Isolation and Characterization of Senescence-induced cDNAs Encoding Deoxyhypusine Synthase and Eucaryotic Translation Initiation Factor 5A from Tomato*

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Full-length cDNA clones encoding deoxyhypusine synthase (DHS) and eucaryotic initiation factor 5A (eIF-5A) have been isolated from a cDNA expression library prepared from tomato leaves (Lycopersicon esculentum, cv. Match) exposed to environmental stress. DHS mediates the first of two enzymatic reactions that activate eIF-5A by converting a conserved lysine to the unusual amino acid, deoxyhypusine. Recombinant protein obtained by expressing tomato DHS cDNA in Escherichia coli proved capable of carrying out the deoxyhypusine synthase reaction in vitro in the presence of eIF-5A. Of particular interest is the finding that DHS mRNA and eIF-5A mRNA show a parallel increase in abundance in senescing tomato flowers, senescing tomato fruit, and environmentally stressed tomato leaves exhibiting programmed cell death. Western blot analyses indicated that DHS protein also increases at the onset of senescence. It is apparent from previous studies with yeast and mammalian cells that hypusine-modified eIF-5A facilitates the translation of a subset of mRNAs mediating cell division. The present study provides evidence for senescence-induced DHS and eIF-5A in tomato tissues that may facilitate the translation of mRNA species required for programmed cell death.

Eucaryotic translation initiation factor 5A (eIF-5A) is deemed to be present in all eucaryotic cells and, following post-translational modification to an activated form, appears to play a role in the initiation of protein synthesis (1). This modification entails the addition of a butylamine residue derived from spermidine to a highly conserved lysine of inactive eIF-5A, resulting in the formation of the unusual amino acid, hypusine, and activated eIF-5A (2). The first reaction leading to the formation of hypusine is catalyzed by deoxyhypusine synthase (DHS; EC 1.1.1.249), an enzyme that adds butylamine to the conserved lysine of inactive eIF-5A to form deoxyhypusine. A sub-

sequent reaction in which deoxyhypusine is converted to hypusine is catalyzed by deoxyhypusine hydroxylase (EC 1.14.99.29) (2, 3). Hypusine modification apparently ensues immediately following translation of eIF-5A inasmuch as there is no accumulation of either eIF-5A precursor (lysine form) or eIF-5A intermediate (deoxyhypusine form) unless cells are treated with inhibitors of either deoxyhypusine synthase or deoxyhypusine hydroxylase (1).

Hypusine-containing eIF-5A is not required for global protein synthesis. Indeed, in eIF-5A-deficient yeast, protein synthesis is only inhibited by ~30%, and this is acompañied by only a slight change in the polysome profile (1, 4, 5). Rather, activated eIF-5A appears to facilitate the translation of specific subsets of mRNA. For example, yeast cells in which DHS has been inactivated are incapable of dividing and simply enlarge (1, 6). These observations have prompted the proposal that hypusine-containing eIF-5A facilitates translation of the subset of mRNAs required for cell division and hence is necessary for cell proliferation (1, 7). Similarly, inhibitors of the synthesis of spermidine, which is required for DHS activity, have been shown to decrease cell division (1, 7, 8).

Although it seems clear that hypusine-containing eIF-5A facilitates protein synthesis, the way in which it does so is not fully understood. The protein was initially identified as a putative translation initiation factor based on its ability to stimulate methionyl-puromycin synthesis under in vitro conditions (9), but this has since been questioned in light of the fact that a similar effect on translation is not observed in situ (10, 11). More recent evidence suggests that eIF-5A facilitates protein synthesis by promoting nuclear export of specific mRNAs (10). It has also been proposed that eIF-5A may be involved in mRNA turnover, acting downstream of decapping (11).

