kinetics, we should increase considerably the current density. Nevertheless, this was not possible at 570°C because of the ohmic resistance of the SCY. We could obtain higher H^+ fluxes by increasing the temperature, but this would also increase the rate of NH_3 decomposition. Further work is needed to determine the optimum operating temperature, so to propose a mechanism at this stage would be primarily speculation.

This process offers an alternative route that permits operation at desired pressures and temperatures without the thermodynamic restrictions imposed on conventional catalytic reactors. The above experimental observations show that H_2 is quantitatively converted into NH_3 regardless of the thermodynamic restrictions for limited conversion. This result does not mean that the present data violate thermodynamics in any aspect. Simply, the final state of high conversion to NH_3 is achieved by the consumption of electrical work by the system. This situation is similar to the case of H_2O dissociation into H_2 and O_2: if this reaction is carried out at 25°C and atmospheric pressure, the thermodynamically calculated mole fractions of H_2 and O_2 at equilibrium are on the order of 10^{-27}. Nevertheless, water is quantitatively dissociated if electrical work is offered (electrolysis).

References and Notes

1. C. N. Satterfield, *Heterogeneous Catalysis in Practice* (McGraw-Hill, New York, 1980), pp. 301–308.
2. M. Stoukides, *Ind. Eng. Chem. Res.* **27**, 1745 (1988).
3. C. G. Vayenas, M. M. Jaksic, S. I. Bebelis, S. G. Neophytides, in *Modern Aspects in Electrochemistry*, J. O'.M. Bockris, B. E. Conway, W. R. E. White, Eds. (Plenum, New York, 1996), vol. 29, pp. 57–202.
4. T. M. Gür and R. A. Huggins, *Science* **219**, 967 (1983).
5. Y. Jiang, I. V. Yentekakis, C. G. Vayenas, *ibid.* **264**, 1563 (1994).
6. C. G. Vayenas and R. D. Farr, *ibid.* **208**, 593 (1980).
7. H. Iwahara, *Solid State Ionics* **86–88**, 9 (1996).
8. E. Panagos, I. Voudouris, M. Stoukides, *Chem. Eng. Sci.* **51**, 3175 (1996).
9. H. Iwahara, T. Esaka, H. Uchida, N. Maeda, *Solid State Ionics* **3–4**, 359 (1981).
10. C. Athanasiou, G. Marnellos, P. Tsiakaras, M. Stoukides, *Ionics* **2**, 353 (1996).
11. S. Hamakawa, T. Hibino, H. Iwahara, *J. Electrochem. Soc.* **140**, 459 (1993).
12. P.-H. Chiang, D. Eng, M. Stoukides, *Solid State Ionics* **61**, 99 (1993).

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An *Arabidopsis* Mutant Defective in the Plastid General Protein Import Apparatus

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Elaborate mechanisms have evolved for the translocation of nucleus-encoded proteins across the plastid envelope membrane. Although putative components of the import apparatus have been identified biochemically, their role in import remains to be proven in vivo. An *Arabidopsis* mutant lacking a new component of the import machinery [translocon at the outer envelope membrane of chloroplasts (Toc33), a 33-kilodalton protein] has been isolated. The functional similarity of Toc33 to another translocon component (Toc34) implies that multiple different translocon complexes are present in plastids. Processes that are mediated by Toc33 operate during the early stages of plastid and leaf development. The data demonstrate the in vivo role of a translocon component in plastid protein import.

The routing of newly synthesized proteins to appropriate subcellular compartments is a fundamental process that is common to all organisms. In plants, chloroplasts (the photosynthetic plastids of green tissues) are the major target of such protein trafficking because they account for >50% of the total soluble protein in leaves and because >80% of the proteins required for their formation are encoded in the nucleus (*1*). The translocation of proteins across the envelope membrane is especially important early on in chloroplast and leaf development, when the photosynthetic apparatus is being assembled for the establishment of photoautotrophic growth (*1*). The capacity of plastids to import proteins is regulated developmentally and is maximal during these early stages of organ expansion (*2*).

