a 50% reduction in *Prlr* gene dose, provides strong evidence for PRL being the mediator of pregnancy-stimulated neurogenesis.

Taken together, our findings indicate that production of new olfactory interneurons is a maternal adaptation initiated early in pregnancy and mediated by PRL. Pregnancy is associated with several other transient alterations in maternal physiological functions, many of which can be affected by PRL and/or the placental PRL-like hormones (placental lactogens), including prolongation of luteal function (*27*), proliferation of pancreatic islets (*39*), mammary gland development (*40*), and immunomodulation (*41*). PRL stimulation of maternal pancreatic islet cell proliferation during pregnancy is intriguing, given that this process shares common signaling properties with the forebrain SVZ, for example, dependence on EGF receptor signaling (*39*).

The increase in new olfactory interneurons by pregnancy or PRL is likely to have important functional consequences. The olfactory bulb is critical in offspring recognition and associated maternal behavior (*22*, *23*), and doubling of olfactory interneurons is sufficient to enhance olfactory function in mice (*21*). Defects in pup-induced maternal behavior are observed in both $Prlr^{-/-}$ and $Prlr^{+/-}$ nulliparous female mice (*38*) and in nulliparous female rats receiving forebrain lateral ventricle (SVZ) infusions of a prolactin receptor antagonist (*42*), proving the physiological link between these processes. PRL may also be considered as a potential therapeutic agent, for example, to augment the intrinsic, redirected SVZ neurogenesis recently observed in a rodent model of stroke (*43*).

Although the neurogenic events of pregnancy are fascinating, a most provocative finding is that forebrain neurogenesis is increased even in female mice that mate but do not become pregnant. Plasma levels of PRL increase markedly following orgasm in humans, in both males and females (*44*, *45*). These observations suggest that PRL-stimulated olfactory neurogenesis following mating may also occur in animals with long gestation periods, such as humans, in order to subserve specific behaviors related to courtship or long-term partnership. Behavioral studies in rodents and primates, which show virtually identical patterns of adult olfactory neurogenesis, will serve to address these intriguing possibilities.

References and Notes

1. J. G. Hildebrand, G. M. Shepherd, *Annu. Rev. Neurosci.* 20, 595 (1997).
2. J. Krieger, H. Breer, *Science* **286**, 720 (1999).
3. K. Mori, H. Nagao, Y. Yoshihara, *Science* **286**, 711 (1999).
4. P. Mombaerts, *Nature Neurosci.* **4** (suppl.), 1192 (2001).
5. M. Cayre, C. Strambi, A. Strambi, *Nature* **368**, 57 (1994).
6. J. Melzig *et al.*, *Curr. Biol.* **8**, 1223 (1998).
7. M. Heisenberg, *Learn. Mem.* **5**, 1 (1998).
8. M. Schmidt, *Brain Res.* **762**, 131 (1997).
9. S. Harzsch, J. Miller, J. Benton, B. Beltz, *J. Neurosci.* **19**, 3472 (1999).
10. J. Altman, G. D. Das, *J. Comp. Neurol.* **124**, 319 (1965).
11. J. Altman, *J. Comp. Neurol.* **137**, 433 (1969).
12. D. R. Kornack, P. Rakic, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 4752 (2001).
13. M. Cayre, J. Malaterre, S. Scotto-Lomassese, C. Strambi, A. Strambi, *Comp. Biochem. Physiol. B* **132**, 1 (2002).
14. B. A. Reynolds, S. Weiss, *Science* **255**, 1707 (1992).
15. C. M. Morshead *et al.*, *Neuron* **13**, 1071 (1994).
16. C. Lois, A. Alvarez-Buylla, *Science* **264**, 1145 (1994).
17. F. Doetsch, I. Caille, D. A. Lim, J. M. Garcia-Verdugo, A. Alvarez-Buylla, *Cell* **97**, 703 (1999).
18. T. Shimazaki, T. Shingo, S. Weiss, *J. Neurosci.* **21**, 7642 (2001).
19. T. Shingo, S. T. Sorokan, T. Shimazaki, S. Weiss, *J. Neurosci.* **21**, 9733 (2001).
20. G. Gheusi *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1823 (2000).
21. C. Rochefort, G. Gheusi, J. D. Vincent, P. M. Lledo, *J. Neurosci.* **22**, 2679 (2002).
22. P. A. Brennan, E. B. Keverne, *Prog. Neurobiol.* **51**, 457 (1997).
23. K. M. Kendrick *et al.*, *Brain Res. Bull.* **44**, 383 (1997).
24. Materials and methods are available as supporting material on *Science* Online.
25. C. M. Morshead, D. van der Kooy, *J. Neurosci.* **12**, 249 (1992).
26. B. Nait-Oumesmar *et al.*, *Eur. J. Neurosci.* **11**, 4357 (1999).
27. G. D. Niswender, J. L. Juengel, P. J. Silva, M. K. Rollyson, E. W. McIntush, *Physiol. Rev.* **80**, 1 (2000).
28. K. L. Hull, K. Fathimani, P. Sharma, S. Harvey, *Comp. Biochem. Physiol. C* **119**, 389 (1998).
29. V. Goffin, N. Binart, P. Touraine, P. A. Kelly, *Annu. Rev. Physiol.* **64**, 47 (2002).
30. M. E. Freeman, B. Kanyicska, A. Lerant, G. Nagy, *Physiol. Rev.* **80**, 1523 (2000).
31. R. Roky *et al.*, *Neuroendocrinology* **63**, 422 (1996).
32. X. J. Pi, D. R. Grattan, *J. Comp. Neurol.* **394**, 462 (1998).
33. M. S. Barkley, G. E. Bradford, I. I. Gerschwind, *Biol. Reprod.* **19**, 291 (1978).
34. T. Shingo, H. Fujikawa, S. Weiss, data not shown.
35. C. Diaz-Ruiz, R. Perez-Tomas, J. Domingo, I. Ferrer, *Neurosci. Lett.* **164**, 44 (1993).
36. A. Ouhtit, G. Morel, P.A. Kelly, *Endocrinology* **133**, 135 (1993).
37. C. J. Ormandy *et al.*, *Genes Dev.* **11**, 167 (1997).
38. B. K. Lucas, C. J. Ormandy, N. Binart, R. S. Bridges, P. A. Kelly, *Endocrinology* **139**, 4102 (1998).
39. J. H. Nielsen, C. Svensson, E. D. Galsgaard, A. Moldrup, N. Billestrup, *J. Mol. Med.* **77**, 62 (1999).
40. M. C. Neville, J. Morton, S. Umemura, *Pediatr. Clin. N. Am.* **48**, 35 (2001).
41. K. Dorshkind, N. D. Horseman, *Bioessays* **23**, 288 (2001).
42. R. Bridges, B. Rigero, E. Byrnes, L. Yang, A. Walker, *Endocrinology* **142**, 730 (2001).
43. J. Arvidsson, T. Collin, D. Kirik, Z. Kokaia, O. Linvall, *Nature Med.* **8**, 963 (2002).
44. M. S. Exton *et al.*, *Psychoneuroendocrinology* **26**, 287 (2001).
45. T. H. Kruger, P. Haake, U. Hartmann, M. Schedlowski, M. S. Exton, *Neurosci. Biobehav. Rev.* **26**, 31 (2002).