Isoforms of the gene encoding eIF-5A have also been isolated from plant tissue. For example, two isoforms of eIF-5A exhibiting tissue-specific expression have been isolated from tobacco (GenBank™ accession numbers X635411 and X635412) (12), and recently DHS was also cloned from tobacco (GenBank™ accession number AJ242017) (13). The eIF-5A gene has also been cloned from humans (GenBank™ accession number I53801), fungi (GenBank™ accession numbers P19211 and P23301), yeasts (GenBank™ accession numbers D83166), Zea mays (GenBank™ accession number Y07920), Solanum tuberosum (GenBank™ accession numbers AB004823 to AB004827), and alfalfa (GenBank™ accession number X59441). However, apart from an expected involvement in translation, no specific function has yet been ascribed to plant DHS and hypusine-containing eIF-5A. The finding that there are isoforms of the plant eIF-5A gene has prompted the proposal that the different isoforms facilitate the translation of subsets of mRNA required for specific physiological functions, including photosynthesis (12). In the present study, we de-
cribe the isolation and characterization of cDNA clones from tomato that encode DHS and eIF-5A. Of particular interest is the finding that these genes are both up-regulated at the onset of natural senescence and in the event of cell death attributable to environmental stress, for this indicates that hypusine-modified eIF-5A may facilitate translation of the suite of mRNAs required for programmed cell death.

EXPERIMENTAL PROCEDURES

Plant Material—Tomato seedlings (Lycopersicon esculentum, cv. Mortgage) were grown in 8-inch pots for 8 weeks under greenhouse condi-
tions. By this stage, the seedlings had developed three pairs of leaves. Cotyledons for Western blot analysis were harvested at 5, 7, 11, 15, and 20 days after sowing. Flowers for Northern blot analysis were harvested at tight bud and open plus senescing stages of development. Fruit for Northern analysis was harvested at breaker (one-tenth of the surface area beginning to turn color), pink, red firm, and red soft stages of development.

RNA Isolation and Construction of a cDNA Library—For preparation of a cDNA library, total RNA was isolated from drought-stressed leaves of 8-week-old tomato seedlings according to Davis et al. (14). Drought stress was induced by removing the roots from the seedlings and placing the cut stems in 2 M sorbitol for 6 h. Poly(A)- RNA was purified using a PolyAtract mRNA Isolation System (Promega). Double-stranded cDNA was prepared using the ZAP express cDNA synthesis kit (Stratagene).

Library Screening—Approximately 5 × 10⁶ clones were screened with a DHS probe obtained by reverse transcriptase-polymerase chain reaction (RT-PCR; PerkinElmer Life Sciences GeneAmp DNA Thermal Cycler, model 2400). Template RNA for RT-PCR was isolated from leaves of 8-week-old tomato plants that had been chill-injured for 2 days at 5 °C in a growth chamber (16-h light/8-h dark photoperiod) and subsequently rewarmed for 6 h in the greenhouse. Reverse transcription was performed by adding 500 ng of total RNA, 50 µl of murine leukemia virus reverse transcriptase (PerkinElmer Life Sciences), 2.5 mM MgCl₂, 1 mM of each dNTP, 1 unit of RNase inhibitor, and 2.5 µM oligo(dT) primer to 50 µl of reaction mixture containing 1× RT-PCR buffer (Stratagene) and heated to 99 °C for 5 min to inactivate the reverse transcriptase. The reaction mixture was incubated at room temperature for 5 min, at 42 °C for 50 min, and then heated to 99 °C for 5 min to inactivate the reverse transcriptase. The cDNA product was amplified with 2.5 units of Taq polymerase (Roche Molecular Biochemicals) in 100 µl of reaction mixture containing 10 µl of reverse transcription primer buffer plus MgCl₂ (Roche Molecular Biochemicals), 1 mM of each dNTP, 1 µM upstream primer (5′-AAGTCCTGACTGTCGGCTCTGCTGAT-3′), and 1 µM oligo(dT) primer. The PCR parameters were 1 min of primer denaturation at 95 °C, 1 min of primer annealing at 58 °C, and 2 min of primer extension at 72 °C for 35 cycles. The PCR products were analyzed on 1% agarose gels and immobilized on Hybon-N nylon membrane (American Blot, Bethesda, MD), and sequence alignments were achieved using the BCM Search Launcher (available on the World Wide Web).

