ic buffer, and a crude particulate fraction was prepared. The particulate fraction was centrifuged through a 20% sucrose cushion to remove cytosolic contaminants and then solubilized in 1% CHAPS, 300 mM NaCl, 5 mM EGTA, 5 mM EDTA, and 10 mM Na-Hepes (pH 7.5) buffer supplemented with phenylmethylsulfonyl fluoride (PMSF; 200 μM) and leupeptin, aprotinin, and pepstatin (2 μg/ml each). Immunoprecipitation was done by addition of a 1:200 dilution of centrifuged ascites fluid for either the AU5-specific or the c-MYC–specific (9E10) monoclonal antibodies (BAbCO, Irvine, CA) with GammBind-G Sepharose (5 μl, Pharmacia). Proteins bound to the washed GammBind-G Sepharose were eluted with 1× SDS-sample buffer and analyzed by 10% SDS–polyacrylamide gel electrophoresis followed by fluorography and autoradiography. In immunoprecipitation assays, the AU5-specific antibody did not cross-react with GIRK2, nor did the c-MYC–specific antibody cross-react with GIRK1. Incubation of *wv*GIRK2- or *wv*GIRK2-GIRK1–expressing COS-M7 cells in low-[Na^+] media [*N*-methyl-D-glucamine (NMDG) substituted for NaCl] increased the amount of detectable *wv*GIRK2 and GIRK1 channel subunits in immunoprecipitates. We presume that the lower extracellular [Na^+] increased the number of cells transfected with *wv*GIRK2 or *wv*GIRK2-GIRK1 that survived. NMDG-containing media did not change the expression of wild-type GIRK2 or GIRK1.

11. The Sac II fragment containing the coding sequence of *wv*GIRK2 or GIRK2 was blunt-ended and subcloned into the Eco RV site of the eukaryotic expression plasmid pCDNA1/amp (Invitrogen) under control of the cytomegalovirus promoter. CHO cells were transfected by the calcium phosphate precipitation method (*2, 3*). Each dish was transfected with appropriate combinations of the following: CD4 (a lymphocyte cell surface antigen) in the L3T4 plasmid construct, as a marker for transfection (4 μg) (*4, 5*); pcDNA1/amp (10 μg); pRBG4-GIRK1 (10 μg); GIRK2 (10 μg); or *wv*GIRK2 (10 μg). Transfected cells were identified by anti-CD4 fluorescence and assayed in the tight seal whole-cell patch clamp configuration. The pipette solution contained 140 mM K^+, 142 mM Cl^-, 10 mM Hepes, 5 mM EGTA, 1 mM Mg^{2+}, 1 μM GTP-γ-S, and 0.1 mM ATP (pH 7.3). Bath solution contained 140 mM K^+, 142 mM Cl^-, 10 mM Hepes, 5 mM EGTA, and 1 mM Mg^{2+} (pH 7.3). Whole-cell currents were recorded at 22° ± 2°C with an Axopatch 200A patch clamp amplifier, filtered at 1 kHz (8-pole Bessel filter), and digitized at 5 kHz. Single channels were recorded in symmetrical 140 mM K^+, filtered at 5 kHz (8-pole Bessel filter), and digitized at 25 kHz (pCLAMP6).
12. L. Heginbotham, Z. Lu, T. Abramson, R. MacKinnon, *Biophys. J.* **66**, 1061 (1994); L. Heginbotham, T. Abramson, R. MacKinnon, *Science* **258**, 1152 (1992). P. A. Slesinger *et al.* [*Neuron* **16**, 321 (1996)] have reported a loss of selectivity of truncated *wv*GIRK2 and *wv*GIRK2-containing channels.
13. F. Duprat *et al.*, *Biochem. Biophys. Res. Commun.* **212**, 657 (1995).
14. We thank A. Aleksandrov for helpful discussions and assistance with data analysis, K. Wickman for comments on the manuscript, G. Krapivinsky for purified $G_{\beta\gamma}$, and S. Manahan for assistance with cell culture. Supported by a Colciencias award to B.N., a National Research Service Award award to M.E.K., a Minnesota American Heart Association award to B.V., and an NIH grant to D.E.C. A.S.P. is a Sandoz scholar and is supported by a grant from the National Down Syndrome Society.

