account for the 400-km discontinuity. The seismic anomaly at 400 km is due mostly to the relatively fast transition from olivine to β-spinel (*19*) because transformations from pyroxene to garnet are comparatively slow and occur over much larger intervals at depth. The xenolith data do not at present permit evaluations of the degree of heterogeneity (centimeter to kilometer scale) in the mantle at these depths, but much heterogeneity is predicted if oceanic slabs pile up in the transition zone (*20, 21*). Kimberlites are thought to be associated with mantle plumes (*21*), perhaps originating in the lower mantle; therefore, xenoliths from, or with chemistries reflecting an origin deeper than, the transition zone should be anticipated.

REFERENCES AND NOTES

1. A. E. Ringwood, *Composition and Petrology of the Earth's Mantle* (McGraw-Hill, New York, 1975).
2. J. D. Bass and D. L. Anderson, *Geophys. Res. Lett.* **11**, 237 (1984).
3. D. L. Anderson and J. D. Bass, *Nature* **320**, 321 (1986).
4. F. I. Birch, *J. Geophys. Res.* **57**, 227 (1952).
5. D. L. Anderson, *Spec. Pap. Mineral. Soc. Am.* **3**, 85 (1970).
6. D. J. Weidner, in *Chemistry and Physics of Terrestrial Planets,* S. K. Saxena, Ed. (Springer-Verlag, New York, 1986), pp. 251–274.
7. T. S. Duffy and D. L. Anderson, *J. Geophys. Res.* **94**, 1895 (1989).
8. S. E. Haggerty and V. Sautter, *Science* **248**, 993 (1990).
9. H. Tsai, H. O. A. Meyer, J. Moreau, H. J. Milledge, in *Proceedings of the Second Kimberlite Conference,* F. R. Boyd and H. O. A. Meyer, Eds. (American Geophysical Union, Washington, DC, 1979), vol. 1, pp. 16–26.
10. R. O. Moore and J. J. Gurney, *Nature* **318**, 553 (1985).
11. M. C. Wilding, B. Harte, J. W. Harris, paper presented at the 28th International Geologic Congress, 9 to 19 July 1989, Washington, DC, vol. 3, pp. 359–360 (abstract).
12. M. Akaogi and S. Akimoto, *Phys. Earth Planet. Inter.* **15**, 90 (1977).
13. For mafic compositions, the Si content in garnet increases concomitantly with Na according to the coupled substitution: (x) Na^{VIII} SI^{VI} = R^{2+} Al. By contrast, for ultramafic compositions the number of Si atoms and M^{2+} (Ca, Mg, Mn, Fe^{2+}) increase in a complementary manner according to a second coupled substitution: (y) $(R^{2+}, Si)^{VI}$ = $(Al, Al)^{VI}$. For compositions having both mafic and ultramafic affinities, coupling of these two substitutional schemes, (x) and (y), gives the following structural formulae: (x) + (y); (Na_xR^{2+}) $(R^{2+}Si_{x+y}R^{3+}{}_{2-2y-x})Si_3O_{12}$. Reconstructed garnets from group 1 having both affinities obey this complex structural formula. The compositional range of the Ca-Na majorite inclusions in diamonds from the Monastary mine (*10*) also corresponds to this double, coupled substitutional scheme.
14. This orientation would match the tetrahedral chains of Si in pyroxenes with the octahedral sites of garnet that contain both Si and Al under very high pressures (>80 kbar).
15. In a displacive transformation, the product phase results generally from shearing of the preexisting mineral without bond breaking as observed in reconstructive transformation. Displacive transformations are fast under a suitable driving stress and are almost independent of temperature (diffusionless).
16. T. Gasparik, *Contrib. Mineral. Petrol.* **103**, 389 (1989).
17. M. Akaogi and S. Akimoto, *Phys. Earth Planet. Inter.* **19**, 31 (1979).
18. T. Irifune, T. Sekine, A. E. Ringwood, W. O. Hibberson, *Earth Planet. Sci. Lett.* **77**, 245 (1987).
19. F. Guyot *et al.*, *Geophys. Res. Lett.* **18**, 89 (1991).
20. A. E. Ringwood, *J. Geol.* **90**, 611 (1982).
21. D. L. Anderson, *Theory of the Earth* (Blackwell Scientific, Oxford, 1989).