DNA Isolation and Sequencing—Plasmid DNA was isolated by alkaline lysis (15) and sequenced at MGBX (McGill University, Hughton, Ontario, Canada). The open reading frame of tomato DHS cDNA was cloned and amplified using the BLAST Search (GenBank™, Bethesda, MD), and sequence alignments were achieved using the BCM Search Launcher (available on the World Wide Web).

Cloning of Eucaryotic Initiation Factor 5A—One full-length Arabi-
dopsis and four full-length tomato cDNA clones encoding eIF-5A were obtained by PCR using an Arabidopsis sensing leaf cDNA library and the environmental stressed tomato leaf cDNA library as templates, respectively. For Arabidopsis eIF-5A, partial-length cDNA clones were obtained using the internal upstream primer (5′-AAARYYCCGCTT-GCAAGCT-3′) with T7 (5′-AATTACGACTCATAATG-3′) as a downstream primer and the internal downstream primer (5′-TCTTGTTCGATCACCC-3′) with T3 (5′-AATTACGACTCACAATG-3′) as a upstream primer. For tomato eIF-5A, partial-length cDNA clones were obtained using the internal upstream primer (5′-AAARYYCCG-CYTGGCAAGCT-3′) with T7 as a downstream primer and the internal downstream primer (5′-AACYTMCACHACCTGCGG-3′) with T3 as an upstream primer. Internal primers were designed from the tobacco eIF-5A sequences (12). The cDNA products were amplified with 2.5 units of Expand High Fidelity polymerase (Roche Molecular Biochemi-
cals) in 50 µl of reaction mixture containing 1.5–2 µl of template cDNA, 1× PCR buffer plus MgCl₂ (Roche Molecular Biochemicals), 1 mM of each dNTP, 1 µM upstream primer, and 1 µM downstream primer. The PCR parameters were 1 min of template denaturation at 95 °C, 1 min of primer annealing at 48 or 52 °C, and 2 min of primer extension at 72 °C for 35 cycles. The PCR products were subcloned into the GST fusion vector, pGEX-5X-3 (Amersham Pharmacia Biotech), using BamHIII and SalI restriction sites and overexpressed in E. coli DH5α. Overnight cultures were maintained for 10 times of volume of fresh 2× YT. After 2 h of initial growth in 2× YT at 37 °C, 0.3 mM isopropyl-β-D-thiogalactoside was added to the cultures, and growth was continued for an additional 4 h at 30 °C before the cells were harvested.

Northern and Western Blot Analysis—For Northern blot analysis, total RNA (10 µg) was fractionated on 1.0% denatured formaldehydeagarose gels and immobilized on Hybon-N nylon membrane (Amer
sham Pharmacia Biotech). Hybridization conditions were as described by Green et al. (16). For Western analysis, homogenate protein (20 µg) was fractionated on 10% SDS-polyacrylamide gels, and the separated proteins were transferred to polyvinylidene difluoride membrane (Bio-Rad). Immunoblotting was carried out according to Wang et al. (18) using antiserum from rabbit as the primary antibody and alkaline phosphatase-conjugated secondary antibody (Roche Molecular Biochemicals).

RESULTS

Isolation of a cDNA Clone Encoding Deoxyhypusine Synthase—A full-length cDNA clone encoding tomato DHS (GenBank™ accession number AF296077) was isolated by screening a cDNA library prepared from sorbitol-treated leaves with a probe obtained by RT-PCR. The cDNA contains 1610 base pairs, including a 53-base pair 5′-noncoding sequence and a 414-base pair 3′-noncoding sequence, and encodes a 381-amino