Genetic screens for loci affecting the expression of nucleus-encoded photosynthetic proteins have identified several mutants with defects in plastid biogenesis (*3, 4*). Here, we describe a new mutant, initially referred to by the number 127-4, which belongs in this category. The 127-4 mutant was identified as having a recessive, pale phenotype in a population of transferred DNA (T-DNA)–mutagenized plants. Although the mutant appeared uniformly pale during the first 2 weeks of its life cycle (Fig. 1, A and B), the oldest leaves of mature plants frequently had an appearance closer to that of the wild type (Fig. 1C) (*5*).

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The gene disrupted by the T-DNA insertion (Fig. 2A) encodes a 33-kD protein that is very similar to Toc34 from *Arabidopsis* (61% amino acid identity) and pea plants (59% amino acid identity) (Fig. 2B); *Arabidopsis* Toc34 and pea Toc34 share 64% amino acid identity (*6–8*). Toc34 is a guanosine triphosphate (GTP)–binding protein of the plastid outer envelope membrane. Toc34, Toc75, and Toc86 are components of the outer envelope segment of the general import apparatus through which the majority of proteins destined for the plastid are believed to pass (*9*). The involvement of Toc34 in protein import is inferred from its interaction with preproteins during import and from its association with Toc75 and Toc86 (*10*). However, its precise role in the import process is not known. The 127-4 mutant was named *ppi1* (for plastid protein import), but we will refer to the protein encoded by the *PPI1* gene as Toc33, in accordance with biochemical nomenclature (*11*). The size (an estimated 13 kb) and location (in intron 2) of the T-DNA insertion, combined with our failure to detect any mRNA in mutant plants (*12*), indicated that the *ppi1* mutation was most likely null.

Given their strong sequence similarity, we tested the hypothesis that Toc33 and Toc34 might correspond to functionally equivalent translocon components. In vitro transcribed and translated Toc33 protein was found to insert itself into the outer envelope membrane of isolated pea chloroplasts in a similar manner to *Arabidopsis* Toc34 (*12, 13*), which suggests that the two proteins are similarly localized in vivo. We subsequently overexpressed Toc33 and Toc34 cDNA clones in *ppi1* plants and found that either protein could complement the chlorophyll deficiency of *ppi1*, which confirms the functional similarity of the two proteins (Fig. 2C) (*14, 15*). The existence of two functionally similar Toc proteins suggests that at least two distinct translocon complexes exist in *Arabidopsis*. This observation is of particular

interest because biochemical studies of pea chloroplasts have revealed the presence of only one Toc complex (*9*).

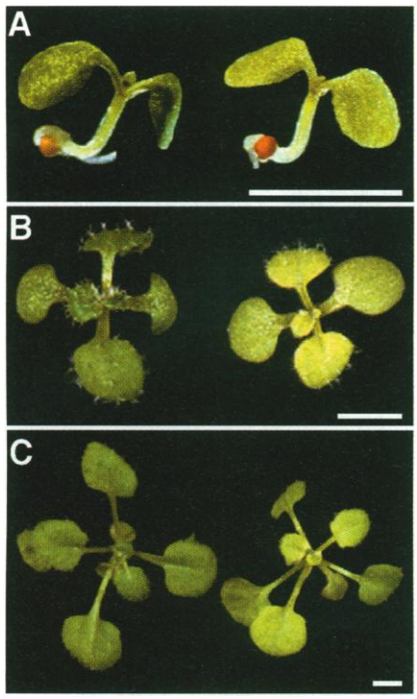

Fig. 1. The visible phenotype of the 127-4 (*ppi1*) mutant. Plants were grown under long-day conditions (16 hours of light and 8 hours of dark) for (**A**) 5 days, (**B**) 13 days, and (**C**) 25 days (*5*). Wild-type plants are shown on the left, and *ppi1* plants are shown on the right. Scale bars, 2 mm.

