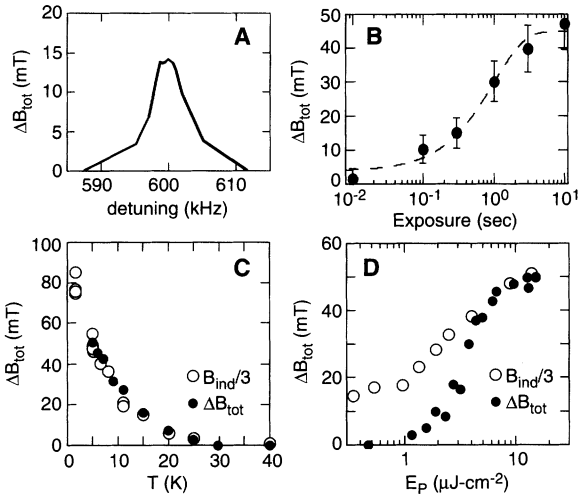

Fig. 3. Relative changes ΔB_{tot} induced by exposure to an additional, statically polarized pump beam, P_2, as a function of (**A**) P_2's detuning at an exposure of 0.3 s and (**B**) P_2's exposure at a detuning of +600.0 kHz. B_{tot} was saturated for 30 min under P illumination before data collection at T = 5 K and B = 5.297 T. The dotted fit in (B) is described in the text. Error bars indicate 90% confidence intervals. (**C**) A comparison of optically induced local fields near B_1 (open circles) and resonant depolarization shifts at B_1 (solid circles) for 1.6 K < T < 40 K. The former were taken from Fig. 1E and scaled by 1/3, and the latter were measured by sweeping B through B_1, as in Fig. 2, (A) and (B), with dB/dt = 0.001 T min^{-1}. (**D**) Same data taken at T = 5 K for various E_p. All data were taken with E_γ = 1.51 eV and E_p = 13 μJ cm^{-2}. In (C) and (D) a single pump laser was used, and in (A) and (B) P_2's energy was 7 μJ cm^{-2}.

obtained using the method of Fig. 2. However, these resonant shifts fail to scale with B_{ind} as the excitation density changes at T = 5 K (Fig. 3D). Such behavior is consistent with optically induced NMR because the tipping field itself is expected to be proportional to the excitation density.

The observed magnetic resonance behavior is both qualitatively and in some ways quantitatively consistent with a scenario of incoherent nuclear depolarization by delocalized electrons. The lower resonance field exhibits a perplexing departure from that expected for any nuclei in the host semiconductor, suggesting that the simple picture of resonance presented here is incomplete. Although one should consider alternative explanations, it is nevertheless difficult to image that B_1 (or B_2) is associated with an electronic moment. Comparably long electronic T_1 times have only been observed for donor localized electrons in silicon (*4*), and in that case depended sensitively on carrier density, donor concentration, and temperature. In contrast, the resonances we observe are somewhat immune to changes in the electronic environment and occur at the same values independent of not only the above parameters but also the excitation density. A hybrid experiment integrating both traditional NMR and this new optical resonance technique may help to clarify the relationship between these two phenomena. Ultimately, we believe that all-optical NMR may combine with local optical probes to address individual donor states whose tipping fields can be considerably stronger. Such a strategy might then enable coherent manipulation of individual nuclear spins, a first step in establishing optical control over solid-state nuclear spin coherence.

Note added in proof: Recent studies in quantum structures indicate that B_1 may be related to a harmonic of the As resonance (*18*).

