*D*) or 1 mM $MgCl_2$ (*E*). Gels were loaded with extracts of the specified wet weight of leaves: lanes 1, 13-day-old primary leaves, light-grown, 375 μg; lanes 2, primary leaves, light-grown 8 days and dark-treated next 5 days, 375 μg; lanes 3, flag leaf, 7 days pre-anthesis, 75 μg; lanes 4, flag leaf, 27 days post-anthesis, 75 μg.

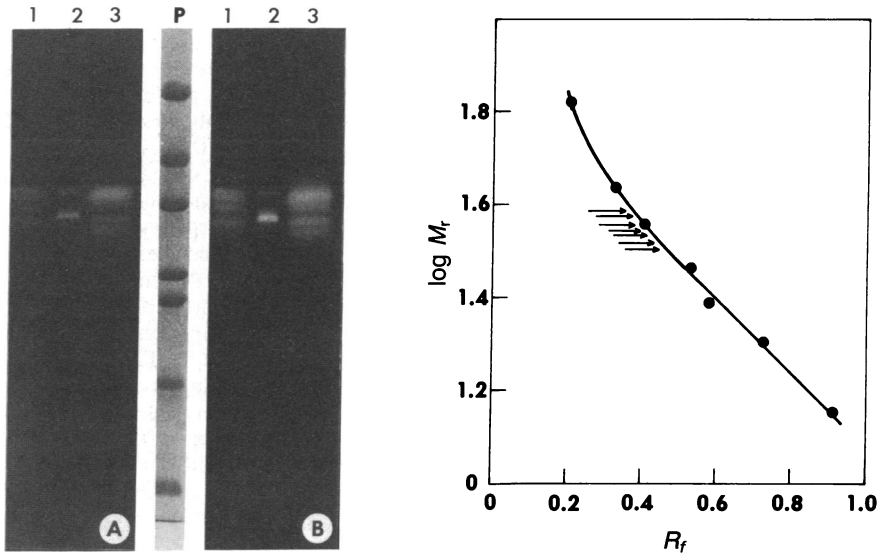

FIG. 2. Molecular weights of nuclease isozymes in $NaDodSO_4$ activity gels (15). (*Left*) Leaf extracts were heated in buffer containing 2% $NaDodSO_4$ and 10 mM dithiothreitol and were subjected to gel electrophoresis and activity staining. Renaturation was as described in *Experimental Procedures*, except that, for *B* only, 1 μM zinc acetate was included in the final 15 min of the 2-propanol wash and in the first 15 min of the succeeding buffer wash. Gels were loaded with extracts of the specified wet weight of leaves: lanes 1, 16-day-old primary leaves, grown in light 8 days and then dark-treated 8 days, 3.1 mg; lanes 2, flag leaf, 7 days pre-anthesis, 2.4 mg; lanes 3, flag leaf, 37 days post-anthesis, 4 mg. A section of the same gel (lane P) was loaded with protein standards (Sigma marker kit) and stained with Coomassie blue. (*Right*) A standard curve relating mobility and log M_r was constructed using the retardation factors (R_f) of the proteins and their molecular weights as follows: bovine serum albumin, 66,000; ovalbumin, 43,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; carbonic anhydrase, 29,000; trypsinogen, 24,000; trypsin inhibitor, 20,100; α-lactalbumin, 14,200. Arrows indicate the R_f values of nuclease bands revealed by activity staining.

model process, we monitored its activity during the senescence and regreening of wheat seedlings. Fig. 3 displays the changes in nuclease activity and chlorophyll content of primary leaves that occurred when 8-day-old seedlings were placed in darkness. Activity rose without apparent lag, increasing to 13 times the control level in 7 days. During this period of nuclease accumulation, chlorophyll content declined to 39% of control. Both the loss of chlorophyll and the increase in nuclease activity accelerated after 2 days in darkness. When plants were placed in light after 4 or 5 days in the dark, nuclease activity returned to normal levels; the decline began without apparent lag and was complete in 2 days. Concomitant resynthesis of chlorophyll occurred, but regreening was relatively slow. Notably, the dark-induced increase in nuclease activity was largely reversible even after 6 days. To verify that the changes in activity measured in assays were due to SSP nuclease, extracts were examined in $NaDodSO_4$ activity gels. Activity bands having the mobility and electrophoretic pattern of SSP nuclease increased and decreased in intensity in parallel with the changes measured in solution (Fig. 3 *Inset*). No other activity appeared in the gels. The foregoing data demonstrate that SSP nuclease activity is correlated with dark-induced senescence. It is important to emphasize that (*i*) changes in nuclease activity begin rapidly upon deprivation and restoration of light, (*ii*) the dark-induced increase is large and extended in duration, and (*iii*) the increase, in its early stages, is fully reversible by light. These observations provide evidence for a close association between SSP nuclease and the senescence process and for negative photoregulation of the enzyme.