Analyses of plastids from pea and wheat plants have demonstrated that protein import capabilities can vary substantially through development (*2*). The abundance of Toc75 mRNA was shown in pea plants to be greater in young tissues than in mature leaves (*2*). To assess the role of Toc33 in the regulation of plastid import in *Arabidopsis*, we examined the expression patterns of Toc33 and Toc34 and characterized the *ppi1* phenotype with respect to chlorophyll accumulation, plastid ultrastructure, and plastid protein import. Steady-state levels of Toc33 and Toc34 mRNAs were examined over the course of the *Arabidopsis* life cycle (Fig. 3A). Toc33 was expressed at high levels in very young plants, but its expression declined rapidly as the age of the plant increased. Although the expression of Toc34 appeared to follow a similar trend, Toc34 mRNA remained at a relatively low level throughout development. The expression of both genes was about three times higher in the young, expanding tissues of 25-day-old plants than in the mature, fully expanded leaves of the same plants; this suggests that the expression of Toc33 in very young plants (Fig. 3A) is elevated because these plants have more young, expanding tissues.

Mutant plants contained reduced levels of chlorophyll at all developmental stages (Fig. 3B) (*15*). However, they were extremely pale at the earliest measured time (day 2), and except for a period between days 5 and 10, chlorophyll levels improved steadily throughout development (Fig. 3C). The first observation indicates that Toc33 functions very early in development (before day 2) in the expanding cotyledons. The second observation suggests that Toc33 has a role during the emergence of the primary leaves (between days 5 and 10) and not during other periods; it is likely that the effect of *ppi1* on chlorophyll accumulation during the emergence of later leaves is masked in this assay by the presence of older leaves. Together, these data imply an involvement of Toc33 in chloroplast development that occurs concomitantly with the expansion of newly formed tissues.

The ultrastructure of mutant plastids from different stages of development was examined [electron microscopy was carried out according to described procedures (*3*)]. The most pronounced defect of etioplasts was the markedly reduced size of the prolamellar body (an estimated 50% reduction in cross-sectional area) (Fig. 4, A and B). The nucleus-encoded protochlorophyllide oxidoreductase (POR) enzyme is the primary proteinaceous component of the prolamellar body (*16*). The mutant chloroplasts were smaller than those in the wild type and contained fewer thylakoid membranes with smaller granal stacks. Chloroplast defects appeared far more pronounced in 5-day-old plants (Fig. 4, C and D) than in 4-week-old plants (Fig. 4, E and F), and thus, the defects parallel the visible phenotype of the mutant (Fig. 1, A and C). These data again indicate that Toc33 functions during the early stages of plastid development. At no stage do *ppi1* plastids appear aberrant to a degree that is indicative of a complete block in their development; this observation can be accounted for by the observed