23 February 1996; accepted 22 April 1996

A Chemoautotrophically Based Cave Ecosystem

Serban M. Sarbu, Thomas C. Kane, Brian K. Kinkle*

Microbial mats discovered in a ground-water ecosystem in southern Romania contain chemoautotrophic bacteria that fix inorganic carbon, using hydrogen sulfide as an energy source. Analysis of stable carbon and nitrogen isotopes showed that this chemoautotrophic production is the food base for 48 species of cave-adapted terrestrial and aquatic invertebrates, 33 of which are endemic to this ecosystem. This is the only cave ecosystem known to be supported by in situ autotrophic production, and it contains the only terrestrial community known to be chemoautotrophically based.

Nearly all biological communities derive their energy and organic carbon from photosynthesis. In the late 1970s, however, diverse communities were discovered near deep sea hydrothermal vents (*1*). The food base for organisms in this aphotic habitat is supplied by chemoautotrophic organisms that derive their energy from reduced inorganic compounds (*2*). Communities of obligate cave-dwelling organisms often inhabit the aphotic regions of limestone caves (*3*). However, these cave communities rely on organic material transported from the surface as a food base and are thus ultimately dependent on photosynthetic production.

In 1986 access was obtained to a ground-water ecosystem in southern Romania (Fig. 1) having thermal waters of 21°C that are rich in H_2S (*4*). This cave, Movile Cave, contains an abundant and diverse fauna with a terrestrial community composed of 30 obligate cave-dwelling invertebrate species, including 24 endemic species, and an aquatic community of 18 species with 9 endemics (*5*). Air pockets ("airbells") (Fig. 2) in the submerged portion of the cave contain microbial mats composed of fungi and bacteria that float on the water surface and grow on the limestone walls (*6*). Incubation of mat samples with radiolabeled [^{14}C]bicarbonate (HCO_3^-) resulted in the incorporation of radioactive carbon into microbial lipids, indicating chemoautotrophic carbon fixation (*7*). Further, ribulose 1,5-bisphosphate carboxylase–oxygenase, an enzyme characteristic of certain types of autotrophic organisms, is present and active in homogenates made from the mat and in crude cell lysates of bacteria isolated from cave water and cultured in the laboratory (*8*).

In our study we set out to determine whether the chemoautotrophic production observed in the Movile Cave system is also the food base for the invertebrate species inhabiting this ecosystem. We used stable isotope ratio analysis (SIRA) of carbon and nitrogen to determine the relations of selected species to the chemoautotrophic production in the cave. This method is a valuable technique for studying food webs because organisms fractionate isotopes of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) in predictable ways. Consumers are typically enriched by approximately 1 to 2 per mil in ^{13}C and by 3 to 5 per mil in ^{15}N, relative to their food source (*9*). Samples of microbial mat and of 10 of the 48 species of invertebrates were taken from Movile Cave for isotopic analysis. Some of these cave-limited species

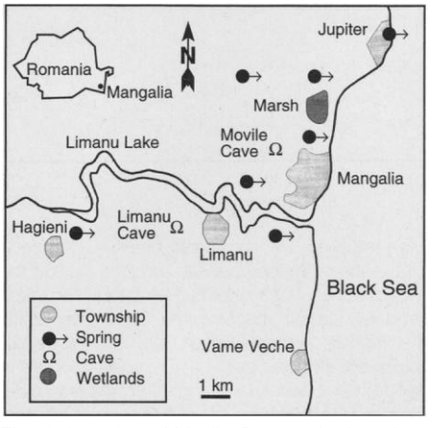

Fig. 1. Location of Movile Cave and other sites from which samples for stable isotope analysis were collected.