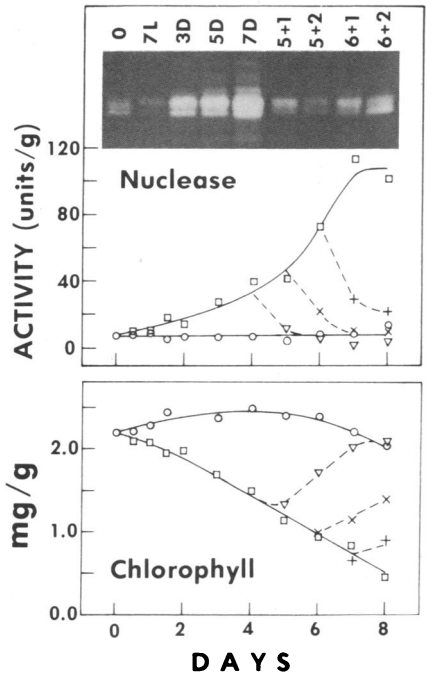

FIG. 3. SSP nuclease activity in primary leaves of seedlings during dark-induced senescence and regreening. Eight-day-old seedlings, grown in light, were either kept in light (○) or placed in darkness (□). After 4 (▽), 5 (×), or 6 (+) days, dark-treated seedlings were reexposed to light to assess the reversibility of dark-induced changes. (*Upper*) Nuclease activity in assays and gel (*Inset*). Samples representing 375 μg (wet weight) of leaves in the gel are identified as follows: 0, day 0; 7L, 7 days light; 7D, 7 days dark; 5+2, 5 days dark followed by 2 days light. (*Lower*) Chlorophyll content.

Nuclease Activity in Natural Senescence of Flag Leaves. To ascertain whether SSP nuclease is involved in natural as well as in dark-induced senescence, we monitored nuclease activity in the flag leaves of maturing wheat (Fig. 4). Activity was low for the first 2 weeks after anthesis, during which time chlorophyll content rose to a maximum. Nuclease activity then increased to reach a 16-fold elevation at 5 weeks post-anthesis, while chlorophyll content declined 53%. Activity gels (Fig. 4, *Inset*) showed enhanced activity of bands previously identified as SSP nuclease (Fig. 1), and no other bands were observed. Notably, extracts of more-senescent leaves showed diffuse banding patterns and band intensity did not closely parallel activity in assays. These findings are

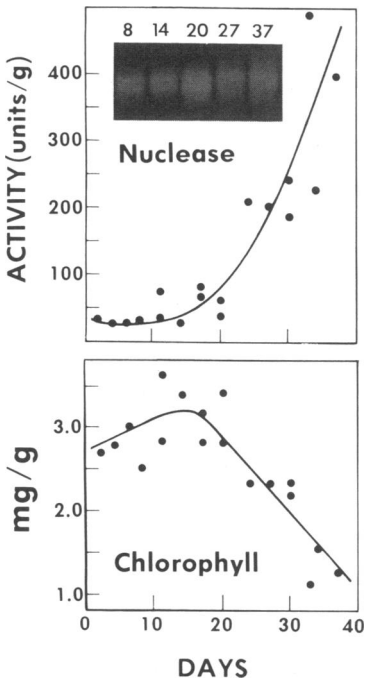

FIG. 4. SSP nuclease activity in senescing flag leaves. Beginning at anthesis (day 0), individual flag leaves were harvested from wheat plants growing in a greenhouse. (*Upper*) Nuclease activity in leaf extracts. Aliquots of extracts, representing 75 μg (wet weight) of leaves taken on various days after anthesis, were subjected to electrophoresis and activity staining (*Inset*). (*Lower*) Chlorophyll content of leaf homogenates.

consistent with increased proteolysis in senescing leaves (16). Proteolysis could generate nicked polypeptides that retain secondary structure and activity in solution assays but migrate heterogeneously and renature poorly in $NaDodSO_4$/polyacrylamide gels. We conclude that an increase in nuclease activity is correlated with the progression of flag-leaf senescence.