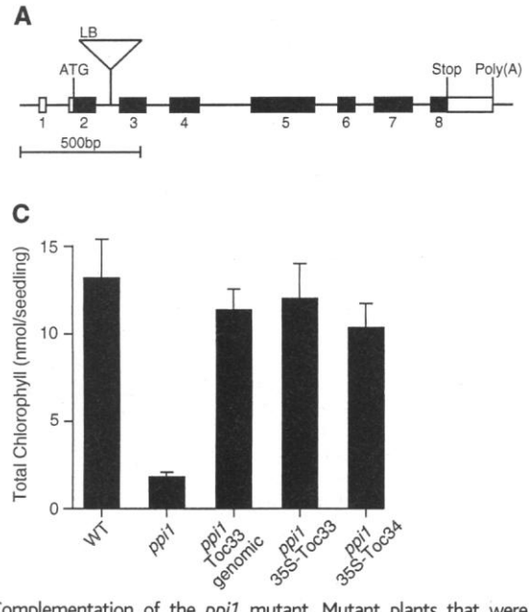

Fig. 2. Identity of the 127-4 (*PPI1*) gene. (**A**) Schematic representation of the gene disrupted in the 127-4 (*ppi1*) mutant (*8*). The depicted gene encodes the Toc33 protein. ATG, translation initiation codon; LB, left border of T-DNA insertion (not to scale); stop indicates translation termination codon; and poly(A), polyadenylation site. (**B**) Alignment of the Toc33, *Arabidopsis thaliana* Toc34 (atToc34), and pea (*Pisum sativum*) Toc34 (psToc34) amino acid sequences (*8, 19*). Dashes indicate gaps introduced to maximize alignment; residues conserved in at least two of the three sequences are shaded. (**C**) Complementation of the *ppi1* mutant. Mutant plants that were transformed with a wild-type (WT) copy of the *PPI1* gene (Toc33 genomic), with a Toc33 overexpression construct (35S-Toc33), or with a Toc34 overexpression construct (35S-Toc34) were assayed for their chlorophyll content after 13 days of growth in continuous white light (*14, 15*). The presented data are average values from at least six transformants in each case. Error bars indicate 1 SE.

B

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Toc33     1 MGSL---VREWVGFQQFPAATQEKLIEFF 26
atToc34   1 MAALQ-TLREWIGIQQFPPATQSKLLEIL 28
psToc34   1 MASQQQTVREWSGINTFAPATQTKLLELL 29

Toc33    27 GKLKQKDMNSMTVLVLGKGGVGKSSTVNS 55
atToc34  29 GKYKEEDVSSLTVLVMGKGGVGKSSTVNS 57
psToc34  30 GNLKQEDVNSLTILVMGKGGVGKSSTVNS 58

Toc33    56 LIGEQVVRVSPFQAEGLRPVMVSRTMGGF 84
atToc34  58 VIGEKAAAVSTFQSEGLRPTLVSRTRSGF 86
psToc34  59 IIGERVVSISPFQSEGPRPVMVSRSRAGF 87

Toc33    85 TINIIDTPGLVEAGYVNHQALELIKGFLV 113
atToc34  87 TLNIIDTPGLIEGGYVNDQAINIIKRFLL 115
psToc34  88 TLNIIDTPGLIEGGYINDMALNIIKSFLL 116

Toc33   114 NRTIDVLLYVDRLDVYRVDELDKQVVIAI 142
atToc34 116 NMTIDVLLYVDRLDVYRVDDLDRQVVGAI 144
psToc34 117 DKTIDVLLYVDRLDAYRVDNLDKLVAKAI 145

Toc33   143 TQTFGKEIWCKTLLVLTHAQFSPPDELSY 171
atToc34 145 TDAFGKEIWKKSALVLTHAQFSPPDGLNY 173
psToc34 146 TDSFGKGIWNKAIVALTHAQFSPPDGLPY 174

Toc33   172 ETFSSKRSDSLLKTIRAGSKMRKQEFEDS 200
atToc34 174 NHFVSKRSNALLKVIQTGAQLKKQDLQGF 202
psToc34 175 DEFFSKRSEALLQVVRSGASLKKDA-QAS 202

Toc33   201 AIAVVYAENSGRCSKNDKDEKALPNGEAW 229
atToc34 203 SIPVILVENSGRCHKNESDEKILPCGTSW 231
psToc34 203 DIPVVLIENSGRCNKNDSDEKVLPNGIAW 231

Toc33   230 IPNLVKAITDVATNQRKAIHVDKKMVDGS 258
atToc34 232 IPNLFNKITEISFNGNKAIHVDKKLVEGP 260
psToc34 232 IPHLVQTITEVALNKSESIFVDKNLIDGP 260

Toc33   259 YSDDKGKKLIPLTIGAQYLIV-KMIQGAI 286
atToc34 261 NPNERGKKLIPLMFAFQYLLVMKPLVRAI 289
psToc34 261 NPNQRGKLWIPLIFALQYLFLAKPIEALI 289

Toc33   287 RNDIKTSGKPL---------- 297
atToc34 290 KSDVSRESKLAWELRDSGLASRRS 313
psToc34 290 RRDIATETKPAWETRDVG---DRK 310
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basal expression of Toc34 (Fig. 3A).