22. We thank the DeBeers Mining Company for access to Jagersfontein and logistical support. We acknowledge the comments by I. D. MacGregor and the anonymous reviewers. Supported under NSF grant EAR89-054046 (to S.E.H.) and the Centre National de la Recherche Scientifique for a grant from the INSU-DBT program, Theme 4: Fluids, minerals, and kinetics (to V.S.), contribution 252.

2 January 1991; accepted 5 March 1991

In Situ Biodegradation: Microbiological Patterns in a Contaminated Aquifer

EUGENE L. MADSEN, JAMES L. SINCLAIR,* WILLIAM C. GHIORSE

Conventional approaches for proving in situ biodegradation of organic pollutants in aquifers have severe limitations. In the approach described here, patterns in a comprehensive set of microbiological activity and distribution data were analyzed. Measurements were performed on sediment samples gathered at consistent depths in aquifer boreholes spanning a gradient of contaminant concentrations at a buried coal tar site. Microbial adaptation to polyaromatic hydrocarbons (PAHs) was demonstrated by mineralization of naphthalene and phenanthrene in samples from PAH-contaminated, but not adjacent pristine, zones. Furthermore, contaminant-stimulated in situ bacterial growth was indicated because enhanced numbers of protozoa and their bacterial prey were found exclusively in contaminated subsurface samples. The data suggest that many convergent lines of logically linked indirect evidence can effectively document in situ biodegradation of aquifer contaminants.

THE RELEASE OF ORGANIC CHEMICALS to waters and soils can have dire consequences for wildlife, ecosystem integrity, and water quality (*1*). Alleviation of environmental pollution by stimulating native microbiological populations to effect biodegradation processes is promising (*2*), but such "bioremediation technologies" are far from proven. Although indigenous microorganisms in samples from many natural settings have been shown to have the potential to effect pollutant elimination (*3, 4*), the extent to which biodegradation potential is expressed in situ usually is a matter for speculation. Proof of in situ biodegradation must show that the mass of pollutant compounds has decreased and that microorganisms are the causative agents. These two pieces of information are exceedingly difficult to obtain in a field setting because mass balances may be precluded by the open complexity of the site and because other abiotic attenuating processes (dilution, migration, volatilization, sorption) may occur simultaneously with biodegradation (*5*). In situ biodegradation has been documented successfully in field studies of ponds and soil plots (*6*), in which specific responses of microorganisms were distinguished from abiotic responses. In contrast, such studies have not been possible in ground-water aquifers because their inaccessibility and variability prevent implementation of replicated, statistically valid experimental designs. Thus, studies that directly and unequivocally demonstrate in situ biodegradation in aquifers are rare (*7*).

The majority of attempts to document in situ biodegradation in aquifers have used indirect observations. Typically an imperfect mass balance, based on computer modeling, is cited to show loss of a pollutant in water pumped from the aquifer (*8*); but this approach does not distinguish unambiguously between biotic and abiotic processes. Chemical data from ground-water or sediment samples also may suggest that putative biodegradation activity is accompanied by changes in reactants (for example, oxygen and nutrients) and products (for example, CO_2 or intermediary metabolites) which are indicative of known microbiological processes (*2, 9*). The case is strengthened if high numbers of microorganisms, especially of pollutant-degrading bacteria are found (*2, 10*). Further support may be garnered from laboratory biotransformation assays indicating that the pollutant is chemically modified or converted completely to CO_2 in samples from the site (*2, 4, 11*). However, no established combination of these measures is robust enough to constitute absolute proof of in situ biodegradation in aquifer sediments. Methodological improvements are needed. We used patterns of microbiological activity

Section of Microbiology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853.

*Present address: NSI Technology Services, R. S. Kerr Laboratory, P.O. Box 1198, Ada, OK 74820.

and distribution to indicate in situ biodegradation in a shallow aquifer contaminated by buried coal tar.

