germinated on Al-containing media (pH 4.3), we germinated seeds from the CSb and control lines on media containing 0.1 to 1 mM Al. At concentrations higher than 300 μM, control seeds germinated but did not develop a root system (Fig. 3A), and at low Al concentrations (50 to 75 μM) root growth was only slightly affected, but root-hair development was severely impaired (Fig. 3B). CSb lines germinated on Al-containing medium were more resistant to these effects (Fig. 3C). Root growth and root hair development of CSb lines exposed to Al correlated with their level of CS activity and citrate content (Fig. 3, D and E). Tolerant and susceptible phenotypes segregated with a 3:1 ratio for the lines containing a single transferred DNA (T-DNA) insertion (Fig. 3H). Al tolerance cosegregated with the kanamycin resistance gene also present in the T-DNA of the binary vector used to produce the CSb lines (*14*).

To analyze Al uptake, we exposed roots from 14-day-old homozygous CSb plantlets for 1 hour to a 100 μM solution of Al (pH 4.3), then washed the roots extensively with water and stained them with hematoxylin (*18*). CSb lines showed less staining than did controls (Fig. 3, F, G, I, and J), indicating that lower amounts of Al penetrated the root tip and root hairs of CSb lines (hematoxylin turns violet only in the presence of Al).

The 35S-CSb construct was also introduced into the genome of papaya plants by particle bombardment (*19*). CSb transgenic papaya lines contained two- to threefold higher levels of citrate synthase compared with controls transformed with the vector alone (*14*). Twenty regenerated plants from each papaya CSb line were transferred to rooting media (pH 4.3) containing different concentrations of Al. Root development failed in control plants exposed to 50 μM or higher concentrations of Al, whereas CSb lines were able to form roots and grow normally on concentrations of Al of up to 300 μM (Fig. 4).

Fig. 4. Control and citrate-overproducing papaya plants after 30 days of culture in the presence of 300 μM Al. (Left) CSb transformant; (right) transformant containing the vector without the CS coding sequence.

Our data provide a direct demonstration that organic acid excretion is indeed a mechanism of Al tolerance in higher plants and that this trait can be engineered transgenically. This finding opens the possibility of applying this technology to important crop plants, such as maize, rice, and sorghum, which are often grown in acidic soils in which Al toxicity is a major problem.

REFERENCES AND NOTES.