DISCUSSION

Leaf senescence is the terminal, genetically controlled developmental process in the life of a leaf. Senescence can be triggered by endogenous mechanisms and by environmental factors, such as reduction in light, and is reversible in its early stages (16). During senescence, the leaf gradually ceases to serve as a source of photosynthate and becomes, instead, a source of mobilized carbon, nitrogen, and minerals that are transported elsewhere in the plant. Senescence thus assures reutilization, rather than loss, of chemical constituents of the aging leaf. Though concerned with tissue destruction, programmed leaf senescence is, in essence, an adaptive process that promotes survival of entire plant populations (monocarpic or endogenously triggered senescence) and of individual plants in unfavorable, unpredictable environmental conditions (tactical or strategic senescence). The reversibility of early senescence enhances adaptiveness, adding flexibility to the plant's initial responses to changing growth/survival imperatives (16).

Dark-induced senescence of seedlings (17) appears to be a useful model of naturally occurring leaf senescence, although the extent to which the two processes share constituent biochemical events is not fully known. Our data establish that in wheat a large increase in SSP nuclease activity is common to both naturally occurring senescence of mature flag leaves and dark-induced senescence of primary leaves. In primary leaves, the increase is reversible upon restoration of light. The increase in nuclease activity of leaf extracts is evident in $NaDodSO_4$ activity gels as well as in solution assays, so it is likely that the increase reflects enhanced amounts of the catalytically active protein rather than dissociation of the nuclease from a noncovalently bound inhibitor. Moreover, the increase in activity is seen when whole-leaf homogenates, instead of extracts, are applied to gels (unpublished data). This observation indicates that the increased activity in extracts reflects larger amounts of nuclease in leaf tissue rather than enhanced extractability of the enzyme from fragile senescent cells and organelles.

Our demonstration of an increase in SSP nuclease activity during wheat leaf senescence was foreshadowed by the early observation that the EDTA sensitivity of total RNase activity increases during aging of detached wheat leaves (18). In fact, our findings concerning the wheat leaf nuclease may be generally applicable to cereals. For example, in primary leaves of 1-week-old oat seedlings, nucleolytic activity on DNA represents a relatively minor component of that on RNA, but it is a major component in 3-week-old leaves. Also, illumination of etiolated oat seedlings results in a decrease in nuclease activity (19). Moreover, we have found that barley seedlings held in the dark for 5 days exhibit a 31% loss of chlorophyll and a 3.6-fold increase in nuclease activity; DNA-containing $NaDodSO_4$ activity gels show increased intensity of a band that migrates slightly faster than the major nuclease bands in wheat seedlings (unpublished data).

In wheat then, and perhaps in cereals generally, SSP nuclease activity is regulated both by light and by an endogenous factor(s) associated with senescence. Light, acting through photoreceptors, regulates the activity of diverse plant enzymes (20, 21). However, in the great majority of cases the effect of light is to increase activity. SSP nuclease is thus one of a few plant proteins (e.g., phytochrome, protochlorophyllide reductase, lipoxygenase) known to be under negative photoregulation. The detailed mechanisms by which light regulates enzyme activities are not well understood but are currently under scrutiny (20–24). The mechanisms that regulate the biochemical events of endogenously signalled senescence are not known. However, it has been hypothesized that these mechanisms are encompassed by a genetic program that controls the initiation and progression of senescence (16). Our finding that an increase in SSP nuclease activity coincides closely with the onset of senescence, measured as loss of chlorophyll, suggests that control of nuclease expression is closely linked to the regulatory mechanisms that initiate both endogenously signaled and dark-induced senescence. Study of the mechanisms that control SSP nuclease expression should provide insights concerning the genetic regulation of senescence and its interconnection with photoregulation.

We believe that SSP nuclease may have at least two major roles in wheat leaf senescence. First, the nuclease may mediate changes in gene expression that underlie the biochemical events of senescence. This suggestion reflects the broader idea that SSP nuclease could be widely involved in mediating the altered genetic expression characteristic of many developmental processes. Not only has increased SSP nuclease activity been observed in the reversible stage of dark-induced wheat seedling senescence and in early flag-leaf senescence, but high levels of the enzyme have been found in very young seedlings (11) and in expanding vs. mature leaves (unpublished data). One specific function of the nuclease in altering genetic expression may be to facilitate changes in transcription, perhaps by promoting a more open, accessible chromatin structure (25–27) and/or increasing the number of nonspecific entry points (nicks) for RNA polymerase (28–30). Consistent with this view, nuclease activity has been found in association with barley chromatin (31, 32), though the activity remains to be definitively characterized.