We measured the potential of subsurface microorganisms to degrade organic compounds in aquifer sediment core samples obtained from a buried coal tar site (*12*) (Fig. 1) by aseptic subcoring procedures (*13*). Evolution of $^{14}CO_2$ from the ^{14}C-labeled PAHs (naphthalene and phenanthrene), and *p*-hydroxybenzoate (PHB) (*14*) was measured (*15*). The abundance and distribution of bacteria (including actinomycetes), fungi, and protozoa were assessed by established methods (*16, 17*). Core samples were obtained from the unsaturated, water table, shallow saturated, and deep saturated subsurface zones in each borehole (*13*) (Fig. 1). On the basis of previous site characterization data (*12*), borehole locations were selected for collecting sediments that spanned a range of contaminant concentrations both horizontally and vertically. Naphthalene and phenanthrene were detected in samples taken from boreholes drilled inside the plume (Fig. 1 and Table 1), but not in samples from a pristine borehole outside the plume.

Mineralization of PHB was evident in sediments from all three boreholes, but the samples from the upgradient borehole nearest the coal tar were most active (Fig. 2, A to C). In the upgradient borehole samples, there was no detectable lag period (*18*) before the onset of biodegradation activity (Fig. 2A). Downgradient in the plume PHB mineralization also was detected in all samples, but microbial metabolism was most rapid and extensive in the sample from the water table zone (Fig. 2B). A slight lag period was noted in the deepest sample from the saturated zone. Three of the four samples from the pristine borehole showed appreciable mineralization of PHB, albeit with lag periods prior to $^{14}CO_2$ evolution (Fig. 2C). The water table sample was most active. No mineralization was observed in the deepest saturated zone sample from the pristine borehole.

Mineralization of naphthalene and phenanthrene was detected only in sediments taken from inside the contaminant plume (Fig. 2, D to G). All sediment samples from the pristine borehole failed to mineralize these PAHs during the 3-week incubation period. In samples from upgradient in the plume, naphthalene and phenanthrene were mineralized in all cases. The water table and deep saturated zones were most active (Fig. 2, D and F). Naphthalene was mineralized in three of the four samples taken from the downgradient borehole; again the water table zone sample was most active (Fig. 2E). Phenanthrene was mineralized only in the water table sample from the downgradient borehole (Fig. 2G).

There was often an inverse relation between the PAH concentration in sediments and PAH biodegradation activity for a given sample. For instance, neither of the PAHs were detected in the water table zone of the downgradient borehole (Table 1), yet PAH mineralization activity was high in these samples (Fig. 2, E and G). Furthermore, elevated levels of PAHs were detected in all shallow saturated zone samples (Table 1) where PAH mineralization activities were low (Fig. 2, D to G). These findings might be explained by small-scale sample heterogeneity or other sampling problems. However, it is also possible that the absence of detectable PAHs reflected zones of rapid in situ biodegradation (*4*) and that the presence of measurable PAHs reflected zones where rates of contaminant influx exceeded rates of microbiological mineralization.

Sediment samples were examined for the numbers and types of microorganisms pres-

Table 1. Concentrations of PAHs. No naphthalene or phenanthrene detected in the pristine borehole; BD, below detection.

Zone	Plume			
	Upgradient		Downgradient	
	Naphthalene (mg kg^{-1})	Phenanthrene (mg kg^{-1})	Naphthalene (mg kg^{-1})	Phenanthrene (mg kg^{-1})
Unsaturated	0.06	1.6	BD	BD
Water table	BD	0.86	BD	BD
Shallow saturated	2.3	0.33	0.24	0.35
Deep saturated	0.06	BD	0.05	BD

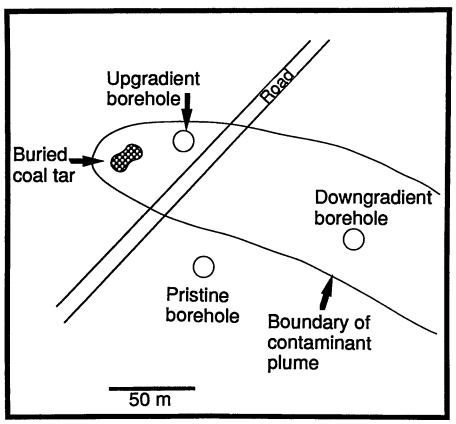

Fig. 1. Plan view of field site showing boundary of contaminant plume and borehole locations. Coal tar was buried 30 years ago (*12*). Groundwater flow has distributed coal tar components, including naphthalene and phenanthrene, through sedimentary strata at depths between 1 and 10 m.