1. C. D. Foy, R. L. Chaney, M. C. White, *Annu. Rev. Plant Physiol.* **29**, 511 (1978).
2. V. L. Kochian, *Plant. Mol. Biol.* **46**, 237 (1995); E. Delhaize and P. R. Ryan, *Plant Physiol.* **107**, 315 (1995).
3. V. C. Baliger *et al.*, *Plant Soil* **116**, 257 (1989).
4. V. C. Baligar, H. L. Dos Santos, G. V. E. Pitta, A. F. de C.Bahia Filho, *Plant Soil* **150**, 271 (1993).
5. P. R. Ryan, J. M. Di Tomaso, L. V. Kochian, *J. Exp. Bot.* **44**, 437 (1993).
6. J. D. Ownby and H. R. Popham, *J. Plant Physiol.* **135**, 588 (1990).
7. P. R. Ryan, E. Delhaize, J. P. Randall, *Planta* **196**, 103 (1995).
8. E. Delhaize *et al.*, *Plant Physiol.* **103**, 685 (1993).
9. E. Delhaize, P. R. Ryan, P. J. Randall, *ibid.*, p. 695; D. M. Pellet, D. L. Grunes, L.V. Kochian, *Planta* **196**, 788 (1995).
10. S. C. Miyasaka, J. Buta, R. K. Howell, C. D. Foy, *Plant Physiol.* **91**, 737 (1991).
11. N. V. Hue, G. R. Craddock, F. Adams, *Soil Sci. Soc. Am. J.* **50**, 28 (1986); R. J. Barlett and D. C. Riego, *Plant Soil* **37**, 419 (1972).
12. *Pseudomonas aeruginosa* CS gene and antiserum were reported by J. L. Donald *et al.* [*J. Bacteriol.* **171**, 5542 (1989)].
13. L. Herrera-Estrella and J. Simpson, in *Plant Molecular Biology: A Practical Approach*, C. Shaw, Ed. (IRL, Oxford, UK, 1988), pp. 131–160.
14. J. M. de la Fuente, V. Ramírez-Rodríguez, J. L. Cabrera-Ponce, L. Herrera-Estrella, data not shown.
15. CS activity was measured as described by P. Srere [*Methods Enzymol.* **13**, 3 (1969)].
16. For measurement of citrate content, 1 g of roots was ground in liquid nitrogen and extracted with 10 ml of boiling 80% ethanol. The homogenate was centrifuged in a tabletop centrifuge at 1000 rpm for 10 min and the supernatant filtered through a 0.45-μm Millipore filter. Organic acid content was analyzed by HPLC as described [H. D. Picha, *J. Agric. Food Chem.* **33**, 743 (1985)].
17. One hundred seeds were germinated on plates placed in a vertical position inside a container with nutrient solution. When the plantlets were exposed to Al-containing Blaydes solution [W. A. Parrot and H. J. Bouton, *Crop Sci.* **30**, 387 (1990)] (adjusted to pH 4.3 by addition of 0.1 N KOH), the plates containing the seedlings were rotated 90°, where a change in the direction of root growth occurred due to gravitropism, allowing the measurement of root growth after exposure to Al. This technique was described by L. Taiz and A. Murphy [*Plant Physiol.* **108**, 29 (1995)]. The pH of the nutrient solution was monitored throughout the experiment and found to vary by no more than 0.2 pH units.
18. E. Polle, C. Kozak, A. J. Kittrik, *Crop Sci.* **30**, 389 (1978).
19. J. Cabrera-Ponce, A. Vegas-Garcia, L. Herrera-Estrella, *Plant Cell Rep.* **15**, 1 (1995).

20. We are grateful to W. Duckworth for providing the *P. aureginosa* CS gene and antiserum to CS. We thank G. Olmedo and J. Simpson for critically reviewing our work. Supported in part by grants from the Howard Hughes Medical Institute (75191-526901) and the European Commission (ERBIC-18CT-960089).

11 December 1996; accepted 8 April 1997

Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethene to Ethene

Xavier Maymó-Gatell, Yueh-tyng Chien, James M. Gossett, Stephen H. Zinder*

Tetrachloroethene is a prominent groundwater pollutant that can be reductively dechlorinated by mixed anaerobic microbial populations to the nontoxic product ethene. Strain 195, a coccoid bacterium that dechlorinates tetrachloroethene to ethene, was isolated and characterized. Growth of strain 195 with H_2 and tetrachloroethene as the electron donor and acceptor pair required extracts from mixed microbial cultures. Growth of strain 195 was resistant to ampicillin and vancomycin; its cell wall did not react with a peptidoglycan-specific lectin and its ultrastructure resembled S-layers of Archaea. Analysis of the 16S ribosomal DNA sequence of strain 195 indicated that it is a eubacterium without close affiliation to any known groups.

The solvent tetrachloroethene [perchloroethylene (PCE)] is a common groundwater pollutant (*1*, *2*) that is highly toxic and is suspected to be a human carcinogen. It is nonbiodegradable by aerobes but can be reductively dechlorinated by natural microbial communities and mixed microbial enrichment cultures under anaerobic conditions according to the reaction sequence shown in Fig. 1 (*3*). The formation of nontoxic products such as ethene (ETH) (*4*) and ethane (*5*) indicates the potential for complete anaerobic detoxification of chloroethenes in situ.

Slow reductive dechlorination of chlo-

X. Maymó-Gatell, Y.-T. Chien, S. H. Zinder, Section of Microbiology, Wing Hall, Cornell University, Ithaca, NY 14853, USA.