http://www.jbc.org/content/273/25/17542.full.pdf+html
Acid polypeptide with a calculated molecular mass of 42.1 kDa (Fig. 1). The derived amino acid sequence of tomato DHS cDNA has high homology with the amino acid sequences of human DHS (GenBank® accession number 1352267), yeast DHS (*Saccharomyces cerevisiae*, GenBank® accession number 731670), fungal DHS (*Neurospora crassa*, GenBank® accession number 1352268), Archaeobacteria DHS (*Methanococcus janaschii*, GenBank® accession number 2498303), and tobacco DHS (GenBank® accession number AJ242017) (Fig. 2). The tomato DHS sequence also has high homology with an *Arabidopsis* genomic clone (GenBank® accession number AB017060) that has not been functionally annotated. Using primers designed from this sequence and the tomato DHS sequence, PCR products encoding *Arabidopsis* DHS, carnation DHS, and banana DHS were cloned using senescing *Arabidopsis* leaf, carnation flower, and ripening banana fruit cDNA libraries as templates. Full-length cDNA clones encoding *Arabidopsis* DHS (GenBank® accession number AF296078), carnation DHS (GenBank® accession number AF296079), and banana DHS (GenBank® accession number AF296080) were subsequently obtained by screening these libraries with the PCR products, and the derived amino acid sequences proved to have high homology with the tomato DHS amino acid sequence (Fig. 2).

**Up-regulation of Deoxyhypusine Synthase Expression during Environmental Stress**—Northern blot analyses indicated that expression of the tomato DHS gene is up-regulated in osmotically stressed leaves. In these experiments, 8-week-old plants bearing 3–4 pairs of leaves were derooted by cutting the stem at the soil surface, and the aerial part of the plant was placed in 2 M sorbitol, a treatment known to induce osmotic stress (17). The abundance of DHS transcript in preparations of total RNA increased substantially within 6 h of treatment with sorbitol but showed no increase over the same period in leaf RNA preparations from control derooted plants placed in water (Fig. 3A). Indeed, no increase in DHS transcript level was apparent even after 18 h for the water control plants, indicating that the up-regulation of DHS expression observed for leaves of sorbitol-stressed plants was specific to osmotic stress.

**Fig. 1.** Nucleotide and inferred amino acid sequences of the cDNA for senescence-induced tomato DHS. Nucleotide sequence is shown as follows. Lowercase letters, 5′- and 3′-noncoding cDNA sequence; uppercase letters, open reading frame; underline, oligonucleotides used for RT-PCR; shaded letters, mismatched nucleotides; boldface letters, restriction enzyme sites for subcloning PCR fragments; asterisk, stop codon. Numbers of nucleotides and amino acids are indicated.
treated plants reflected osmotic stress rather than a wound effect arising from the derooting procedure. This was further confirmed by simply withholding water from derooted plants. Within 6 h, the water-deprived plants were wilted, and there was an increase in DHS transcript level comparable with that induced by treatment with sorbitol (data not shown). In addition, treatment of the plants for up to 20 h with 1 ppm ethylene had no effect on DHS transcript level, indicating that the up-regulation of DHS in response to sorbitol treatment or withholding water is not induced by the increase in ethylene production that normally accompanies drought stress (17).

Expression of the tomato leaf DHS gene is also up-regulated in response to chilling injury. In these experiments, total RNA for Northern blots was isolated from the second leaf pairs of 8-week-old potted plants that had been exposed to 5 °C in a growth chamber for varying periods of time and subsequently allowed to rewarm at ambient temperature in the greenhouse. The degree of chilling injury was assessed by determining the conductivity of leaf diffusates, which is a measure of membrane leakiness (21). For plants chilled for 2 days at 5 °C with no subsequent rewarming (C2W0 plants), there was no up-regulation of DHS expression and no change in leaf diffusate conductivity (Fig. 4, lanes 1 and 2). However, in keeping with the fact that chilling injury is more strongly manifested during subsequent rewarming (22), there was a substantial increase in DHS transcript abundance coincident with a slight increase in membrane leakage within 6 h of rewarming (C2W6 plants) (Fig. 4, lane 3). The C2W6 plants also exhibited slight wilting symptoms. Within 24 h of rewarming (C2W24 plants), wilting symptoms had completely disappeared, diffusate conductivity had returned to normal, and DHS transcript abundance was at background levels, indicating that expression of the gene had been down-regulated (Fig. 4, lane 4). Thus, DHS expression was up-regulated coincident with the onset of symptoms of chilling injury and down-regulated during recovery from the low temperature episode.