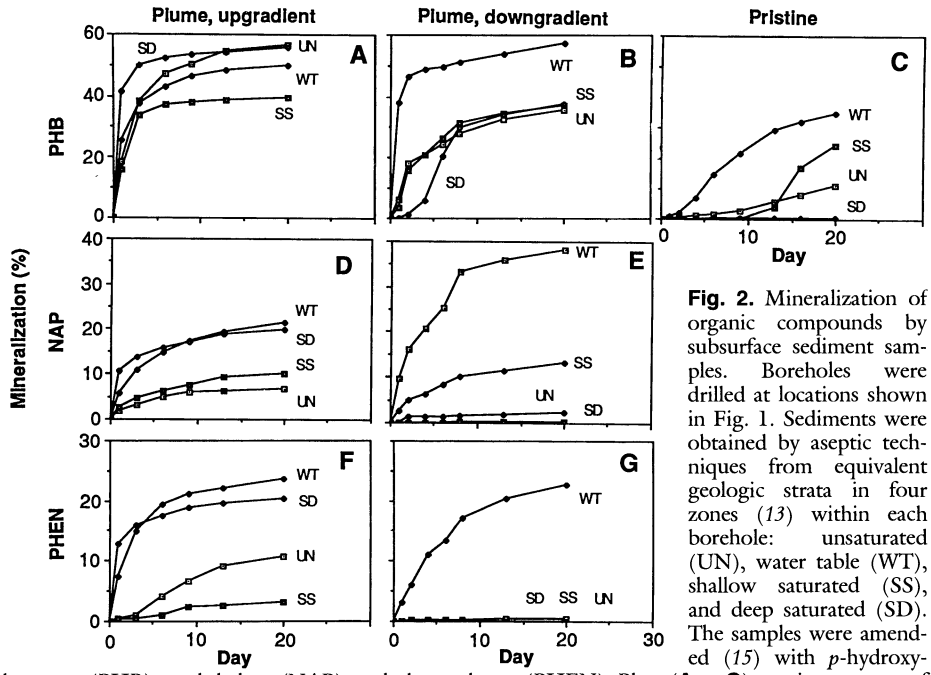

Fig. 2. Mineralization of organic compounds by subsurface sediment samples. Boreholes were drilled at locations shown in Fig. 1. Sediments were obtained by aseptic techniques from equivalent geologic strata in four zones (*13*) within each borehole: unsaturated (UN), water table (WT), shallow saturated (SS), and deep saturated (SD). The samples were amended (*15*) with *p*-hydroxybenzoate (PHB), naphthalene (NAP), and phenanthrene (PHEN). Plots (**A** to **G**) are time courses of cumulative $^{14}CO_2$ trapped in triplicate flasks. No $^{14}CO_2$ was evolved in control flasks containing autoclaved, $HgCl_2$-poisoned sediments.

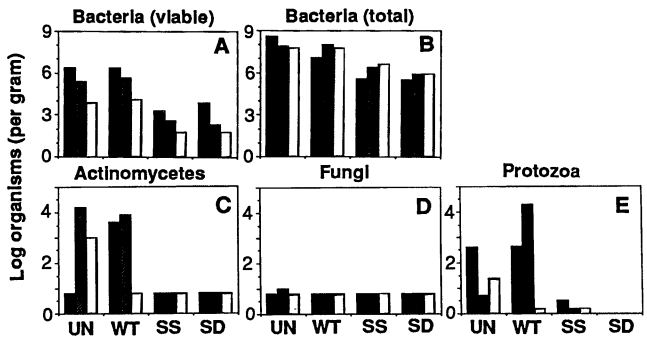

Fig. 3. Comparison of microbiological abundances in sediments at four depths within three boreholes. The four depths examined were the unsaturated (UN), water table (WT), shallow saturated (SS), and deep saturated (SD) zones. Each cluster of three bars represents numbers of microorganisms from samples inside the plume, upgradient (left); inside the plume, downgradient (center); and in the pristine area (right). (**A**) Viable bacteria, (**C**) actinomycetes, and (**D**) fungi were determined by the plate-count method (*16*). (**B**) Total bacteria were determined by epifluorescence microscopy (*16*). (**E**) Protozoa were enumerated using *Enterobacter aerogenes* as prey bacteria (*17*).