J. M. Gossett, School of Civil and Environmental Engineering, Cornell University, Ithaca, NY 14853, USA.

*To whom correspondence should be addressed. E-mail: shz1@cornell.edu

roethenes and other haloorganic compounds can be carried out by a cometabolic process by organisms rich in reduced transition-metal cofactors such as methanogens and acetogens (*6*, *7*). However, some organisms can use haloorganic compounds as electron acceptors for energy conservation and growth (sometimes called dehalorespiration) (*8*), and several anaerobes that grow by dechlorinating PCE partially to *cis*-dichloroethene (*cis*-DCE) have been described recently (*9*).

We have studied a set of enrichment cultures that dechlorinate PCE to ETH, using methanol, H_2, or butyrate as electron donors (*10–13*). A partially purified culture was derived from a 10^{-6} dilution of a methanol-PCE culture inoculated into medium with H_2 and PCE (*14*). This culture contained no methanogens or acetogens and could be transferred indefinitely into H_2-PCE medium supplemented with a mixture called ABSS [2 mM acetate, 0.05 mg of vitamin B_{12} per liter, and 25% (v/v) anaerobic digester sludge supernatant], but it could not be transferred if either H_2 or PCE was omitted. The amount of H_2 consumed was stoichiometric to the chlorine eliminated through reductive dechlorination of PCE. The two main morphotypes in this culture were an irregular coccus and a short rod.

PCE dechlorination with H_2 as the electron donor was resistant to vancomycin (100 mg/liter), an inhibitor of eubacterial peptidoglycan cell wall synthesis (*11*). Therefore, we transferred the partially purified H_2-PCE culture [2% (v/v) inoculum] to medium containing vancomycin (100 mg/liter) or ampicillin (another peptidoglycan-synthesis inhibitor) at up to 3 g/liter. PCE was dechlorinated and only the coccoid morphotype was present under these conditions (*15*). No PCE dechlorination was detected in cultures transferred to medium containing tetracycline (a eubacterial protein-synthesis inhibitor) at 20 mg/liter.

Ampicillin- or vancomycin-treated cultures could not be transferred a second time into antibiotic-containing medium. On the basis of previous results (*11*), it was likely that other organisms present in the culture were producing a nutrient or nutrients required by the PCE dechlorinator and not present in ABSS. Therefore, we amended the ABSS medium of second-generation ampicillin-treated cultures with either the filter-sterilized supernatant or sonicated pellet fractions of mixed cultures (Table 1). Both culture supplementations stimulated dechlorination, with the pellet fraction extract being more stimulatory. Moreover, only cultures amended with the pellet extract could be transferred again. These results indicate that one or more cell components of contaminating organisms were responsible for the stimulation.

Other potential nutrient amendments were examined for their effect on dechlorination (Table 1). Cholesterol and horse serum, two nutrients that are required by many mycoplasmas (eubacteria lacking a cell wall), did not stimulate dechlorination, whereas yeast extract and a mixture of volatile fatty acids, which are required by certain anaerobes, had a slight stimulatory effect. Casamino acids (Difco Laboratories, Detroit, Michigan) had a stimulatory effect similar to that produced by the supernatant fraction of mixed cultures. Nevertheless, none of these amendments allowed further transfer. Growth stimulation was not conferred by cell extracts of the Gram-negative aerobic eubacterium *Escherichia coli* or of

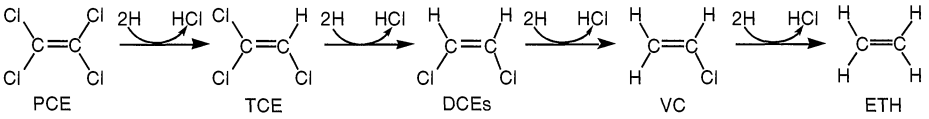

Fig. 1. Reductive dechlorination of chloroethenes. TCE, trichloroethene; DCEs, dichloroethene isomers (represented by *cis*-DCE); 2H, electron pair derived from the electron donor.