Endogenous endonuclease action has also been observed at hypersensitive sites in potentially active transcription regions (33). Nicking of chromatin has been reported to occur during differentiation (34–36) and exogenous nuclease can transform the higher-order chromatin structure of undifferentiated cell nuclei to a structure resembling that of chromatin in differentiated cells (37), leading to the inference that DNA-nicking may be associated with changing genetic expression. Integrity of such nicked DNA would have to be restored by DNA-repair systems that seal the single-strand breaks formed, for example, during differentiation (35, 36). Failure of such repair would logically promote the genomic dissolution characteristic of irreversible leaf senescence (38, 39). Cell death resulting from inadequate DNA repair is known in animal cells (40) and aged, germinating cereal seeds (41). Alternatively to acting on DNA, SSP nuclease could facilitate altered gene expression by mediating RNA turnover. Although the action of the nuclease on RNA *in vitro* can be nonselective, targeting of nuclease cleavage to specific RNA molecules *in vivo* could be accomplished through differential accessibility of potential substrates. There is precedent, in fact, for selective turnover of rRNA and mRNA by broad-specificity RNases—e.g., the α-sarcin toxic factor and RNase L (42, 43). In addition to mediating changes in gene expression, SSP nuclease may have a second major role in leaf senescence, which is to promote recycling of the nitrogenous bases in RNA and DNA. This role in the mobilization of nutrients is in accord with a traditional view of nucleolytic enzymes as instruments of mass degradation and scavenging (4).

Historically, nucleic acid-degrading enzymes have been widely associated with plant senescence and death. Once-prevalent hypotheses include the ideas that nucleolytic enzymes may be a major factor triggering senescence and that their very presence leads inexorably to disintegration of DNA and RNA and to cell death. However, our data show that elevated levels of SSP nuclease are compatible with integrity of the genome and with cell survival, and this elevation, like leaf senescence itself, is reversible. These findings are consistent with the current view that the action of hydrolytic enzymes upon their substrates can be controlled by various mechanisms, and that it is the genetically programmed demise of such control mechanisms [e.g., compartmentalization (44)] that marks irreversible senescence and commitment to cell death.

We wish to thank Ms. Emily Grey for typing the manuscript and Mr. Robert David Wong for photographing gels and preparing figures. We thank the U.S. Department of Agriculture–Agricultural Research Service Research Associate Program for the 1984 T. W. Edminster Award, which supported A.B.