ent (Fig. 3). Viable counts of aerobic heterotrophic bacteria showed a consistent pattern; they were highest upgradient, inside the plume, closest to the source of contamination and lowest in the pristine borehole (Fig. 3A). Microscopic counts for total numbers of bacteria were 100- to 1000-fold higher than the viable counts (*19*) and showed the same declining trend with depth, but only small differences were observed between pristine and contaminated samples (Fig. 3B). Actinomycetes were found in significant numbers ($\geq 10^3$ per gram of sediment; Fig. 3C) in two-thirds of the unsaturated and water table zone samples. Low numbers of actinomycetes were detected in saturated zone samples. Low numbers of fungi also were present in subsurface sediments; only small differences were found between samples regardless of depth or borehole location (Fig. 3D). Similar low numbers of fungi and actinomycetes have been found in other shallow and deep subsurface sites (*16, 17*). In contrast to fungi, protozoa (amoebae and flagellates) showed a striking range in abundance (Fig. 3E). High protozoan numbers were found in several samples from unsaturated and water table zones within the contaminant plume. The upgradient plume borehole contained more than 400 protozoa per gram in both the unsaturated and water table zone samples. This is a relatively high population density for subsurface protozoa (*17*). In the downgradient borehole, the water table zone sample contained more than 19,000 protozoa per gram, a number far above those normally encountered in shallow aquifer sediments (*17*), but comparable to those found in activated sewage sludge facilities (*20*). The water table zone sample that supported a high density of protozoa was highly active in mineralizing the three compounds examined (Fig. 2, B, E, and G). Another recent study has also reported the occurrence of large numbers of protozoa in subsurface sediments where jet fuel vapors commingled with atmospheric oxygen (*21*). Typically low numbers of protozoa (<50 per gram) were present in all samples from the pristine borehole and in saturated sediments from just below the water table within the plume of contamination. Sediments from deeper in the saturated zone were not examined for protozoa.

The mineralization activity and microbial distribution patterns observed in this study are likely to be controlled by spatial heterogeneity of sediment properties such as texture and hydraulic conductivity (*22*), as well as by the presence or absence of carbon and energy sources provided by coal tar components in the ground water. Even though sedimentary characteristics of this study site were relatively uniform (*12*), it is impossible to be certain that the sediment samples obtained from four depths of each borehole were derived from hydrogeologically equivalent strata. In an attempt to separate the influence of aquifer sediment heterogeneity from that of PAH contamination, we obtained vertically and horizontally separated samples from zones of high and low PAH concentration and sought patterns in the microbiological data. The significant patterns were as follows: (i) PHB mineralizing microorganisms were present throughout the site; however PAH mineralization activity was restricted to samples from within the plume of contamination; (ii) samples from all depths in the upgradient borehole mineralized both PAHs, whereas several samples from the downgradient borehole were inactive; (iii) lag periods prior to mineralization were observed only in downgradient and pristine samples; (iv) viable bacteria were detected in greatest abundance in samples from the upgradient plume borehole, whereas the lowest abundance was found in samples from the pristine borehole; and (v) elevated numbers of protozoa were found in unsaturated and water table zone sediment samples from within the contaminant plume which contained active populations of PAH-degrading microorganisms. The obvious conclusion from these results is that microbial distribution and adaptational biodegradation activity (*23*) in this polluted aquifer system were governed by proximity of the source of PAH contamination.