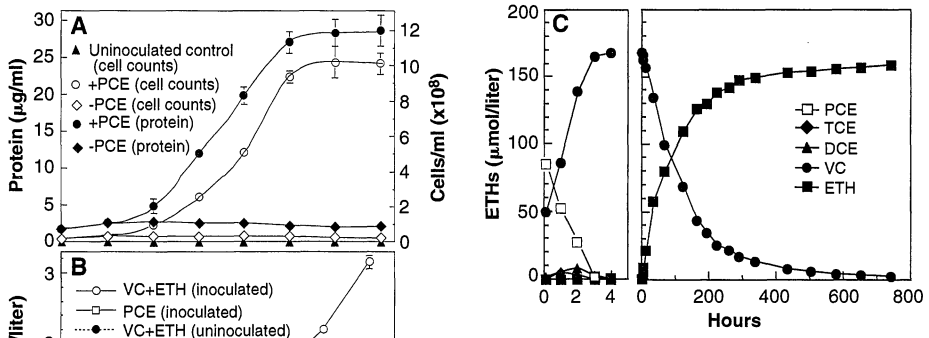

Fig. 2. (**A**) Growth of strain 195 on H_2-PCE as measured by particulate protein or cell counts. Growth medium was supplemented with ABSS and 5% (v/v) extract from a mixed culture. Protein was quantified with the NanoOrange kit (Molecular Probes, Eugene, OR); cells were counted in a Petroff Hauser counting chamber with the use of an epifluorescence microscope after staining with acridine orange (4 mg/liter, final concentration). (**B**) PCE utilization and product formation by the same cultures as in (A). Data points represent means ± SD, unless values are smaller than the symbols, from triplicate tubes. (**C**) Conversion of PCE to ETH (about 120 μmol/liter) by a pure culture of strain 195. The culture received several doses of PCE followed by flushing with N_2 and CO_2, which removed all ETHs except for about 50 μmol of VC per liter. PCE was underestimated in early data points because of its slow dissolution into the aqueous phase (*13*).

Table 1. Effect of nutrient addition on product formation from PCE by cultures transferred a second time into ABSS-supplemented medium containing ampicillin (0.3 g/liter). Products were measured 23 days after transfer. Values less than 1 μmol/liter are denoted as 0.

Addition	Product (μmol/liter)			
	TCE	DCEs	VC	ETH
None	0	0	35	0
Mixed culture supernatant [20% (v/v)]*	0	20	1400	55
Mixed culture pellet extract [10% (v/v)]†	0	37	2130	172
Cholesterol (10 mg/liter)	0	0	9	0
Horse serum [15% (v/v)]	0	0	28	0
Volatile fatty acids‡	0	0	133	3
Yeast extract (2 g/liter)	203	83	288	0
Casamino acids (0.5 g/liter)	0	30	1600	70
E. coli extract [15% (v/v)]§	20	10	32	0
C. pasteurianum extract [15% (v/v)]§	50	120	60	3

*Addition of 20% (v/v) supernatant of a mixed PCE dechlorinating culture incubated without ampicillin. †Extract from a cell pellet from a mixed culture equivalent to 10% (v/v) of the culture. ‡Final concentrations: acetate, 29.7 mM; propionate, 8 mM; butyrate 3.2 mM, valerate, 0.92 mM; isovalerate, 0.91 mM; isobutyrate, 1.09 mM, 2-methyl butyrate, 0.82 mM. §Total amount of cell biomass is unknown but is greater than the culture pellet extract.

the Gram-positive anaerobic eubacterium *Clostridium pasteurianum* strain W5, which indicates that the growth factor is not ubiquitous in bacteria.

Using a growth medium supplemented with filter-sterilized extract from mixed H_2-PCE cultures and ABSS (*14*), we isolated the PCE-dechlorinating organism (strain 195) by a 10^{-7} dilution to liquid H_2-PCE medium containing ampicillin (0.3 g/liter). These cultures, when transferred several times in the absence of ampicillin, showed no morphotypes other than irregular cocci. No visible growth was detected by tests for contamination (with a sensitivity of about 10 organisms per milliliter) with basal growth medium amended with lactate, sulfate, or thiosulfate to detect sulfate reducers, yeast extract (0.2 g/liter) to detect fermentative heterotrophs, or Brewer's thioglycollate medium (Difco) to detect fermentative heterotrophs.