To determine the precise nature of the defect in *ppi1* plants, we assayed the import of several proteins into mutant chloroplasts isolated from plants grown for either 10 or 28 days (*13*). Chloroplasts isolated from 28-day-old plants were largely derived from mature, fully expanded leaves, whereas a substantial proportion of chloroplasts isolated from 10-day-old plants were derived from newly expanding primary leaves. We found that eight tested proteins (RBCS, PORA, PORB, CAB, OEP14, Toc33, Toc34, and Toc75) were imported into the plastids of mature *ppi1* plants with wild-type efficiencies (*12*). However, the translocation efficiencies of all four tested proteins (RBCS, PORA, PORB, and CAB) into the plastids of 10-day-old mutant plants were reduced (Fig. 5A); the amounts of each protein imported into mutant plastids, which were expressed as percentages of the protein amounts imported into wild-type plastids, were 59% (RBCS), 68% (PORA), 61% (PORB), and 75% (CAB). To corroborate the findings of these studies in organelles, we conducted immunoblot experiments that were designed to assess plastid protein import defects in vivo. Five-day-old etiolated mutant plants contained reduced levels of the mature POR form (POR; 36 kD) and a pronounced accumulation of nonprocessed POR preprotein (pPOR; 44 kD) (Fig. 5B). The accumulated pPOR was stable for at least 48 hours after the transfer of etiolated plants to continuous light, which implied that the pPOR had not been imported because a chloroplast-specific protease degrades both POR and pPOR (*12, 17*).

The data suggest that the changing import capabilities of maturing plastids result from variations in the number of translocon complexes, which are brought about in turn through changes in translocon gene expression. Given the functional similarity between Toc33 and Toc34, the reason for the presence of both proteins is unclear. One possibility is that two separate genes, whose transcription can be regulated inde-

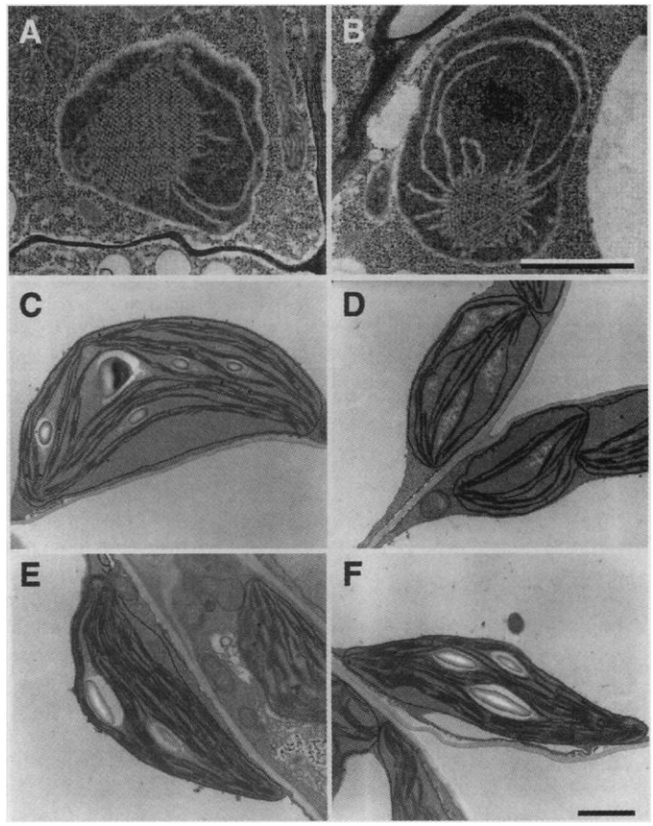

Fig. 4. Ultrastructure of *ppi1* plastids at different stages of development. Representative plastids from (**A**) 5-day-old etiolated wild-type cotyledons; (**B**) 5-day-old etiolated *ppi1* cotyledons; (**C**) 5-day-old light-grown wild-type cotyledons; (**D**) 5-day-old light-grown *ppi1* cotyledons; (**E**) 4-week-old light-grown wild-type leaves; and (**F**) 4-week-old light-grown *ppi1* leaves [electron microscopy was carried out according to described procedures (*3*)]. (A) and (B) are at higher magnification than (C) through (F). Scale bars, 1 μm.