Protozoa are important predators in aquatic and terrestrial environments (*20*), but only recently has it been established that protozoa also are widely distributed in subsurface sediments (*17*). The concept of using protozoan abundance as an index of pollution dates from the early part of this century (*24*). In fact, associations between protozoan abundance and high levels of organic carbon in soil or municipal waste water are well established (*25*). However, studies examining interactions between organic contaminants and protozoa are rare (*21*). Data derived from coastal field samples and laboratory-incubated soil cores indicated that crude oil was inhibitory to protozoa (*26, 27*). In contrast, another laboratory study found that a ciliate protozoan enhanced microbial degradation of crude oil (*28*). Until now field evidence for the biogeochemical function of subsurface protozoa, which usually are found at low population density, has not been obtained. The population density of protozoa usually reflects the rate at which they are able to graze on their bacterial prey (*20*). A high protozoan grazing rate is indicated by a high population density. This in turn reflects a high bacterial growth rate rather than increased bacterial biomass. The dependence on bacterial growth rate has been shown in sewage treatment plants where high numbers of protozoa are able to reduce viable counts of bacteria while simultaneously accelerating carbon cycling and increasing their own biomass (*20*). In this study, elevated numbers of protozoa occurred exclusively in sediment samples from upper zones of the subsurface profile where contaminants and oxygen would be expected to mix. The high protozoan numbers are indicative of rapidly growing populations of bacteria in situ. To the extent that prey are growing on contaminant compounds, the elevated protozoan biomass reflects in situ biodegradation activity. Thus, we have compelling indirect evidence for in situ biodegradation of organic contaminants in aquifer sediments: (i) protozoan biomass indicates in situ growth of prey bacteria and (ii) adaptational biodegradation activity indicates that the prey bacteria are growing in response to contaminant compounds.

REFERENCES AND NOTES

1. F. Moriarty, *Ecotoxicology: The Study of Pollutants in Ecosystems* (Academic Press, London, ed. 2, 1988); J. F. Piatt *et al.*, *Auk* **107**, 387 (1990).
2. M. D. Lee *et al.*, *CRC Crit. Rev. Environ. Cont.* **18**, 29 (1988).
3. J. G. Leahy and R. R. Colwell, *Microbiol. Rev.* **54**, 305 (1990).
4. J. T. Wilson *et al.*, *Environ. Toxicol. Chem.* **4**, 721