In order to confirm that changes in expression of DHS are correlated with the onset of chilling injury rather than recov-
ery, tomato plants were subjected to more severe chilling injury by exposing them to 5 °C for 2 days followed by rewarming at ambient temperature for 1 day and a further 3-day 5 °C chilling episode with no subsequent rewarming (C3W0 plants). This resulted in stronger up-regulation of DHS expression and extensive membrane leakage reflecting chilling injury (Fig. 4, lane 5). The C3W0 plants also exhibited strong wilting symptoms. After a 6-h rewarming period (C3W6 plants), there was still high expression of DHS and extensive membrane damage (Fig. 4, lane 6), and the plants were still visibly wilted. However, after a 24-h period of rewarming (C3W24), the plants were beginning to regain turgor; diffusate conductivity, although still high by comparison with background levels, was lower, reflecting repair of membrane damage; and DHS expression was only barely detectable (Fig. 4, lane 7). Indeed, within 48 h of returning the C3W0 plants to ambient temperature, the plants regained full turgor. These observations collectively indicate that DHS expression is up-regulated coincident with the onset of chilling injury but is not required for the recovery phase.

In a final set of experiments, tomato plants were exposed to 5 °C continuously for 6 days with no subsequent rewarming (C6W0 plants). These plants were severely wilted and showed high levels of DHS expression as well as extensive membrane damage as reflected by high leaf diffusate conductivity (Fig. 4, lane 8). During a subsequent 6-h rewarming period (C6W6 plants), DHS expression was further up-regulated, and there was a slight further increase in leakage (Fig. 4, lane 9). After 24 h of rewarming (C6W24 plants), DHS expression had begun to abate, membrane damage was still extensive, and the leaves showed visual symptoms of dying (Fig. 4, lane 10), and after 48 h at ambient temperature, the leaves were crinkly and dead. Thus, 6 days of continuous exposure to 5 °C with no intermittent opportunity for recovery proved to be a lethal episode of chilling during which there was strong expression of DHS.

**Up-regulation of Deoxyhypusine Synthase Expression during Natural Senescence**—DHS expression is also up-regulated during natural senescence. This was demonstrated by probing Northern blots of total RNA from tomato flowers and fruit at different stages of development with radiolabeled DHS cDNA. Levels of DHS transcript were barely detectable in flower buds and greatly enhanced in preparations of total RNA isolated from a mixture of open and senescing flowers (Fig. 5A). The
abundance of DHS transcript also proved to be very low in breaker tomato fruit, pink fruit, and fully ripe red firm fruit but increased substantially as fully ripe fruit began to soften coincident with the onset of senescence (Fig. 5B). These observations indicate that DHS expression is not required for fruit ripening and is only up-regulated when the fully ripened fruit begins to senesce.

In order to confirm that up-regulated expression of the DHS gene reflected increased expression of its cognate protein, Western blots were probed with polyclonal antibodies raised against tomato DHS recombinant protein; each lane contained 20 μg of protein. Northern blot of total RNA probed with tomato DHS cDNA; each lane contained 10 μg of RNA. B, chlorophyll levels.

FIG. 6. Changes in DHS protein and mRNA levels and in chlorophyll levels of developing tomato cotyledons. A, Western blot of total protein probed with polyclonal antibodies raised against tomato DHS recombinant protein; each lane contained 20 μg of protein. Northern blot of total RNA probed with tomato DHS cDNA; each lane contained 10 μg of RNA. B, chlorophyll levels.