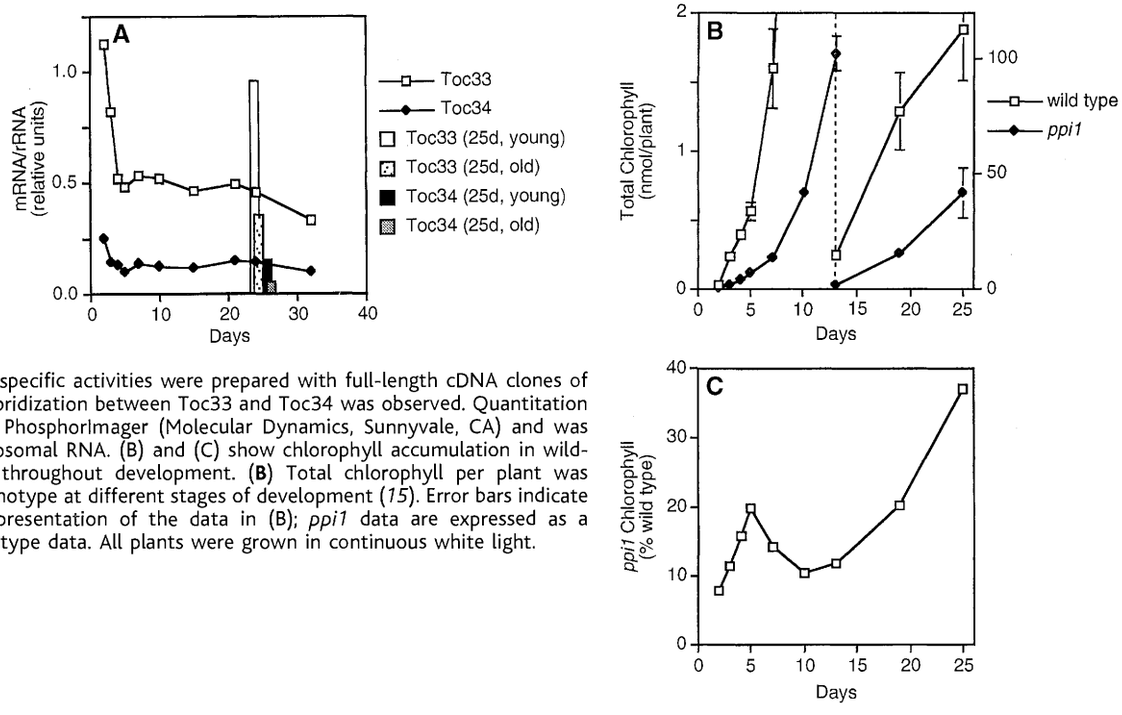

Fig. 3. Developmental time-course analyses of gene expression and chlorophyll accumulation. (**A**) Northern (RNA) blot analyses of Toc33 and Toc34 expression at different stages in the development of wild-type *Arabidopsis*. RNA was extracted from whole plants or from the young and old aerial tissues of 25-day-old plants (d, day). Probes of similar specific activities were prepared with full-length cDNA clones of each gene; no cross-hybridization between Toc33 and Toc34 was observed. Quantitation was performed with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) and was normalized for 18*S* ribosomal RNA. (B) and (C) show chlorophyll accumulation in wild-type and *ppi1* plants throughout development. (**B**) Total chlorophyll per plant was determined for each genotype at different stages of development (*15*). Error bars indicate 1 SE. (**C**) Alternative presentation of the data in (B); *ppi1* data are expressed as a percentage of the wild-type data. All plants were grown in continuous white light.