References and Notes

1. C. P. Slichter, *Principles of Magnetic Resonance* (Springer-Verlag, New York, ed. 2, 1978).
2. *Optical Orientation*, F. Meier and B. P. Zakharchenya, Eds. (North-Holland, New York, 1984).
3. J. A. Marohn *et al.*, *Phys. Rev. Lett.* **75**, 1364 (1995).
4. G. Feher, *Phys. Rev.* **114**, 1219 (1959); G. Feher and E. A. Gere, *Phys. Rev.* **114**, 1245 (1959).
5. D. Paget, G. Lampel, B. Sapoval, V. I. Safarov, *Phys. Rev. B* **15**, 5780 (1977); D. Paget, *Phys. Rev. B* **24**, 3776 (1981); *Phys. Rev. B* **25**, 4444 (1982).
6. R. Tycko, S. E. Barrett, G. Dabbagh, L. N. Pfeiffer, K. W. West, *Science* **268**, 1460 (1995); N. N. Kuzma, P. Khandelwal, S. E. Barrett, L. N. Pfeiffer, K. W. West, *Science* **281**, 686 (1998).
7. M. Seck, M. Potemski, P. Wyder, *Phys. Rev. B* **56**, 7422 (1997).
8. D. C. Dixon, K. R. Wald, P. L. McEuen, M. R. Melloch, *Phys. Rev. B* **56**, 4743 (1997); H. Cho *et al.*, *Phys. Rev. Lett.* **81**, 2522 (1998); S. Kronmuller *et al.*, *Phys. Rev. Lett.* **81**, 2526 (1998).
9. B. E. Kane, *Nature* **393**, 133 (1998).
10. S. A. Crooker, J. J. Baumberg, F. Flack, N. Samarth, D. D. Awschalom, *Phys. Rev. Lett.* **77**, 2814 (1996).
11. J. M. Kikkawa, I. P. Smorchkova, N. Samarth, D. D. Awschalom, *Science* **277**, 1284 (1997).
12. J. M. Kikkawa and D. D. Awschalom, *Phys. Rev. Lett.* **80**, 4313 (1998).
13. J. M. Kikkawa and D. D. Awschalom, *Phys. Today* **52**, 33 (1999) and references therein.
14. V. L. Berkovitz, C. Hermann, G. Lampel, A. Nakamura, V. I. Safarov, *Phys. Rev. B* **18**, 1767 (1978).
15. A. W. Overhauser, *Phys. Rev.* **92**, 411 (1953); A. Abragam, *Phys. Rev.* **98**, 1729 (1955).
16. A. Abragam, *The Principles of Nuclear Magnetism* (Clarendon Press, Oxford, 1961).
17. V. P. Zakharchenya, M. E. Kompan, V. G. Fleisher, *Zh. ETF Pis. Red.* **19**, 734 (1974) [*J. Exp. Theor. Phys. Lett.* **19**, 378 (1974)].
18. G. Salis *et al.*, unpublished data.
19. S. Kronmuller *et al.*, *Phys. Rev. Lett.* **82**, 4070 (1999).
20. V. A. Novikov, V. G. Fleisher, *Zh. Eksp. Teor. Fiz.* **74**, 1026 (1978) [*Sov. Phys. J. Exp. Theor. Phys.* **47**, 539 (1978)].
21. V. K. Kalevich *et al.*, *Pis'ma Zh. Eksp. Teor. Fiz.* **35**, 17 (1982) [*J. Exp. Theor. Phys. Lett.* **35**, 20 (1982)].

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Trienoic Fatty Acids and Plant Tolerance of High Temperature

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The chloroplast membrane of higher plants contains an unusually high concentration of trienoic fatty acids. Plants grown in colder temperatures have a higher content of trienoic fatty acids. Transgenic tobacco plants in which the gene encoding chloroplast omega-3 fatty acid desaturase, which synthesizes trienoic fatty acids, was silenced contained a lower level of trienoic fatty acids than wild-type plants and were better able to acclimate to higher temperatures.

In some desert and evergreen plants, an increase in the growth temperature leads to a reduction in trienoic fatty acids α-linolenic acid (18:3) and hexadecatrienoic acid (16:3) (*1*, *2*). In order to investigate the physiological effect of these fatty acids in plants grown at high temperatures, we constructed transgenic tobacco plants in which the expression of the chloroplast trienoic fatty acid synthetase gene was inhibited.