Southern Analysis of Genomic Deoxyhypusine Synthase—The Northern blot data illustrated in Figs. 3–6 were obtained using full-length tomato DHS cDNA as a radiolabeled probe. The same patterns of DHS expression in chill-injured and drought-stressed leaves and in naturally senescing flowers and fruit were obtained when Northern blots of total RNA were probed with cDNA corresponding to the 3′-noncoding region of tomato DHS (data not shown). This could mean that there is a

Coexpression of DHS and Eucaryotic Translation Initiation Factor 5A—Full-length cDNA clones for four isoforms of tomato eIF-5A (eIF-5A1, GenBank™ accession number AF296083; eIF-5A2, GenBank™ accession number AF296084; eIF-5A3, GenBank™ accession number AF296085; eIF-5A4, GenBank™ accession number AF296086) were isolated by PCR using the cDNA library prepared from sorbitol-treated leaves as a template and primers designed from tobacco eIF-5A (GenBank™ accession numbers X635411 and X635412). The tomato eIF-5A sequences are 89–92% identical at the amino acid level and 70–80% identical at the nucleotide level, and they are also closely similar (80–97% identity at the amino acid level to other plant eIF-5A sequences (Table I). The extent of sequence identity is lower (50–60% at the amino acid level) when the tomato eIF-5A sequences are compared with those of human, fungi, and yeast (Table I).

Full-length cDNA for one of the tomato isoforms, eIF-5A1, was used to probe Northern blots of total RNA isolated from environmentally stressed tissue and naturally senescing tissue. In keeping with the fact that DHS activates eIF-5A, the genes encoding these proteins were found to be up-regulated in parallel in drought-stressed leaf tissue (Fig. 3, A and B), chill-injured leaf tissue (Fig. 4), senescing tomato blossoms (Fig. 5A), and senescing (red soft) tomato fruit (Fig. 5B). It is noteworthy, however, that DHS expression exhibits more plasticity than eIF-5A expression during cycles of sublethal chilling stress with intervening periods of recovery applied to tomato leaves (Fig. 4).

Enzyme Activity of Recombinant Deoxyhypusine Synthase Protein—In order to confirm the identity of the isolated tomato DHS gene, the enzymatic activity of the recombinant protein obtained by overexpression of DHS cDNA in E. coli was assayed. This involved measuring the ability of recombinant tomato DHS protein to transfer butylamine from radiolabeled spermidine to recombinant Arabidopsis eIF-5A (GenBank™ accession number AF296082) protein. Recombinant DHS and eIF-5A were both made as GST fusion proteins. GST was released from the fusion proteins by proteolytic cleavage with factor Xa, and the resultant protein mixtures (GST plus DHS and GST plus eIF-5A) were used for the DHS activity assays. Thus, the standard reaction mixture contained GST plus DHS and GST plus eIF-5A protein mixtures (Fig. 7, lane D). In addition, three control reactions were run: GST alone (Fig. 7A), GST plus DHS (B), and GST plus eIF-5A (C), and in each case sufficient free GST was added to make the protein concentrations of the control reaction mixtures equivalent to that of the standard reaction mixture (Fig. 7, A–C). Catalytic activity was discerned by autoradiography after SDS-polyacrylamide gel electrophoresis of the reaction mixtures. The standard reaction mixture yielded a radioactive band at 18 kDa (Fig. 7D). This corresponds to the radiolabeled intermediate form of eIF-5A, which is formed when DHS transfers butylamine from radiolabeled spermidine to a conserved lysine residue of inactive eIF-5A (2). No 18-kDa proteins were detectable in the control reaction mixture containing radiolabeled spermidine and GST alone (Fig. 7A) or radiolabeled spermidine and GST plus eIF-5A alone (Fig. 7C). Unused radiolabeled spermidine ran off the gel (Fig. 7, A and C). These observations collectively indicate that recombinant DHS exhibits its expected catalytic activity and, further, that recombinant eIF-5A is capable of being deoxyhypusine-modified.