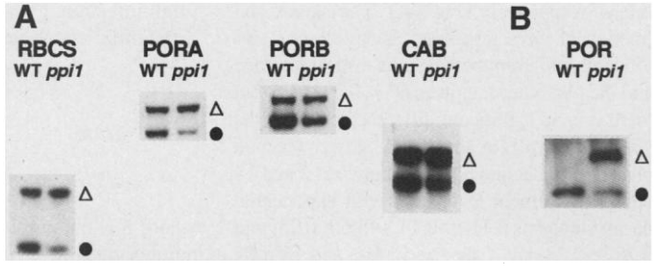

Fig. 5. Analyses of plastid protein import in the *ppi1* mutant. **(A)** In-organelle analysis of protein import into wild-type and mutant plastids isolated from 10-day-old plants. The import of four different protein precursors was investigated: RBCS, PORA, PORB, and CAB. Chloroplasts were repurified after the import reactions, and equal numbers of chloroplasts were loaded in each lane (*13*). **(B)** In vivo immunoblot analysis of POR protein in 5-day-old etiolated wild-type and mutant plants. Precursor proteins are indicated with open triangles; mature proteins are indicated with solid circles.

pendently, provide the simplest means of achieving the necessary pattern of expression. The fact that the translocation efficiencies of some preproteins vary between different plastid types suggests that the import machinery possesses a certain degree of specificity (*18*). Although our transgenic data indicate functional similarity between the two proteins (Fig. 2C), it is possible that, under normal conditions, subtle functional differences exist.

References and Notes

1. J. E. Mullet, *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 475 (1988); G. K. Lamppa, G. Morelli, N.-H. Chua, *Mol. Cell. Biol.* **5**, 1370 (1985); C. Dean and R. M. Leach, *Plant Physiol.* **69**, 904 (1982); M. Viro and K. Kloppstech, *Planta* **150**, 41 (1980).
2. C. Dahlin and K. Cline, *Plant Cell* **3**, 1131 (1991); P. J. Tranel, J. Froehlich, A. Goyal, K. Keegstra, *EMBO J.* **14**, 2436 (1995).
3. H.-m. Li, K. Culligan, R. A. Dixon, J. Chory, *Plant Cell* **7**, 1599 (1995).
4. E. López-Juez, R. P. Jarvis, A. Takeuchi, A. M. Page, J. Chory, *Plant Physiol.*, in press.
5. Plants were grown on 0.5 × Murashige and Skoog medium (Gibco-BRL) containing 1.5% sucrose. Incubator temperatures were maintained at 20°C; white light fluency rates were between 200 and 300 μE m^{-2} s^{-1} (an einstein, E, is the energy of 1 mol of photons). All plants described are of the Columbia ecotype.
6. H.-m. Li and L.-J. Chen, *J. Biol. Chem.* **272**, 10968 (1997).
7. M. Seedorf, K. Waegemann, J. Soll, *Plant J.* **7**, 401 (1995); F. Kessler, G. Blobel, H. A. Patel, D. J. Schnell, *Science* **266**, 1035 (1994).
8. The close linkage of a single-locus T-DNA insertion to the mutant locus was established by analyzing the cosegregation of the kanamycin-resistant (T-DNA–associated) and pale phenotypes in the progeny of a segregating population of 68 second-generation transformants. Genomic DNA flanking the T-DNA left border was isolated with a thermal asymmetric interlaced polymerase chain reaction [Y.-G. Liu, N. Mitsukawa, T. Oosumi, R. F. Whittier, *Plant J.* **8**, 457 (1995)] and found to correspond to a completely sequenced bacterial artificial chromosome (BAC) clone. Its genomic sequence and that of a full-length expressed sequence tag (EST) enabled the delineation of the structure of the disrupted gene. Database searches were performed at the U.S. National Center for Biotechnology Information (Bethesda, MD) with the BLAST program [S. F. Altschul, W. Gish, W. Miller, E. W. Myers, D. J. Lipman, *J. Mol. Biol.* **215**, 403 (1990)]. The genomic sequence and the sequence annotation were provided by Stanford University (Stanford, CA). BAC T7I23 and EST 190I17T7 were obtained from the *Arabidopsis* Biological Resource Center (Columbus, OH). The amino acid sequence alignment was performed with the MegAlign program (DNASTAR, Madison, WI), using the clustal method.
9. B. Fuks and D. J. Schnell, *Plant Physiol.* **114**, 405 (1997); K. Cline and R. Henry, *Annu. Rev. Cell Dev. Biol.* **12**, 1 (1996).
10. A. Kouranov and D. J. Schnell, *J. Cell Biol.* **139**, 1677 (1997); M. Akita, E. Nielsen, K. Keegstra, *ibid.* **136**, 983 (1997); M. Seedorf and J. Soll, *FEBS Lett.* **367**, 19 (1995); D. J. Schnell, F. Kessler, G. Blobel, *Science* **266**, 1007 (1994).
11. D. Schnell *et al.*, *Trends Cell Biol.* **7**, 303 (1997).
12. P. Jarvis, L.-J. Chen, H.-m. Li, J. Chory, data not shown.
13. The methods used during the in-organelle analysis of plastid protein import were as described [S. E. Perry, H.-m. Li, K. Keegstra, *Methods Cell Biol.* **34**, 327 (1991)].
14. The Toc33 genomic construct comprised a 6.5-kb Xba I restriction fragment of BAC T7I23, which was inserted into the plant transformation vector pPZP221 [P. Hajdukiewicz, Z. Svab, P. Maliga, *Plant Mol. Biol.* **25**, 989 (1994)]. Overexpression constructs comprised Toc33 and Toc34 cDNA clones, which were inserted into a pPZP221-based plant expression vector that carried the cauliflower mosaic virus 35S promoter and a pea ribulose 1,5-bisphosphate carboxylase/oxygenase terminator (pCHF2) [C. Fankhauser, K. Hanson, J. Chory, unpublished data].
15. Chlorophyll was extracted with *N,N*-dimethylformamide, and its concentration was determined spectrophotometrically [R. J. Porra, W. A. Thompson, P. E. Kriedemann, *Biochim. Biophys. Acta* **975**, 384 (1989)].
16. S. Reinbothe, C. Reinbothe, N. Lebedev, K. Apel, *Plant Cell* **8**, 763 (1996).
17. C. Reinbothe, K. Apel, S. Reinbothe, *Mol. Cell. Biol.* **15**, 6206 (1995).
18. J. Wan, S. D. Blakely, D. T. Dennis, K. Ko, *J. Biol. Chem.* **271**, 31227 (1996); K. Fischer *et al.*, *ibid.* **269**, 25754 (1994).
19. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, The; V, Val; W, Trp; and Y, Tyr.