Growth of strain 195 on H_2 and PCE was measured by direct microscopic cell counts and cell protein during metabolism of PCE to vinyl chloride (VC) and ETH (Fig. 2, A and B). Cultures continued to grow until day 5, with a doubling time of about 19.2 hours. After day 5, growth ceased but PCE dechlorination continued, which suggests uncoupling of growth and dechlorination. Cultures receiving H_2 but not PCE showed only slight growth, and PCE dechlorination products were not detected in uninoculated cultures. The amount of VC and ETH produced represented more than 90% of the PCE added to inoculated cultures. The protein yield for days 1 through 5 was 4.8 ± 0.3 g of protein per mole of chloride released; a specific activity of 69.0 ± 10.5 nmol of chloride released per minute per milligram of protein was determined.

Analysis of the conversion of PCE to ETH by a culture of strain 195 that had received five previous doses of PCE showed that PCE was metabolized to VC at a rate of 40 μmol per hour per liter of culture medium, with little buildup of intermediates (Fig. 2C). VC dechlorination to ETH commenced after PCE depletion and could be fit by first-order kinetics with a half-life of about 80 hours for the first 300 hours and of about 150 hours thereafter. This indicated a decay with time in the ability of the culture to metabolize VC. These results resemble those for the mixed methanol-PCE culture from which it was derived (*13*), except that the mixed culture dechlorinated VC more rapidly relative to PCE. If strain 195 is responsible for VC dechlorination in those mixed cultures, then some factor, perhaps nutritional, limits the rate of VC dechlorination in the pure culture. It is also possible that there is another organism or strain present in the mixed culture that is capable of more rapid VC metabolism.

Physiological characterization of strain 195 revealed that it required H_2 for PCE reduction and that it grew only when both H_2 and PCE were present (Fig. 2). Potential electron donors that supported neither PCE dechlorination, nor growth in the absence of PCE, were methanol, pyruvate, lactate, ethanol, formate, glucose, and yeast extract. Potential electron acceptors that did not support growth or were not reduced when H_2 was provided as the electron donor included sulfate, sulfite, thiosulfate, nitrate, nitrite, fumarate, and oxygen (2 or 21%). The culture could reductively dechlorinate 1,2-dichloroethane and 1,2-dibromoethane to ETH, as did the original enrichment culture (*13*).

Electron microscopic examination (*16*) of strain 195 (Fig. 3, A and B) revealed small, irregular coccoid cells with an unusual cell wall ultrastructure that resembled the S-layer protein subunit type of cell walls found in many Archaea (*17*). To test for the presence of a peptidoglycan cell wall, we used fluorescently labeled wheat germ agglutinin, which specifically binds to *N*-acetylglucosamine and *N*-acetylneuraminic acid (*18*). This stain bound to whole cells of the Gram-positive eubacterium *C. pasteurianum*