Transgenic tobacco plants harboring transferred DNA (T-DNA) with the chloroplast-localized ω-3 desaturase gene (*FAD7*) from *Arabidopsis thaliana* under the control of the cauliflower mosaic virus 35*S* promoter were generated (*3*). Gene-silencing and reduction of trienoic–fatty acid content were observed in four transgenic lines (*4*). Of the four lines, two lines (T15 and T23) exhibited a 3 : 1 segregation ratio of kanamycin resistance versus nonresistance in the next generation, suggesting that the T-DNA was inserted in one position in the genome. The T15 line was backcrossed twice to

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produce a homozygous line (*5*). The T23 line was self-pollinated to produce another homozygous line.

The amount of trienoic fatty acids in the chloroplasts of homozygous T15 and T23 plants was lower than that in the chloroplasts of the wild-type plants (Table 1). This reduction in trienoic fatty acid content was associated with an increase in the corresponding dienoic fatty acid precursors, linoleic acid (18:2) and hexadecadienoic acid (16:2). The fatty acid composition of nonchloroplast lipids, such as phosphatidylcholine and phosphatidylethanolamine, was less affected by the absence of the chloroplast trienoic fatty acid synthetase gene (Table 1). The levels of monounsaturated fatty acids remained unaffected. Thus, the activity of chloroplast ω-3 fatty acid desaturase was suppressed in the T15 and T23 homozygous lines. The lipid ratios (Table 1) indicate that the overall flux through the prokaryotic and eukaryotic pathways of glycerolipid synthesis (*6*) was not affected by the T-DNA. These characteristics were stably inherited through backcrosses.

Photosynthesis is one of the most heat-sensitive functions of plant cells. Temperatures in the range of 35° to 45°C tend to inhibit photosynthesis (*7*, *8*). In order to assess the effect of high temperatures on the photosynthetic machinery, intact leaves from transgenic tobacco plants and *Arabidopsis* mutants (*9*) were pretreated at various temperatures between 25° and 55°C, and the level of photosynthetic activity was measured using O_2 evolution as the index of activity (*10*). At 40°C, the photosynthetic activity of the wild-type tobacco plants was significantly diminished, whereas the activity of the transgenic T15 and T23 plants was higher than that at the normal growing temperature of 25°C (Fig. 1A).

In order to confirm that the relation between the trienoic fatty acid level and the thermal stability of the photosynthetic machinery is the same in *Arabidopsis*, we examined the *Arabidopsis fad7fad8* double mutant, which lacks two chloroplast-localized ω-3 fatty acid desaturases (*11*). The fatty acid composition of the *fad7fad8* mutant was similar to that of the tobacco T15 and T23 lines. The chloroplast lipids of the *fad7fad8* mutant consisted of small amounts of trienoic fatty acids and large amounts of dienoic fatty acids (*11*). The *fad7fad8* mutant tolerated higher temperatures, as did the tobacco transgenic lines (Fig. 1B). On the other hand, the *fad3* mutant, which lacks ω-3 fatty acid desaturase localized in the endoplasmic reticulum (*12*), showed wild-type photosynthetic activity (Fig. 1C). Thus, trienoic fatty acids in chloroplast lipids affect the high-temperature tolerance of the photosynthetic machinery more than the trienoic fatty acids in nonchloroplast lipids.

The primary sites of thermal damage are thought to be components of the photosynthetic system located in the thylakoid membrane, such as photosystem II (PSII) (*7*). The potential quantum efficiency of PSII under dark-adapted conditions after exposure to high temperature was assessed by measuring the fluorescence parameter F_v/F_m (quantum yield of PSII in dark-adapted) of the samples used in the O_2 evolution experiments. However, heat treatment did not affect the F_v/F_m of the transgenic tobacco lines and *Arabidopsis fad7fad8* mutants (*13*). Thus, the trienoic fatty acid level in chloroplast lipids does not directly contribute to the resistance of PSII to high temperatures.

In most plants using the C_3 photosynthetic pathway, the optimal temperature for CO_2 assimilation is far below the thermal tolerance limit. Even within the limits, the photosynthetic productivity at high temperatures is rather low. The low rate of assimilation at high temperatures is caused, in part, by photorespiration and an imbalance in the regulation of carbon metabolism (*8*). A reduced trienoic fatty acid level in chloroplast lipids may alleviate such dissipative processes.