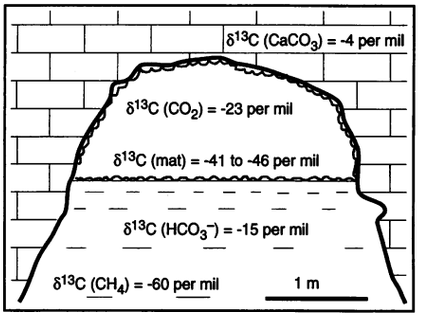

Fig. 2. Cross-sectional view of an airbell in Movile Cave. The atmosphere in the airbells is depleted in O_2 (7 to 10%) and enriched in CO_2 (2.5 to 3.5%) and contains 0.5 to 1.0% CH_4. We determined the atmospheric composition using Draeger tubes.

Department of Biological Sciences, University of Cincinnati, Cincinnati, OH 45221, USA.

*To whom correspondence should be addressed.

were also collected from three hand-excavated wells that intercept the sulfidic aquifer in the area. For purposes of comparison, samples of plant material, and of invertebrates ecologically similar to those sampled from the cave system, were taken in the vicinity from a surface terrestrial community, from a nonsulfidic surface aquatic community, and from the Black Sea. Additional samples of invertebrates were taken from Limanu Cave, a nearby cave system (Fig. 1) that does not communicate with the sulfidic aquifer and receives inputs of organic matter from the surface. The SIRA technique was also applied to samples of CO_2 from the surface and to samples of CO_2, HCO_3^-, CH_4, and $CaCO_3$ (limestone) from the cave. The only form of biologically available nitrogen detected in the waters of Movile Cave was ammonium (NH_4^+) (*7*, *10*, *11*).

All forms of inorganic carbon in the cave are isotopically light (Fig. 2). The CO_2 in the cave atmosphere is a mixture of heavier CO_2 from the dissolution of limestone ($CaCO_3$ $\delta^{13}C = -4$ per mil) by sulfuric acid (*12*) and lighter CO_2 presumably resulting from oxidation of isotopically light CH_4 ($\delta^{13}C = -60$ per mil; Fig. 2) ascending from the deep aquifer. The HCO_3^- in the water ($\delta^{13}C = -15$ per mil; Fig. 2) is at isotopic equilibrium with CO_2 in the cave atmosphere (*13*). If we assume a fractionation of approximately -25 per mil between inorganic carbon and the biomass of chemoautotrophs (*14*), the carbon isotope values for the microbial mat ($\delta^{13}C = -41$ to -46 per mil; Fig. 2) are as expected. Atmospheric CO_2 at the surface is isotopically heavier ($\delta^{13}C = -7.6$ per mil), and the $\delta^{13}C = -28$ per mil observed in aquatic macrophytes from the surface aquatic habitat (point A4, Fig. 3) is consistent with an expected isotopic fractionation of carbon of -20 per mil by C_3 plants (plants in which the first product of photosynthesis is a three-carbon acid) (*15*).

The NH_4^+ in the cave water is isotopically heavy ($\delta^{15}N = 19.9$ per mil). However, nitrogen in the microbial mat is isotopically lighter (mean $\delta^{15}N = -9.11$ per mil). A fractionation of stable nitrogen isotopes of -10 to -20 per mil has been reported for NH_4^+ uptake by microbes when NH_4^+ is not limited (*16*). Further, nitrification (that is, $NH_4^+ \rightarrow NO_3^-$) can deplete ^{15}N on the order of -10 to -30 per mil (*17*). Whether either of these factors is the cause of the observed nitrogen isotope differences between NH_4^+ in the water and nitrogen in the microbial mats requires a better understanding of the nitrogen cycle in Movile Cave.