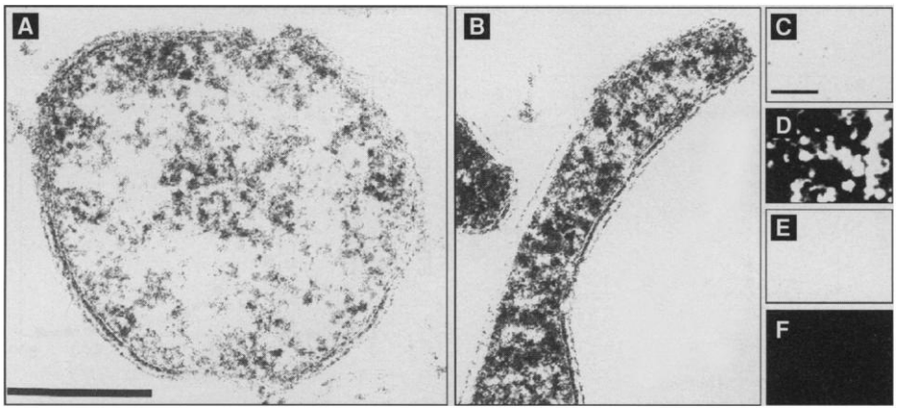

Fig. 3. Thin-section electron micrographs of coccoid (**A**) and flattened (**B**) cells of strain 195 stained with uranyl acetate. Scale bar, 0.2 μm. Phase-contrast (**C**) and epifluorescence (**D**) micrographs of cell wall preparations of *E. coli* DH5α stained with fluorescein-labeled wheat germ agglutinin (100 mg/liter) (Molecular Probes) (*18*). Phase-contrast (**E**) and epifluorescence (**F**) micrographs of cell wall preparations of strain 195 stained with wheat germ agglutinin. Cell wall samples for (C) through (F) were prepared by lysing the cells in boiling 4% SDS in 25 mM phosphate buffer (pH 7) (*20*) followed by heat fixation to a microscope slide and washing with distilled water to remove SDS and other chemicals before staining. Scale bar in (C) [for (C) through (F)], 5 μm.

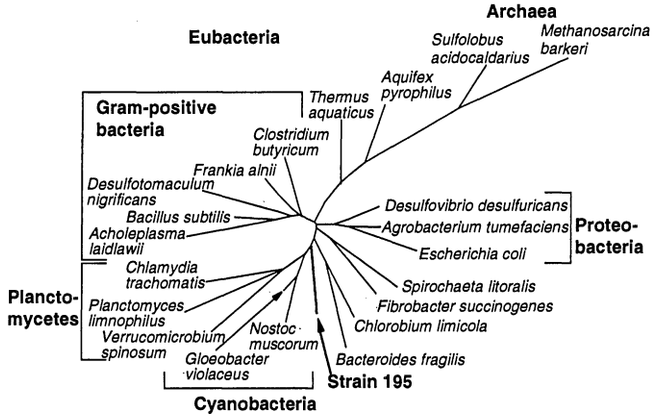

Fig. 4. Unrooted phylogenetic tree generated for the 16S ribosomal DNA sequence (GenBank database number AF004928) from strain 195 with the use of the SUGGEST TREE maximum-likelihood program provided by the Ribosome Database Project (RDP) (*21*). DNA was extracted from strain 195 as described (*22*). The sequence was amplified as a polymerase chain reaction product with the use of primers 27f and 1522r under standard conditions (*23*), followed by cloning with the Invitrogen (San Diego, California) TA cloning kit and sequencing with an Applied Biosystems model 373 analyzer operated by the Cornell Biotechnology Institute. Eight sequencing primers were used (*23*), including two against the vector, resulting in only a single ambiguous base in the entire sequence. For simplicity, some organisms included in the original analyses have been deleted from the figure. Other analyses of these sequences were performed by manually aligning the sequence of strain 195 to other prealigned sequences from the RDP, followed by the use of the PHYLIP 3.5c package (*24*), including DNAML (maximum-likelihood analysis), and DNADIST (Kimura model) coupled to either FITCH or NEIGHBOR.

WF (*15*) and to cell wall preparations of the Gram-negative eubacterium *E. coli* DH5α (Fig. 3, C and D) (*18*). No binding was detected for whole cells (*15*) or for cell wall preparations (Fig. 3, E and F) of strain 195.

The phylogenetic position of strain 195 was determined on the basis of its 16S ribosomal DNA sequence (Fig. 4). The PCE dechlorinator grouped within the eubacteria in all analyses but did not cluster within any of the known phylogenetic lines. Although the maximum likelihood analysis presented places strain 195 on a branch that includes cyanobacteria and planctomycetes, DNA distance analyses placed it closer to *Clostridium butyricum* and its relatives (*19*) but with little affiliation for other members of the Gram-positive branch. Thus, its relationship with the presently described eubacterial branches is unclear at this time. It is clearly distinct from other recent isolates that reduce PCE to *cis*-DCE (*9*), which are affiliated with the ε and γ branches of the Proteobacteria or with the Gram-positive sulfate-reducing bacteria. Because strain 195 does not appear to belong to any presently known genus or species, we suggest naming it *Dehalococcoides ethenogenes* strain 195, pending a more thorough taxonomic description.