The effect of humidity on plant growth must be taken into account in determining the sensitivity of plants to the growing temperature. To ensure constant humidity in the fol-

Table 1. Fatty acid composition of individual membrane lipids from leaves of wild-type (WT) and transgenic tobacco (T15, T23) plants. The major classes of membrane lipids were isolated from the total lipid extracted from mature leaves, and the fatty acid composition was determined (*15*). Each value represents the mean of two independent experiments. Dash (–) indicates trace amounts (<1.0%).

Lipid class	Percent of total polar lipids	Fatty acid (mol%)							
		16:0	16:1	16:2	16:3	18:0	18:1	18:2	18:3
Monogalactosyldiacylglycerol									
WT	46.2	2.0	–	–	12.1	–	–	2.5	82.0
T15	48.3	2.7	1.6	10.2	2.2	–	2.1	53.7	27.1
T23	45.3	3.9	2.8	10.5	4.1	–	2.2	38.6	37.4
Digalactosyldiacylglycerol									
WT	35.2	33.0	1.1	–	–	2.5	2.7	6.4	51.9
T15	36.3	21.8	4.4	1.3	–	1.7	4.1	42.8	23.3
T23	39.0	31.2	–	–	–	4.1	6.0	34.7	21.9
Phosphatidylglycerol									
WT	7.3	26.6	36.9	–	–	2.3	7.4	11.1	15.8
T15	7.0	25.2	34.3	–	–	4.5	10.9	22.5	2.2
T23	6.6	24.8	33.6	–	–	4.6	13.4	19.8	3.0
Phosphatidylcholine									
WT	8.9	26.0	1.5	–	–	4.1	4.1	41.6	23.0
T15	6.7	24.5	1.1	–	–	5.2	5.2	52.5	11.7
T23	7.3	21.3	1.3	–	–	5.9	5.9	53.2	12.9
Phosphatidylethanolamine									
WT	2.5	31.5	1.8	–	–	7.0	2.7	42.2	14.4
T15	2.4	25.3	–	–	–	2.6	2.7	56.0	12.1
T23	3.6	31.6	–	–	–	4.9	3.7	47.0	11.9

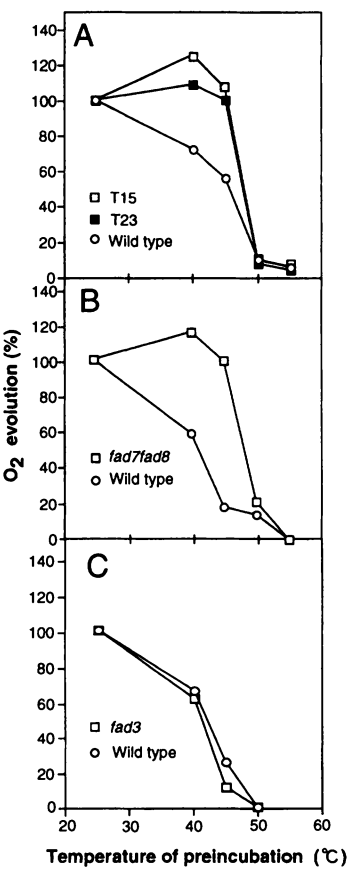

Fig. 1. Oxygen evolution from transgenic tobacco and mutant *Arabidopsis* leaves that had been preincubated at various temperatures for 5 min. (**A**) T15, T23, and wild-type tobacco. (**B**) *fad7fad8* double mutant and wild-type *Arabidopsis*. (**C**) *fad3* mutant and wild-type *Arabidopsis*. For each plant line, the O_2 evolution at 25°C was set at 100%. The O_2 evolution at 25°C was 2.4, 2.5, and 2.5 mmol O_2 m^{-2} s^{-1} in T15, T23, and WT tobacco, respectively, in (A); 1.4 and 1.7 mmol O_2 m^{-2} s^{-1} in *fad7fad8* and WT *Arabidopsis*, respectively, in (B); and 3.1 and 2.9 mmol O_2 m^{-2} s^{-1} in *fad3* and WT *Arabidopsis*, respectively, in (C). Each data point represents the mean value from four independent experiments.