Factor 5A—
single isoform of DHS in tomato. In an attempt to clarify this, Southern blots of tomato genomic DNA digested with restriction enzymes were probed with full-length DHS cDNA. Genomic DNA was digested with XbaI and EcoRI, which cut in the open reading frame of DHS, and with EcoRV and HindIII, for which there are no open reading frame cut sites. In each case, several restriction fragments were detected, suggesting the presence of multiple DHS isoforms (Fig. 8). However, these data are not conclusive because the tomato DHS genomic sequence(s) is not known, and it is possible that the restriction enzymes are cutting within one or more introns.

**DISCUSSION**

DHS catalyzes the first reaction in a two-step conversion of inactive eIF-5A to its activated form. The DHS-mediated reaction entails the transfer of a butylamine residue from spermidine to a conserved lysine of inactive eIF-5A, forming the unusual amino acid, deoxyhypusine, which is then converted to hypusine by deoxyhypusine hydroxylase (1). Hypusine-modified eIF-5A, the active form of the protein, is the only cellular
protein known to contain hypusine. It is present in all eucaryotic cells, and although its precise function has not been elucidated, it appears to facilitate mRNA translation (1). It was initially designated as a translation initiation factor based on in vitro experiments indicating that it stimulates the formation of methionyl-puromycin, a dipeptide analogue (9). However, more recent experiments have demonstrated that eIF-5A is not involved in the initiation of global protein synthesis. For example, mutating the single isoform of DHS or both isoforms of eIF-5A in yeast results in only marginal changes in total protein synthesis (1, 4, 5). The DHS mutants are, however, incapable of cell division, yet they remain alive and simply enlarge (1, 6). Inactivation of both isoforms of yeast eIF-5A produces the same phenotype. Indeed, the isoforms appear to be functionally redundant in that inactivation of either alone does not produce the phenotype (6). A correlation between growth arrest and a reduction in hypusine formation has also been observed in spermidine-depleted mammalian cells (23).

These observations have been interpreted as indicating that eIF-5A facilitates translation of the suite of mRNAs required for cell division (1) and have prompted the more general view that the various isoforms of eIF-5A promote translation of specific subsets of mRNA required for selected cell functions (4). Moreover, recent data indicate that eIF-5A functions as a nucleocytoplasmic shuttle protein that facilitates translation by mediating the translocation of specific mRNAs from the nucleus to the cytoplasm. For example, immunofluorescence and immunogold labeling studies with mammalian cells have demonstrated that eIF-5A is localized in both the cytoplasmic and nuclear compartments, interacts with the general nuclear export receptor, CRM1, and is transported from the nucleus to the cytoplasm (24). Also, expression of eIF-5A mutant protein has been shown to inhibit HIV-1 replication in human T-cells (16, 25). The selective nature of eIF-5A involvement in mRNA translation is supported by the finding that inhibitors of the hypusine modification of eIF-5A cause the disappearance of only certain mRNAs from polysomes (26). Also, intracellular depletion of eIF-5A in yeast has been shown to cause a marked accumulation of specific mRNAs in the nuclear compartment (27).

Two isoforms of eIF-5A have been isolated from tobacco (12), and the sequences for several other eIF-5A genes including those from Z. mays (GenBank™ accession number Y07920), S. tuberosum (GenBank™ accession numbers AB004823 to AB004827), and alfalfa (GenBank™ accession number X59441) are known. In the present study, cDNAs encoding four isoforms of tomato eIF-5A (GenBank™ accession numbers AF29683 to AF29686), eIF-5A from Arabidopsis (GenBank™ accession number AF296082), and eIF-5A from carnation (GenBank™ accession number AF296081) were isolated. It is clear from a comparison of eIF-5A sequences that, in keeping with the fact that it is involved in regulation, this gene is highly conserved among plant species (80–97% identity at the amino acid level) but is less conserved across the plant and animal kingdoms (50–60% identity at the amino acid level). DHS genes from tomato (GenBank™ accession number AF296077), Arabidopsis DHS (GenBank™ accession number AF296078), carnation DHS (GenBank™ accession number AF296079), and banana DHS (GenBank™ accession number AF296080) were also cloned in the present study, and the sequence for DHS from tobacco has also recently been reported (28). DHS also appears to be highly conserved among plant species (70–90% identity at the amino acid level) but is less well conserved across the plant and animal kingdoms (40–60% identity at the amino acid level). It has been proposed that plant DHS, like its mammalian and yeast counterparts, mediates the first step in the hypusine modification of eIF-5A required for its activation (1), but to date no specific function has been ascribed to plant eIF-5A.