In summary, we isolated an organism that is capable of respiratory reductive dechlorination of PCE completely to ETH with H_2 as an electron donor. Previous isolates reduce PCE only as far as *cis*-DCE. It is of interest that at many PCE-contaminated sites, dechlorination proceeds only as far as *cis*-DCE, whereas at other sites VC and ETH are produced (*3*). It is not clear whether incomplete dechlorination at a given site is due to suboptimal physiochemical conditions, deficiencies in electron donors or nutrients present, or a lack of appropriate organisms.

REFERENCES AND NOTES

1. P. H. Abelson, *Science* **250**, 73 (1990).
2. J. J. Westrick, J. W. Mello, R. F. Thomas, *J. Am. Water Works Assoc.* **76(5)**, 52 (1984).
3. R. E. Hinchee, A. Leeson, L. Semprini, Eds., *Bioremediation of Chlorinated Solvents* (Battelle, Columbus, OH, 1995).
4. D. L. Freedman and J. M. Gossett, *Appl. Environ. Microbiol.* **55**, 2144 (1989).
5. W. P. de Bruin, M. J. J. Kotterman, M. A. Posthumus, G. Schraa, A. J. B. Zehnder, *ibid.* **58**, 1996 (1992).
6. C. J. Gantzer and L. P. Wackett, *Environ. Sci. Technol.* **25**, 715 (1991).
7. B. D. Ensley, *Annu. Rev. Microbiol.* **45**, 283 (1991).
8. W. W. Mohn and J. M. Tiedje, *Microbiol. Rev.* **56**, 482 (1992).
9. C. Holliger, G. Schraa, A. J. M. Stams, A. J. B. Zehnder, *Appl. Environ. Microbiol.* **59**, 2991 (1993); H. Scholz-Muramatsu, A. Neumann, M. Messmer, E. Moore, G. Diekert, *Arch. Microbiol.* **163**, 48 (1995); P. K. Sharma and P. L. McCarty, *Appl. Environ. Microbiol.* **62**, 761 (1996); J. Gerritse *et al.*, *Arch. Microbiol.* **165**, 132 (1996); L. R. Krumholz, R. Sharp, S. S. Fishbain, *Appl. Environ. Microbiol.* **62**, 4108 (1996).
10. T. D. DiStefano, J. M. Gossett, S. H. Zinder, *Appl. Environ. Microbiol.* **57**, 2287 (1991).
11. ———, *ibid.* **58**, 3622 (1992).
12. D. E. Fennell, M. A. Stover, S. H. Zinder, J. M. Gossett, in (*3*), pp. 9–16.
13. V. Tandoi, T. D. DiStefano, P. A. Bowser, J. M. Gossett, S. H. Zinder, *Environ. Sci. Technol.* **28**, 973 (1994).
14. X. Maymó-Gatell, V. Tandoi, J. M. Gossett, S. H. Zinder, *Appl. Environ. Microbiol.* **61**, 3928 (1995).
15. X. Maymó-Gatell, Y.-t. Chien, J. M. Gossett, S. H. Zinder, data not shown.
16. J. J. Bozzola and L. D. Russell, *Electron Microscopy: Principles and Techniques for Biologists* (Jones and Bartlett, Boston, MA, 1992), pp. 16–37.
17. O. Kandler, *Syst. Appl. Microbiol.* **16**, 501 (1994).
18. R. K. Sizemore, J. J. Caldwell, A. S. Kendrick, *Appl. Environ. Microbiol.* **56**, 2245 (1990).
19. M. D. Collins *et al.*, *Int. J. Syst. Bacteriol.* **44**, 812 (1994).
20. G. D. Sprott, S. F. Koval, C. A. Schnaitman, in *Methods for General and Molecular Microbiology*, P. Gerhardt, Ed. (American Society for Microbiology, Washington, DC, 1994), pp. 72–103.
21. B. L. Maidak *et al.*, *Nucleic Acids Res.* **22**, 3485 (1994).
22. D. C. Goodwin and S. B. Lee, *Biotechniques* **15**, 438 (1993).
23. J. L. Johnson, in *Methods for General and Molecular Microbiology*, P. Gerhardt, Ed. (American Society for Microbiology, Washington, DC, 1994), pp. 683–700.
24. J. Felsenstein, Phylogenetic inference program (PHYLIP) manual version 3.5c (University of Washington, Seattle, WA, 1993). http://evolution.genetics.washington.edu/phylip.html