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Hepatitis C Viral Dynamics in Vivo and the Antiviral Efficacy of Interferon-α Therapy

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To better understand the dynamics of hepatitis C virus and the antiviral effect of interferon-α-2b (IFN), viral decline in 23 patients during therapy was analyzed with a mathematical model. The analysis indicates that the major initial effect of IFN is to block virion production or release, with blocking efficacies of 81, 95, and 96% for daily doses of 5, 10, and 15 million international units, respectively. The estimated virion half-life ($t_{1/2}$) was, on average, 2.7 hours, with pretreatment production and clearance of 10^{12} virions per day. The estimated infected cell death rate exhibited large interpatient variation (corresponding $t_{1/2}$ = 1.7 to 70 days), was inversely correlated with baseline viral load, and was positively correlated with alanine aminotransferase levels. Fast death rates were predictive of virus being undetectable by polymerase chain reaction at 3 months. These findings show that infection with hepatitis C virus is highly dynamic and that early monitoring of viral load can help guide therapy.

Chronic infection with hepatitis C virus (HCV) is alarmingly prevalent (2 to 15%) throughout the world; 20 to 30% of infected people may develop cirrhosis and 1 to 3% may develop liver cancer (*1*). Unfortunately, the current treatment with interferon (IFN) is successful in only 11 to 30% of cases (*1*), and the mechanism of action is not well understood (*2*). In other viral infections, such as human immunodeficiency virus (HIV) (*3*) and hepatitis B virus (HBV) (*4*), analysis of viral dynamics during antiviral therapy has been helpful in understanding pathogenesis and in guiding therapy. The kinetics of HCV during IFN therapy has been described recently (*5–7*), but the underlying viral dynamics and the effect of IFN are not yet well understood.

In a preliminary study (*6*), we observed a dose-dependent acute HCV RNA decline in serum over a 1-day period after a single injec-