Organisms sampled from Movile Cave are isotopically lighter in both carbon and nitrogen than organisms sampled from the three surface habitats (Fig. 3). Isotopic ratios observed in surface-dwelling organisms (Fig. 3) reflect dependence on photosynthetic production, whereas values for organisms from Movile Cave suggest dependence on the chemoautotrophic production occurring in the cave (Fig. 3). This includes three pairs of cave-surface congeners (*Cryptops* T4 and C5, *Haemopis* A3 and C2, and *Nepa* A2 and C1; Fig. 3) for which isotopic values reflect the habitats in which the species occur.

If the Movile Cave ecosystem were dependent on allochthonous input of organic material from the surface, we would predict that organisms in the cave would be isotopically heavier for both carbon and nitrogen than comparable organisms from the surface. This is the case for the two organisms sampled in Limanu Cave (Fig. 3, L1 and L2), which are isotopically heavier than all (for nitrogen) or nearly all (for carbon) of the species sampled from the surface habitats. Limanu Cave does not communicate with the sulfidic aquifer and is nonthermal (temperature approximately 14°C). None of the endemic species of Movile Cave has ever been observed or collected in Limanu Cave. Organic matter of surface origin in Limanu Cave includes wood, plant detritus, and vertebrate feces; the isotope data suggest that these materials provide the food base for organisms in this cave.

Two species of amphipods, *Niphargus dobrogicus* and *Pontoniphargus racovitzai*, and one species of aquatic isopod, *Asellus aquaticus*, collected from the three hand-excavated sulfidic wells are also known from Movile Cave. All three samples are enriched in the heavy isotopes of both carbon and nitrogen (Fig. 3) relative to samples from Movile Cave, including *N. dobrogicus* (Fig. 3, G3 and W1), which we collected from both locations. The carbon and nitrogen isotope values from the wells are intermediate between the values observed in Movile Cave and values for samples from surface habitats. We often noted surface invertebrates on the well walls and found debris, such as wood, in the water. The isotopic values for the wells suggest, therefore, that the organisms sampled use a mixed diet of chemoautotrophically produced organic material and organic material of surface origin.

Isotopic differences among Movile Cave samples correspond with the presumptive trophic status of the organisms sampled. The mean $\delta^{15}N$ value for the mat, which includes the primary producers (P group, Fig. 3), is -9.11 per mil ($n = 8$); for grazers (G group) presumably feeding on the mat, the value is