25. Supported by the U.S. Air Force Armstrong Laboratory, Environmental Quality Directorate, Tyndall Air Force Base, FL; the Cornell Center for Advanced Technology in Biotechnology; and the "La Caixa" Foundation, Catalonia, Spain (X.M.-G.). We thank T. Anguish, S. McKay, and C. Bakermans for technical assistance; W. C. Ghiorse, B. Eglesham, and R. Garen for assistance in electron and fluorescence microscopy; and D. Fennell for providing culture material.

22 April 1996; accepted 14 April 1997

Membrane and Morphological Changes in Apoptotic Cells Regulated by Caspase-Mediated Activation of PAK2

Thomas Rudel* and Gary M. Bokoch†

Apoptosis of Jurkat T cells induced the caspase-mediated proteolytic cleavage of p21-activated kinase 2 (PAK2). Cleavage occurred between the amino-terminal regulatory domain and the carboxyl-terminal catalytic domain, which generated a constitutively active PAK2 fragment. Stable Jurkat cell lines that expressed a dominant-negative PAK mutant were resistant to the Fas-induced formation of apoptotic bodies, but had an enhanced externalization of phosphatidylserine at the cell surface. Thus, proteolytic activation of PAK2 represents a guanosine triphosphatase–independent mechanism of PAK regulation that allows PAK2 to regulate morphological changes that are seen in apoptotic cells.

Apoptosis, or regulated cell death, is a fundamental process in the development of multicellular organisms. Although it is initiated by many physiologic and pathologic stimuli, all apoptotic cells undergo a similar sequence of morphological and biochemical events (*1*). The cascade of ICE/CED-3 family cysteine proteases (termed caspases) (*2*) is a common and critical component of the cell death pathway (*3*, *4*). The identified targets for proteolytic cleavage by caspases are few, and the role of individual targets in mediating particular apoptotic events remains ill-defined.

p21-activated kinases (PAKs) are serine-threonine kinases whose activity is regulated by the small guanosine triphosphatases (GTPases) Rac and Cdc42 (*5*, *6*). PAKs regulate morphological and cytoskeletal changes in a variety of cell types (*7*, *8*), implicating PAKs as downstream mediators of the effects of Rac and Cdc42 on the actin cytoskeleton. Immunoblot analysis (*9*) reveals that Jurkat T cells predominantly express the 62-kD PAK2 isoform. An apparent decrease in the intensity of the 62-kD PAK2 band is observed after induction of Jurkat cell death by Fas receptor cross-linking (*10*). A 34-kD COOH-terminal PAK2 fragment (Fig. 1A) and a 28-kD NH_2-terminal PAK2 fragment (Fig. 1B) appeared after 1 hour, suggesting that PAK2 is cleaved into two defined fragments during Fas-induced apoptosis (*11*). In detailed time-course studies, PAK2 cleavage was detected as early as 30 min after stimulation by immunoglobulin M (IgM) antibody to Fas (anti-Fas) and the cleavage of PAK2 always correlated with the onset of apoptotic cell death. PAK2 cleavage was also observed when apoptosis was induced in Jurkat cells with C2 ceramide or in MCF-7 cells with tumor necrosis factor–α (TNF-α) (Fig. 1C), suggesting that this is a general phenomenon in apoptotic cells.

Department of Immunology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

*Present address: Max-Planck-Institut für Infektionsbiologie, Abt. Molekulare Biologie, Mobijoustrassse 2, D-10117 Berlin, Germany.

†To whom correspondence should be addressed.