lowing experiments, plants were grown on Murashige-Skoog (MS) agar media in closed plastic boxes or petri dishes. In plants grown at a cool temperature (15°C) and a more suitable growth temperature (25°C), there were no differences in the growth of the two transgenic tobacco lines (T15 and T23) and the wild type. After germination and cultivation of these plants for 45 days at 25°C, the fresh weight of the aerial parts of the T15 and T23 plants was 489 ± 71 mg and 513 ± 88 mg, respectively, whereas the fresh weight of the aerial parts of the wild-type plants was 497 ± 43 mg (n = 5). The fresh weight of the aerial parts of the T15, T23, and the wild-type plants cultivated at 15°C for 45 days was 6.2 ± 1.4 mg, 6.9 ± 1.2 mg, and 6.6 ± 0.9 mg (n = 5), respectively. These results in tobacco plants are consistent with the growth of the *Arabidopsis fad7fad8* mutant within the normal cultivation temperature range of 12° to 28°C (*11*). Furthermore, at temperatures below 10°C, the growth of the two transgenic tobacco lines and the growth of the wild type were similarly suppressed.

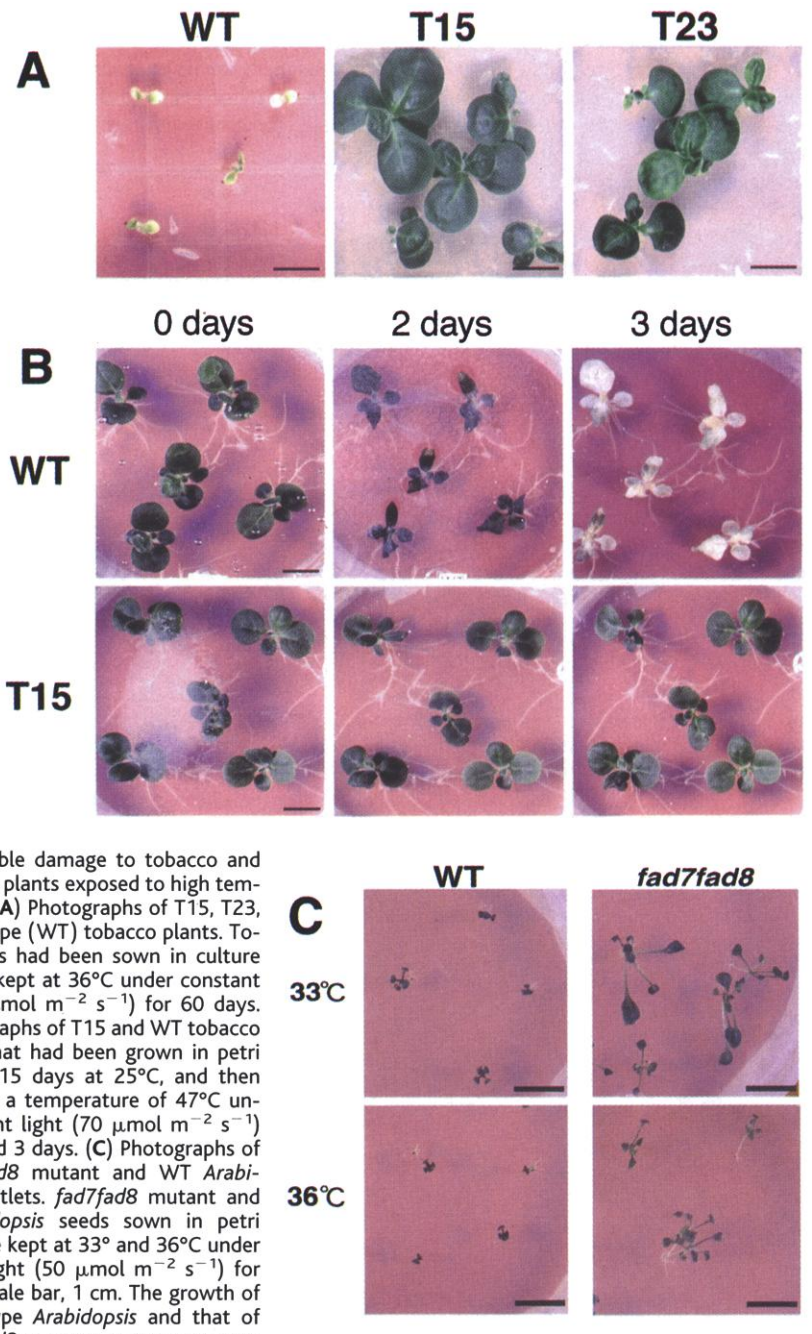

Fig. 2. Visible damage to tobacco and *Arabidopsis* plants exposed to high temperatures. (**A**) Photographs of T15, T23, and wild-type (WT) tobacco plants. Tobacco seeds had been sown in culture boxes and kept at 36°C under constant light (50 μmol m^{-2} s^{-1}) for 60 days. (**B**) Photographs of T15 and WT tobacco plantlets that had been grown in petri dishes for 15 days at 25°C, and then exposed to a temperature of 47°C under constant light (70 μmol m^{-2} s^{-1}) for 0, 2, and 3 days. (**C**) Photographs of the *fad7fad8* mutant and WT *Arabidopsis* plantlets. *fad7fad8* mutant and WT *Arabidopsis* seeds sown in petri dishes were kept at 33° and 36°C under constant light (50 μmol m^{-2} s^{-1}) for 14 days. Scale bar, 1 cm. The growth of the wild-type *Arabidopsis* and that of the *fad7fad8* mutant at temperatures below 30°C, were similar. However, *Arabidopsis* shows heat stress at lower temperatures than tobacco. Most of the wild-type plants grown at 36°C had died by day 45, and all of the wild-type and *fad7fad8* plants grown at a temperature near 40°C died.