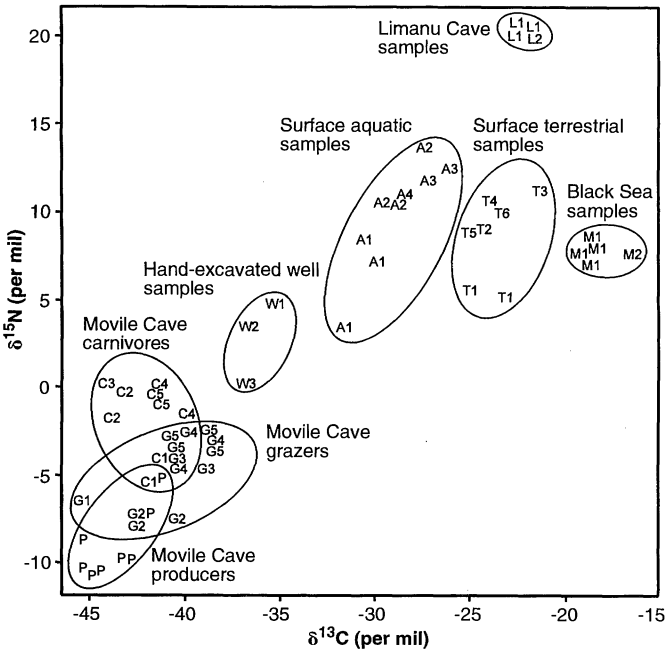

Fig. 3. Stable isotope ratios of carbon and nitrogen for organic samples (*11*). Movile Cave samples include the following: producers P, floating microbial mats; grazers: G1, *Allobophora* sp. (Oligochaeta, Lumbricidae); G2, *Archiboreoiulus* sp. (Diplopoda, Iulidae); G3, *Niphargus dobrogicus* (Amphipoda, Gammaridae); G4, *Trachelipus troglobius* (Isopoda, Trachelipidae); G5, *Armadillidium tabacarui* (Isopoda, Armadillidiidae); carnivores: C1, *Nepa anophthalma* (Hemiptera, Nepidae); C2, *Haemopis caeca* (Hirudinea, Haemopidae); C3, *Medon dobrogicus* (Coleoptera, Staphylinidae); C4, *Agraecina cristiani* (Arachnida, Liocranidae); and C5, *Cryptops anomalans* (Chilopoda, Cryptopidae). Limanu Cave samples include the following: L1, staphylinid beetle (Coleoptera, Staphylinidae), and L2, clubionid spider (Arachnida, Clubionidae). Surface aquatic samples include the following: A1, *Asellus aquaticus aquaticus* (Isopoda, Asellidae); A2, *Nepa cinerea* (Hemiptera, Nepidae); A3, *Haemopis sanguisuga* (Hirudinea, Haemopidae); and A4, aquatic macrophytes. Surface terrestrial samples include the following: T1, *Armadillidium* sp. (Isopoda, Armadillidiidae); T2, *Trachelipus* sp. (Isopoda, Trachelipidae); T3, *Roncus* sp. (Pseudoscorpiones, Neobisiidae); T4, *Cryptops anomalans* (Chilopoda, Cryptopidae); T5, *Scolopendra* sp. (Chilopoda, Scolopendridae); and T6, clubionid spider (Arachnida, Clubionidae). Black Sea marine samples include the following: M1, *Mytilus* sp. (Mollusca, Bivalvia, Mytilidae); and M2, marine amphipods (Crustacea, Amphipoda, Gammaridae). Well samples include the following: W1, *Niphargus dobrogicus* (Amphipoda, Gammaridae); W2, *Pontoniphargus racovitzai* (Amphipoda, Gammaridae); and W3, *Asellus aquaticus* (Isopoda, Asellidae).

−4.6 per mil (n = 13); for presumed predators (C group) $\delta^{15}N$ = −1.58 per mil (n = 9). These differences in $\delta^{15}N$ between successive trophic levels of 4.51 per mil (G group versus the P group) and 3.02 per mil (C group versus the G group) are statistically significant [Tukey's honestly significant difference (hsd) test (*18*), $P < 0.01$] and within the range of 3 to 5 per mil expected.

Results for the C isotope data are less clear. Grazers (mean $\delta^{13}C$ = −40.66 per mil, n = 13) are significantly (Tukey's hsd test, $P < 0.01$) enriched by 3.08 per mil in ^{13}C relative to the mat (mean $\delta^{13}C$ = −43.74 per mil, n = 8), which is a greater degree of enrichment than expected (*10*). However, the mean $\delta^{13}C$ = −42.16 per mil (n = 9) for predators is intermediate between and not significantly different from (Tukey's hsd, $P > 0.05$) the value for the mat and grazers, which is not as expected (*10*). Many cave-dwelling organisms are dietary generalists (*3*), and thus it is possible that these presumptive "predators" are in fact omnivores, perhaps including mat material in their diets. Were this the case, we would also expect the behavior to be reflected in the isotope data for N, which it is not. Cave-dwelling organisms also tend to have attenuated appendages and a general increase in surface-to-volume ratio (*3*). For invertebrates this can increase the proportion of exoskeleton and chitin in the individual. Chitin may be depleted in ^{13}C by ~2 per mil relative to the rest of the organism (*19*). Thus, our carbon values for predators may reflect this "chitin effect."