(1985); J. M. Thomas *et al.*, *J. Ind. Microbiol.* **4**, 109 (1989).
5. J. J. Cooney, in *Petroleum Microbiology*, R. M. Atlas, Ed. (Macmillan, New York, 1984), pp. 399–433; G. Floodgate, in *ibid.*, pp. 355–397; D. M. Karl, in *Bacteria in Nature*, J. S. Poindexter and E. R. Leadbetter, Eds. (Plenum, New York, 1986), pp. 85–176.
6. J. C. Spain *et al.*, *Appl. Environ. Microbiol.* **48**, 944 (1984); R. L. Raymond, J. O. Hudson, V. W. Jamison, *Appl. Environ. Microbiol.* **31**, 522 (1976).
7. The field study by L. Semprini *et al.* [*Ground Water* **28**, 715 (1990)] is exceptional. Its success can be attributed to field instrumentation, a shallow confined aquifer, and the simultaneous metabolism of oxygen, methane, and chlorinated ethenes.
8. R. C. Borden and P. B. Bedient, *Water Res. Bull.* **23**, 629 (1987); C. Y. Chiang *et al.*, *Ground Water* **27**, 823 (1989).
9. P. V. Cline and D. R. Viste, *Waste Manage. Res.* **3**, 351 (1985); F. H. Chapelle and D. R. Lovley, *Appl. Environ. Microbiol.* **56**, 1865 (1990).
10. T. W. Federle and G. M. Pastwa, *Ground Water* **26**, 761 (1988); H. F. Ridgeway *et al.*, *Appl. Environ. Microbiol.* **56**, 3565 (1990). Microbial counts do not necessarily reflect in situ metabolic activity.
11. G. M. Klecka *et al.*, *Ground Water* **28**, 534 (1990); W. C. Ghiorse and J. T. Wilson. *Adv. Appl. Microbiol.* **33**, 107 (1988). Laboratory data describing biodegradation of various organic chemicals [M. Alexander, *Science* **211**, 132 (1981); W. C. Evans, *Nature* **270**, 17 (1977); J. F. Quensen, J. M.Tiedje, S. A. Boyd, *Science* **242**, 752 (1988); S. Dagley, *Surv. Prog. Chem.* **8**, 121 (1977)] show that microorganisms in soil and water have the potential to catalyze chemical reactions. However, microbial activity in disturbed environmental samples contained by laboratory vessels cannot be equated to in situ activity (*5*).
12. Coal tar is a black material produced from coal gasification processes [J. F. Villaume, in *Hazardous and Toxic Wastes: Technology Management and Health Effects*, S. K. Majumdar and E. W. Miller, Eds. (Pennsylvania Academy of Science, Easton, PA, 1984), pp. 362–375]. A single truckload of coal tar was buried in a forested area in the northeastern United States. This site has been characterized under EPRI research projects 2879-1 and 2879-12 [B. B. Taylor *et al.*, in *Proceedings: Environmental Research Conference on Groundwater Quality and Waste Disposal* (Electric Power Research Institute, EN-6749, Palo Alto, CA), pp. 26-1–26-12]. Sediments were 80 to 99% sand. Subsamples of materials used in biodegradation assays were analyzed for PAHs by gas chromatography (GC) and GC/mass spectrometry by Cambridge Analytical Associates, Inc., Boston, MA, under EPRI contract 2879-1.
13. Aseptic coring procedures followed established principles (*2, 4, 16, 17*). Depths selected for analyses were 1 to 2 m (unsaturated zone), 2.3 to 3 m (water table interface), 4 to 5, and 5 to 6 m (shallow and deep saturated zones). Additional details appear in EPRI Final Report RP 2879-5.
14. PHB is an intermediary metabolite in lignin biodegradation [J. P. Martin and K. Haider, in *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*, T. K. Kirk, T. Higuchi, H. Chang, Eds. (CRC Press, Boca Raton, FL, 1980), vol. 1, pp. 77–100] that is metabolized by many aerobic soil bacteria.
15. Conversion of organic compounds to inorganic compounds (mineralization) was measured by standard $^{14}CO_2$ trapping methods. Radiolabeled [1-^{14}C]naphthalene (80 mCi/mmol, >98% radiopurity), [9-^{14}C]phenanthrene (10.4 mCi/mmol, >99% radiopurity), and *p*-hydroxybenzoate (ring UL 7.7 mCi/mmol, >99% radiopurity) were purchased from Sigma Radiochemicals (St. Louis, MO). Compounds were dissolved, filter-sterilized (naphthalene and phenanthrene in acetone before eing diluted 100-fold in distilled water, *p*-hydroxybenzoate in distilled water) and then added to sterile 125-ml flasks containing 4 g of aseptically distributed sediment sample. Each flask received 0.04 μCi of ^{14}C-labeled and unlabeled naphthalene, phenanthrene, or *p*-hydroxybenzoate at concentrations of 1 ppm. An abiotic control flask was prepared in each test by autoclaving the sample for 1 hour and adding 1 ml of 1 M $HgCl_2$ before addition of carbon compounds. The flasks were sealed with lids suspending a small plastic cup (containing 0.6 ml of CO_2 trapping agent) and incubated statically at 23°C. At each sampling time, trapping agent was withdrawn, counted in a scintillation counter, then replenished.
16. D. L. Balkwill and W. C. Ghiorse, *Appl. Environ. Microbiol.* **50**, 580 (1985); J. T. Wilson *et al.*, *Ground Water* **21**, 134 (1983).
17. J. L. Sinclair and W. C. Ghiorse, *Geomicrobiol. J.* **7**, 15 (1989); *Appl. Environ. Microbiol.* **53**, 1157 (1987).
18. T. D. Brock and M. T. Madigan, *Biology of Microorganisms* (Prentice-Hall, Englewood Cliffs, NJ, ed. 5, 1988). Lag periods occur when enzyme synthesis or population growth precede biodegradation; their absence indicates a state of metabolic readiness.
19. Total counts normally exceed viable counts by 2 to 3 orders of magnitude [D. B. Roszak and R. R. Colwell, *Microbiol. Rev.* **51**, 365 (1987); (*16, 17*)].
20. T. Fenchel, *Ecology of Protozoa* (Science Tech, Madison, WI, 1987); W. Foissner, *Prog. Protistol.* **2**, 69 (1987); J. D. Stout, *Adv. Microbiol. Ecol.* **4**, 1 (1980).
21. J. L. Sinclair, in *Proceedings of the First International Symposium on Microbiology of the Deep Subsurface*, C. B. Fliermans and T. C. Hazen, Eds. (WSRC Information Services, Aiken, SC, 1991), pp. 3-35–3-45.
22. S. N. Levine and W. C. Ghiorse, in *ibid*, pp. 5-31–5-45; J. K. Fredrickson *et al.*, *Geomicrobiol. J.* **7**, 53 (1989).
23. Adaptational biodegradation is indicated by an acceleration in microbiological destruction of chemicals after their introduction into a given environment (*3, 4*). Adaptation has been reported in subsurface microbial communities to PAHs (*4*) and in other settings (*3*).
24. R. Kolwitz and M. Marsson, *Int. Rev. Hydrobiol. Hydrogr.* **2**, 126 (1909) [cited in Fenchel (*20*)].
25. H. T. Tribe, *Soil Sci.* **92**, 61 (1961); C. R. Curds, *Annu. Rev. Microbiol.* **36**, 27 (1982).
26. E. Hartwig, *Senckenbergiana Marit.* **16**, 121 (1984) [cited in Foissner (*20*)].
27. J. R. Vestal *et al.*, in *Petroleum Microbiology*, R. M. Atlas, Ed. (Macmillan, New York, 1984), pp. 475–505.
28. A. Rogerson and J. Berger, *J. Gen. Appl. Microbiol.* **29**, 41 (1983).
29. We thank B. B. Taylor and J. A. Ripp for collaborative field assistance and analytical data that were obtained under EPRI contract 2879-1. This investigation was carried out under EPRI project RP 2879-5. Technical assistance from L. Anguish, S. Best, C. Thomas, and A. Winding is gratefully acknowledged.