An elevated trienoic fatty acid level as a result of overexpression of the *Arabidopsis FAD7* ω-3 desaturase gene (*14*) has a minor protective effect against chilling-induced damage to young transgenic tobacco seedlings (*15*). Furthermore, the *Arabidopsis* triple mutant, *fad3fad7fad8*, which lacks trienoic fatty acids in all membranes, can grow at temperatures as low as 6°C, although with reduced photosynthesis capacity (*16*). Thus, trienoic fatty acids are not critical for growth at low temperatures.

On the other hand, the resistance of a plant to high temperature depends on the trienoic fatty acid content. In plants cultivated at 30°C for 45 days after germination, the fresh weight of the aerial parts of the T15, T23, and wild-type plants was 492 ± 81 mg, 445 ± 62 mg, and 399 ± 69 mg (n = 5), respectively. At a higher temperature (36°C), marked differences in the growth of the transgenic tobacco lines and the wild type were seen (Fig. 2A). After cultivating plants at 36°C for 45 days, the fresh weight of the aerial parts of the T15 and T23 lines and the wild type was 124 ± 49 mg, 123 ± 23 mg, and 13 ± 6 mg (n = 5), respectively. Temperature resistance in the transgenic lines was not transient and was unlike the protection conferred by induction of a heat shock protein (*17*). At 47°C, the leaves of the wild-type plants began to wither within 2 days, and necrotic areas developed by the third day (Fig. 2B). However, T15 and T23 plants that were exposed to a temperature of 47°C were uninjured. Although the growth of the T15 and T23 plants was suppressed at 47°C growth resumed when the temperature was reduced to 25°C.

The *Arabidopsis fad7fad8* mutant was also resistant to high temperatures. When *Arabidopsis* wild-type plants and *fad7fad8* mutants were grown at a temperature above 30°C, and particularly near 35°C, a distinct difference in the growth of the wild-type and *fad7fad8* mutant was observed (Fig. 2C). When wild-type plants and *fad7fad8* mutants were grown under the high-temperature condition, the growth of the wild-type plants was significantly reduced and their leaves were wilted, whereas the growth of the *fad7fad8* mutant was only slightly reduced.