The stable isotope data show that the Movile Cave ecosystem derives its organic carbon from in situ chemoautotrophic production. As far as we know, all other limestone cave ecosystems that have been studied require allochthonous inputs of organic material of photosynthetic origin from the surface. In addition, the terrestrial community in Movile Cave, which accounts for over 60% of the animal species and over 70% of the endemic species in the system, appears to be a community with a chemoautotrophic energy base. To our knowledge, all other chemoautotrophically based communities have been described from marine (*20*) and freshwater (*21*) habitats. The Movile Cave system is similar to deep sea vents in having a chemoautotrophic food base and a diverse biota. However, the cave system appears to lack the symbioses between chemoautotrophic microbes and animals so characteristic of deep sea vent communities.

REFERENCES AND NOTES

1. R. D. Ballard, *Oceanus* **20**, 34 (1977); V. Tunnicliffe, *Am. Sci.* **80**, 336 (1992).
2. J. B. Corliss *et al.*, *Science* **203**, 1073 (1979); H. W. Jannasch, *Proc. R. Soc. London Ser. B* **225**, 227 (1985); R. C. Fisher, J. J. Childress, S. A. Macko, J. M. Brooks, *Mar. Ecol. Prog. Ser.* **103**, 45 (1994).
3. D. C. Culver, *Cave Life* (Harvard Univ. Press, Cambridge, MA, 1982).
4. C. Lascu, *Misc. Speolog. Romanica* **1**, 13 (1989).
5. V. Decu, M. Gruia, L. S. Keffer, S. M. Sarbu, *Ann. Entomol. Soc. Am.* **87**, 755 (1994); M. Gruia, V. Iavorschi, S. M. Sarbu, *Proc. Biol. Soc. Wash.* **107**, 599 (1994); S. M. Sarbu and T. C. Kane, *NSS Bull.* **57**, 91 (1995).
6. S. M. Sarbu *et al.*, in *Microbial Mats*, L. J. Stahl and P. Chaumette, Eds. (Springer-Verlag, Berlin, 1994), pp. 45–50.
7. S. M. Sarbu and R. Popa, in *The Natural History of Biospeleology*, A. I. Camacho, Ed. (Monografias Museo Nacional de Ciencias Naturales, Madrid, 1992), pp. 638–666.
8. S. M. Sarbu, B. K. Kinkle, L. Vlasceanu, T. C. Kane, R. Popa, *Geomicrobiol. J.* **12**, 175 (1994).
9. C. K. Paull, A. J. T. Jull, L. J. Toolin, T. Linick, *Nature* **317**, 709 (1985); J. R. Ehleringer, P. W. Rundel, K. A. Nagy, *Trends Ecol. Evol.* **1**, 42 (1986); B. J. Peterson and B. Fry, *Annu. Rev. Ecol. Syst.* **18**, 293 (1987).
10. Organic samples were analyzed by SIRA at the Water Research Center, University of Alaska–Fairbanks. Inorganic carbon samples were analyzed at Geochron Laboratories, Krueger Enterprises, Cambridge, MA.
11. Values of δ (per mil) are calculated as $X = [(R_{sample} - R_{standard})/R_{standard}] \times 10^3$, where X is $\delta^{13}C$ or $\delta^{15}N$ and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Standards are Pee Dee belemnite for carbon and air for nitrogen. The precision of the measurements was 0.2 per mil or better.
12. G. Diaconu and M. Morar, *Karstologia* **22**, 15 (1993).
13. R. Goericke, J. P. Montoya, B. Fry, in *Stable Isotopes in Ecology and Environmental Science*, K. Lajtha and R. H. Michener, Eds. (Blackwell, Oxford, 1994), pp. 187–221.
14. E. T. Degens, in *Organic Geochemistry*, E. Eglinton and M. J. T. Murphy, Eds. (Springer-Verlag, Berlin, 1969), pp. 304–329; E. G. Ruby, H. W. Jannasch, W. G. Deuser, *Appl. Environ. Microbiol.* **53**, 1940 (1987).
15. M. H. O'Leary, *Phytochemistry* **20**, 553 (1981); *BioScience* **38**, 328 (1988).
16. S. A. Macko and N. E. Ostrum, in *Stable Isotopes in Ecology and Environmental Science*, K. Lajtha and R. H. Michener, Eds. (Blackwell, Oxford, 1994), pp. 45–62.
17. T. Yoshinari and I. Koike, *ibid.*, pp. 114–137.
18. R. G. D. Steele and J. H. Torrie, *Principles and Procedures of Statistics* (McGraw-Hill, New York, 1960).
19. S. A. Macko, R. Helleur, G. Hartley, P. Jackman, *Organic Geochem.* **16**, 1129 (1990); G. H. Rau *et al.*, *Deep-Sea Res.* **37**, 1669 (1990).
20. N. M. Conway, M. C. Kennicutt II, C. L. Van Dover, in *Stable Isotopes in Ecology and Environmental Science*, K. Lajtha and R. H. Michener, Eds. (Blackwell, Oxford, 1994), pp. 158–186.
21. T. O. Stevens and J. P. McKinley, *Science* **270**, 450 (1995).