1. Lehman, I. R. (1981) *Enzymes* **14,** 193–201.
2. Shishido, K. & Ando, T. (1982) in *Nucleases*, eds. Linn, S. M. & Roberts, R. J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 155–185.
3. Wilson, C. M. (1982) in *Isozymes: Current Topics in Biological and Medical Research*, eds. Ratizzi, M. C., Scandalios, J. G. & Whitt, G. S. (Liss, New York), Vol. 6, pp. 33–54.
4. Farkas, G. L. (1982) *Encycl. Plant Physiol. New Ser.* **14B,** 224–262.
5. Linn, S. (1982) in *Nucleases*, eds. Linn, S. M. & Roberts, R. J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 291–309.
6. Sierakowska, H. & Shugar, D. (1977) *Prog. Nucleic Acid Res. Mol. Biol.* **20,** 59–130.
7. Johnson, C. M., Stout, P. R., Broyer, T. C. & Carlton, A. B. (1957) *Plant Soil* **8,** 337–353.
8. Arnon, D. I. (1949) *Plant Physiol.* **24,** 1–15.
9. Blank, A., Sugiyama, R. H. & Dekker, C. A. (1982) *Anal. Biochem.* **120,** 267–275.
10. Blank, A., Silber, J. R., Thelen, M. P. & Dekker, C. A. (1983) *Anal. Biochem.* **135,** 423–430.
11. Hanson, D. M. & Fairley, J. L. (1969) *J. Biol. Chem.* **244,** 2440–2449.
12. Chevrier, N. & Sarhan, F. (1980) *Plant Sci. Lett.* **19,** 21–31.
13. Brown, P. & Ho, T. (1986) *Plant Physiol.* **82,** 801–806.
14. Blank, A. & McKeon, T. A. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44,** 473 (abstr. 366).
15. Blank, A. & Dekker, C. A. (1981) *Biochemistry* **20,** 2261–2267.
16. Stoddart, J. L. & Thomas, H. (1982) *Encycl. Plant Physiol. New Ser.* **14A,** 592–636.
17. Wittenbach, V. A. (1977) *Plant Physiol.* **59,** 1039–1042.
18. Sodek, L. & Wright, S. T. C. (1969) *Phytochemistry* **8,** 1629–1640.
19. Wyen, N. V., Erdei, S. & Farkas, G. L. (1971) *Biochim. Biophys. Acta* **232,** 472–483.
20. Lamb, C. J. & Lawton, M. A. (1983) *Encycl. Plant Physiol. New Ser.* **16A,** 213–257.
21. Tobin, E. M. & Silverthorne, J. (1985) *Annu. Rev. Plant. Physiol.* **36,** 569–593.
22. Fluhr, R., Kuhlemeier, C., Nagy, F. & Chua, N.-H. (1986) *Science* **232,** 1106–1112.
23. Thompson, W. F., Kaufman, L. S. & Watson, J. C. (1986) *BioEssays* **3,** 153–159.
24. Nagy, F., Kay, S. A. & Chua, N.-H. (1988) *Trends Genet.* **4,** 37–41.
25. Eissenberg, J. C., Cartwright, I. L., Thomas, G. H. & Elgin, S. C. R. (1985) *Annu. Rev. Genet.* **19,** 485–536.
26. Cartwright, I. L., Keene, M. A., Howard, G. C., Abmayr, S. M., Fleischmann, G., Lowenhaupt, K. & Elgin, S. C. R. (1982) *CRC Crit. Rev. Biochem.* **13,** 1–86 (see pp. 22, 50, 59).
27. Reeves, R. (1984) *Biochem. Biophys. Acta* **782,** 343–393.
28. Chandler, D. W. & Gralla, J. (1980) *Biochemistry* **19,** 1604–1612.
29. Lewis, M. K. & Burgess, R. R. (1980) *J. Biol. Chem.* **255,** 4928–4936.
30. von Hippel, P. H., Bear, D. G., Morgan, W. D. & McSwiggen, J. A. (1984) *Annu. Rev. Biochem.* **53,** 389–446.
31. Srivastava, B. I. S. (1968) *Biochem. Biophys. Res. Commun.* **32,** 533–538.
32. Kalinski, A., Chandra, G. R. & Muthukrishnan, S. (1986) *J. Biol. Chem.* **261,** 11393–11397.
33. Bonven, B. & Westergaard, O. (1982) *Nucleic Acids Res.* **10,** 7593–7608.
34. Farzaneh, F., Shall, S. & Johnstone, A. P. (1985) *FEBS Lett.* **189,** 62–66.
35. Johnstone, A. P. (1984) *Eur. J. Biochem.* **140,** 401–406.
36. Dawson, B. A. & Lough, J. (1988) *Dev. Biol.* **127,** 362–367.
37. Touchette, N. A., Anton, E. & Cole, R. D. (1986) *J. Biol. Chem.* **261,** 2185–2188.
38. Chen, S. & Srivastava, B. I. S. (1986) *Mech. Aging Dev.* **34,** 57–61.
39. Harris, J. B., Schaefer, V. G. & Miksche, J. P. (1984) *Plant Cell Physiol.* **25,** 225–231.
40. Ayusawa, D., Shimizu, K., Koyama, H., Takeishi, K. & Seno, T. (1983) *J. Biol. Chem.* **258,** 12448–12454.
41. Osborne, D. J. (1983) *Can. J. Bot.* **61,** 3568–3577.
42. Endo, Y., Huber, P. W. & Wool, I. G. (1983) *J. Biol. Chem.* **258,** 2662–2667.
43. Wreschner, D. H., James, T. C., Silverman, R. H. & Kerr, I. M. (1981) *Nucleic Acids Res.* **9,** 1571–1581.
44. Thomson, W. W. & Platt-Aloia, K. A. (1987) in *Plant Senescence: Its Biochemistry and Physiology*, eds. Thomson, W. W., Nothnagel, E. A. & Huffaker, R. C. (American Society of Plant Physiologists, Rockville, MD), pp. 20–30.