Saturation of thylakoid membrane lipids by catalytic hydrogenation increases the thermal stability of the membranes (*18*). Increased saturation may raise the temperature at which lipids such as monogalactosyldiacylglycerol phase-separate into nonbilayer structures, which disrupt membrane organization. If so, the resistance of plants to high temperatures might be improved by reducing the content of lower unsaturated fatty acids such as dienoic fatty acids (*19, 20*). The sensitivity of these plants to low temperatures, however, might possibly be increased (*21*).

The only difference between our gene-silenced transgenic plants that were resistant to high temperature and the respective wild-type plants was that the chloroplasts of the transgenic plants contained a reduced level of trienoic fatty acids and an elevated level of dienoic fatty acids, which is controlled by chloroplast ω-3 fatty acid desaturase. Of the six different higher plant desaturases whose genes have been cloned, only the expression of the chloroplast FAD8 ω-3 fatty acid desaturase gene changes in response to a change in ambient temperature (*22*).

The ω-3 fatty acid desaturase enzyme, which is expressed in nearly all plant species, may be widely useful in engineering temperature tolerance in plants.

References and Notes

1. R. W. Pearcy, *Plant Physiol.* **61**, 484 (1978).
2. J. K. Raison, J. K. M. Roberts, J. A. Berry, *Biochim. Biophys. Acta* **688**, 218 (1982).
3. The T-DNA region of pTiDES7 [K. Iba *et al.*, *J. Biol. Chem.* **268**, 24099 (1993)] was introduced into the genome of *Nicotiana tabacum* cv SR1 (*14*). Plants were transformed by the leaf-disc method [R. B. Horsch *et al.*, *Science* **227**, 1229 (1985)].
4. Twenty-nine lines of R_0 plants exhibiting kanamycin resistance were selected. The fatty acid composition of the R_1 plant derived from each line was measured (*15*).
5. The fatty acid composition of a number of individual seeds of the backcrossed T15 line was assayed in order to identify homozygous individuals. This analysis was repeated in one subsequent generation to ensure that the trait was stably inherited.
6. C. Somerville and J. Browse, *Science* **252**, 80 (1991).
7. J. Berry and O. Björkman, *Annu. Rev. Plant Physiol.* **31**, 491 (1980).
8. E. Weis and J. A. Berry, in *Plants and Temperature*, P. S. Long and F. I. Woodward, Eds. (Company of Biologists, Cambridge, 1988), pp. 329–346.
9. *Arabidopsis thaliana* mutant lines LK70 (*fad3*) and SH1 (*fad7-1fad8-1*) were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University (Columbus, OH).
10. Sterilized tobacco and *Arabidopsis* seeds were routinely germinated on MS agar [T. Murashige and F. Skoog, *Physiol. Plant.* **15**, 473 (1962)] and cultivated at 25° and 23°C, respectively, under a white fluorescent lamp at an intensity of 70 μmol m^{-2} s^{-1}. The fully expanded fourth leaves of tobacco plants that had been cultivated for 5 days under continuous irradiation from a metal halide lamp at an intensity of 170 μmol m^{-2} s^{-1} were used as experimental samples. In *Arabidopsis*, the fully expanded fifth and sixth leaves were used. The detached leaves were heat-treated on a thermostatic heating block. A sheet of filter paper moistened with sterilized water was placed on the heating block. The leaf was placed on the sheet with the surface facing upward and was covered with a thin layer of polyethylene film to prevent drying. Heat treatment was applied for 5 min at different temperatures as indicated in the text. The leaf samples were then immersed in a transparent acrylic cuvette filled with 10 mM 2-(*N*-morpholino) ethanesulfonic acid (MES) buffer saturated with CO_2, and the photosynthetic evolution of O_2 was measured with a Clark-type oxygen electrode at 25°C under irradiation by red light as described [Y. Kobayashi and U. Heber, *Photosynth. Res.* **41**, 419 (1994)].
11. M. McConn, S. Hugly, J. Browse, C. Somerville, *Plant Physiol.* **106**, 1609 (1994).
12. B. Lemieux, M. Miquel, C. Somerville, J. Browse, *Theor. Appl. Genet.* **80**, 234 (1990).
13. Y. Murakami *et al.*, unpublished data.
14. H. Kodama, T. Hamada, G. Horiguchi, M. Nishimura, K. Iba, *Plant Physiol.* **105**, 601 (1994).
15. H. Kodama, G. Horiguchi, T. Nishiuchi, M. Nishimura, K. Iba, *Plant Physiol.* **107**, 1177 (1995).
16. M. McConn and J. Browse, *Plant Cell* **8**, 403 (1996).
17. J. H. Lee, A. Hübel, F. Schöffl, *Plant J.* **8**, 603 (1995).
18. P. G. Thomas *et al.*, *Biochim. Biophys. Acta* **849**, 131 (1986).
19. S. Hugly, L. Kunst, J. Browse, C. Somerville, *Plant Physiol.* **90**, 1134 (1989).
20. L. Kunst, J. Browse, C. Somerville, *Plant Physiol.* **91**, 401 (1989).
21. S. Hugly and C. Somerville, *Plant Physiol.* **99**, 197 (1992).
22. C. Somerville, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 6215 (1995).