22. We thank T. Algeo, D. C. Culver, C. R. Fisher, D. R. Francis, W. K. Jones, B. B. Jorgensen, G. W. Kling, C. Lascu, S. A. Macko, D. P. Mindell, and M. C. Miller for valuable discussions and comments on the manuscript. We also thank S. A. Macko for running SIRA of nitrogen in the cave water NH_4^+. Support and assistance were provided by the "E. Racovita" Speleological Institute and the GESS Group for field work in Romania. The Cave Research Foundation, the Fulbright Program, the Romanian Academy of Sciences (grant 3919-95), the National Geographic Society (grants 4639-91 and 5460-95), the NSF (grant DEB-9420033), and the National Speleological Society provided financial support.

8 March 1996; accepted 1 May 1996

CC CKR5: A RANTES, MIP-1α, MIP-1β Receptor as a Fusion Cofactor for Macrophage-Tropic HIV-1

Ghalib Alkhatib, Christophe Combadiere,* Christopher C. Broder,* Yu Feng,* Paul E. Kennedy,* Philip M. Murphy,† Edward A. Berger†

Human immunodeficiency virus–type 1 (HIV-1) entry requires fusion cofactors on the $CD4^+$ target cell. Fusin, a heterotrimeric GTP-binding protein (G protein)–coupled receptor, serves as a cofactor for T cell line–tropic isolates. The chemokines RANTES, MIP-1α, and MIP-1β, which suppress infection by macrophage-tropic isolates, selectively inhibited cell fusion mediated by the corresponding envelope glycoproteins (Envs). Recombinant CC CKR5, a G protein–coupled receptor for these chemokines, rendered CD4-expressing nonhuman cells fusion-competent preferentially with macrophage-tropic Envs. CC CKR5 messenger RNA was detected selectively in cell types susceptible to macrophage-tropic isolates. CC CKR5 is thus a fusion cofactor for macrophage-tropic HIV-1 strains.

Individual isolates of HIV-1 display markedly distinct tropisms for infection of primary macrophages as compared with $CD4^+$ T cell lines (*1–4*). The viral determinants for this cytotropism reside in the Env (*1*, *2*). Direct assays of fusion mediated by recombinant Envs suggest that the cytotropism of different isolates is largely a consequence of the inherent membrane fusion selectivities of the corresponding Envs for various $CD4^+$ target cell types (*5*).

Insight into the cellular determinants of this fusion selectivity derives from the fact that CD4 must be expressed on a human

G. Alkhatib, C. C. Broder, Y. Feng, P. E. Kennedy, E. A. Berger, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD 20892, USA
C. Combadiere and P. M. Murphy, Laboratory of Host Defenses, NIAID, NIH, Bethesda, MD 20892, USA.

*These authors contributed equally to the research.
†To whom correspondence should be addressed.