It is clear from the present study that tomato DHS and eIF-5A are up-regulated in parallel in response to drought and chilling stress and coincident with the onset of flower and fruit senescence. Plant DHS has been shown to have high sequence homology with the plant gene encoding homospermidine synthase, an enzyme that converts spermidine to homospermidine in a key reaction of alkaloid biosynthesis (28, 29). However, two lines of evidence in addition to sequence similarity with mammalian, yeast, and tobacco DHS clones indicate that the tomato gene encodes DHS. First, overexpression of tomato DHS cDNA in E. coli produced a recombinant protein that proved capable of mediating the transfer of a butylamine residue to eIF-5A, the reaction catalyzed by DHS in situ. Second, tomato DHS and eIF-5A expression are up-regulated in parallel at the onset of senescence and in response to environmental stress, which is consistent with the contention that the cognate protein of the DHS gene is activating eIF-5A. Indeed, these observations suggest that tomato DHS activates an isoform of eIF-5A that facilitates the nucleocytoplasmic transport of mRNAs required for natural senescence as well as premature senescence induced by environmental stress. That up-regulation of DHS expression was observed by probing Northern blots with either full-length or 3'-noncoding cDNA suggests that a single isoform of DHS may be involved in regulating natural senescence as well as stress-induced senescence. However, the possibility that additional isoforms of tomato DHS are involved in the regulation of other physiological events is not precluded.

Inasmuch as senescence is an active process requiring gene expression and synthesis of new proteins, it is classified as a form of programmed cell death. A number of senescence-induced genes have been identified for leaves and flowers. These include genes encoding ferritin and metallothionein as well as lipase, a cysteine protease, aspartate protease, and a vacuolar processing protease (30–32). Indeed, the translation products of senescence-induced genes invariably include hydrolytic enzymes that mediate the breakdown of cell structure, especially membranes, as well as catabolism of macromolecules, and this results in a progressive decline in protein and lipid (33). The expression of genes required for growth and development but not programmed cell death, such as those encoding the small subunit of ribulose-bisphosphate carboxylase in leaves, is suppressed at the onset of senescence, whereas housekeeping genes required for cell viability, such as those supporting respiration, show continued expression throughout development and senescence until the cells actually die (31, 34). There are also tissue-specific differences in senescence. Paramount among these is the fact that natural senescence of leaves and flowers is accompanied by mobilization of nutrients out of the senescing tissue to other parts of the plant, whereas this is not the case for fruit senescence. In a similar vein, rapidly induced cell death in leaves caused by pathogen ingress or severe environmental stress is normally not accompanied by nutrient mobilization. This type of programmed cell death in leaves is sometimes referred to as necrosis rather than senescence, because chlorosis, which defines the progressive yellowing of leaves reflecting chlorophyll degradation during natural leaf senescence, is not observed. It is clear from the present study that DHS and eIF-5A are up-regulated at the onset of natural senescence, whether or not there is mobilization of nutrients out of the senescing tissue, and also in the event of necrosis. Specifically, both genes showed increased expression in senescing tomato flowers from which nutrients are mobilized and in senescing tomato fruit from which nutrients are not remobilized. In addition, DHS and eIF-5A were up-regulated in par-
allel in tomato leaves under conditions in which rapid cell death in the absence of chlorosis was induced by a continuous 6-day exposure to 5 °C, a temperature that induces chilling injury in tomato. It seems likely, therefore, that plant DHS and eIF-5A facilitate the translation of mRNAs required for programmed cell death regardless of how it is initiated, but they may not be involved in the regulation of the mobilization of nutrients that accompanies some types of programmed cell death.

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