13 November 1990; accepted 13 March 1991

Control of *doublesex* Alternative Splicing by *transformer* and *transformer-2* in *Drosophila*

KAZUYUKI HOSHIJIMA, KUNIO INOUE, IKUKO HIGUCHI, HIROSHI SAKAMOTO, YOSHIRO SHIMURA*

Sex-specific alternative processing of *doublesex* (*dsx*) precursor messenger RNA (pre-mRNA) regulates somatic sexual differentiation in *Drosophila melanogaster*. Cotransfection analyses in which the *dsx* gene and the female-specific *transformer* (*tra*) and *transformer-2* (*tra-2*) complementary DNAs were expressed in *Drosophila* Kc cells revealed that female-specific splicing of the *dsx* transcript was positively regulated by the products of the *tra* and *tra-2* genes. Furthermore, analyses of mutant constructs of *dsx* showed that a portion of the female-specific exon sequence was required for regulation of *dsx* pre–messenger RNA splicing.

SOMATIC SEXUAL DIFFERENTIATION IN *Drosophila melanogaster* is accomplished by a hierarchy of regulatory genes that act in response to the number of X chromosomes relative to the number of sets of autosomes in a cell (the X:A ratio) (*1*). One of these regulatory genes, *dsx*, is required for terminal sexual differentiation in both male and female flies (*2*). Molecular analyses have shown that the *dsx* transcript undergoes sex-specific RNA processing (splicing and cleavage-polyadenylation reactions), which leads to the production of two distinct sex-specific polypeptides (Fig. 1A) (*3*). The male- and female-specific *dsx* products regulate sexual differentiation by repressing the female- and male-specific terminal differentiation functions, respectively (*2*). Genetic analyses have shown that the *tra* and *tra-2* genes are required for regulation of sex-specific *dsx* expression (*4*). In males, *tra* produces a nonfunctional product, whereas the female-specific *tra* product is functional and is produced by alternative splicing of *tra* pre-mRNA (*5, 6*). The *tra-2* product is also required for proper differentiation of male germ line cells (*7*). The predicted polypeptide encoded by *tra-2* (*8*) contains a domain of 90 amino acids that is also found in RNA binding proteins (*9*). In addition, the predicted protein sequences encoded by *tra-2* (*8*) and *tra* (*10*) contain arginine- and serine-rich regions that are characteristic of proteins that participate in RNA processing (*9*). Although these findings suggest that the products of *tra* and *tra-2* function in the regulation of alternative processing of *dsx* pre-mRNA, direct evidence has been lacking.

To decipher the mechanism of alternative processing of *dsx*, we constructed a plasmid

Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan.

*To whom correspondence should be addressed.