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One Polypeptide with Two Aminoacyl-tRNA Synthetase Activities

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The genome sequences of certain archaea do not contain recognizable cysteinyl–transfer RNA (tRNA) synthetases, which are essential for messenger RNA–encoded protein synthesis. However, a single cysteinyl–tRNA synthetase activity was detected and purified from one such organism, *Methanococcus jannaschii*. The amino-terminal sequence of this protein corresponded to the predicted sequence of prolyl–tRNA synthetase. Biochemical and genetic analyses indicated that this archaeal form of prolyl–tRNA synthetase can synthesize both cysteinyl-$tRNA^{Cys}$ and prolyl-$tRNA^{Pro}$. The ability of one enzyme to provide two aminoacyl-tRNAs for protein synthesis raises questions about concepts of substrate specificity in protein synthesis and may provide insights into the evolutionary origins of this process.

The insertion of cysteine into nascent peptides during protein synthesis is dependent on the interaction of cysteine codons with cysteinyl-tRNA (Cys-tRNA) in the ribosomal A site. Cys-tRNA is synthesized from cysteine and $tRNA^{Cys}$ in an adenosine 5′-triphosphate (ATP)–dependent reaction catalyzed by cysteinyl–tRNA synthetase (CysRS). Genes encoding CysRS, *cysS*, have been detected in over 40 organisms encompassing all the living kingdoms (*1*). The only known exceptions are the thermophilic methanogens *Methanococcus jannaschii* and *Methanobacterium thermoautotrophicum*, the complete genome sequences of which contain no open reading frames encoding *cysS* homologs (*2*), raising the question of how these archaea synthesize Cys-tRNA for protein synthesis. It was initially suggested that Cys-$tRNA^{Cys}$ might be synthesized by a pathway involving modification of a mischarged tRNA such as Ser-$tRNA^{Cys}$, using a mechanism reminiscent of those previously described for the synthesis of asparaginyl-tRNA (Asn-tRNA), glutaminyl-tRNA (Gln-tRNA) and, more specifically, selenocysteinyl-tRNA (Sec-tRNA) (*3*). Biochemical analyses revealed no evidence for such a pathway (*4*) but instead showed that Cys-tRNA is synthesized directly from cysteine and tRNA in an ATP-dependent reaction (*5*). The identity of the enzyme responsible for Cys-tRNA synthesis in *M. jannaschii* and *M. thermoautotrophicum* is unknown. The recent finding that some aminoacyl–tRNA synthetase (AARS)–encoding genes may be dispensable for cell viability also raised the possibility that *cysS* genes might be absent altogether from the genomes of *M. jannaschii* and *M. thermoautotrophicum* (*6*).

To investigate how Cys-tRNA is synthesized in *M. jannaschii*, we attempted to purify from cell-free extracts a protein with CysRS activity. Conventional chromatographic procedures (*7*) led to the isolation of a single protein with normal CysRS activity (Fig. 1, A and B). Protein analysis revealed an 18–amino acid peptide sequence matching the predicted NH_2-terminal sequence of *M. jannaschii* prolyl–tRNA synthetase (ProRS)

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