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Supporting Online Material

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Fluids from Aging Ocean Crust That Support Microbial Life

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Little is known about the potential for life in the vast, low-temperature (<100°C) reservoir of fluids within mid-ocean ridge flank and ocean basin crust. Recently, an overpressured 300-meter-deep borehole was fitted with an experimental seal (CORK) delivering crustal fluids to the sea floor for discrete and large-volume sampling and characterization. Results demonstrate that the 65°C fluids from 3.5-million-year-old ocean crust support microbial growth. Ribosomal RNA gene sequence data indicate the presence of diverse Bacteria and Archaea, including gene clones of varying degrees of relatedness to known nitrate reducers (with ammonia production), thermophilic sulfate reducers, and thermophilic fermentative heterotrophs, all consistent with fluid chemistry.

The possibility of a biosphere extending throughout the immense volume of aging crust under the global system of mid-ocean ridge (MOR) flanks and ocean basins is controversial. Because most MOR flank and ocean basin crust is buried under thick, impermeable layers of sediment, the fluids circulating within the underlying ocean crust are usually inaccessible for direct studies. A CORK (Circulation Obviation Retrofit Kit) observatory (Fig. 1) (*1*) affixed to the overpressured Ocean Drilling Program (ODP) Hole 1026B (*2*) on the flanks of the Juan de Fuca Ridge (JFR), in the northeast Pacific Ocean, offered an unprecedented opportunity to study biogeochemical properties and mi-

crobial diversity in circulating fluids from 3.5-million-year-old (My) ocean crust. Here, we show that fluids escaping from ODP hole 1026B are derived from the oceanic crust and that the chemical characteristics and temperature (65°C) of these fluids are conducive to microbial growth.

Borehole 1026B (ODP leg 186) was drilled through 247 m of sediments and penetrates 48 m into the underlying basaltic ocean crust. It is sheathed by an impervious steel liner, which prevents direct contribution from sediment pore water; fluid exiting the borehole is derived directly from the basaltic crust. A BioColumn (Fig. 1) was attached to the CORK observatory to collect samples for water chemistry, biomass, ribosomal RNA (rRNA) gene sequencing, and lipid analysis. The borehole was well flushed (>1 year) before the first BioColumn deployment. Average flow rate recorded from fall 1997 to summer 1999 was ~4.8 liters min^{-1}. Estimated basement crustal (formation) fluid temperature, derived from instrument string measurements of borehole temperature gradients, is ~64°C (*2*, *3*). Rapid flow rates minimized cooling during fluid transit from CORK valve to internal BioColumn thermisters (54° to 58°C). The 1026B borehole positive pressure (*4*) results in a borehole flushing rate of only 0.66 days. The tidal signal in borehole pressure and fluid discharge rate (*2*, *4*, *5*), and the relative young age (~4.3×10^3 years) (*6*) of 1026B basement fluids, are consistent with off-axis recharge and enhanced connectivity between crustal fluids and the ocean. The resulting short residence time in the borehole should minimize potential chemical and biological alteration of formation fluids during their flow through the borehole (*7*).

Relative to nearby near-bottom seawater, 1026B fluids are depleted in SO_4^{2-} (0.64×), K^+ (0.65×), and especially Mg^{2+} (0.07×), but greatly enriched in NH_4^+ (142×), Si (5.9×), Ca^{2+} (5.4×), and somewhat enriched in Sr (1.2×) (Table 1). The strong depletion in Mg^{2+}, enrichment in Si, Ca^{2+}, and Sr^{2+}, and basaltic Sr isotopic signature (*7*) indicate that the fluids have reacted extensively with host crustal rock (*8*). The depleted SO_4^{2-} and elevated NH_4^+ suggest microbial sulfate reduction and possibly NO_3^- and NO_2^- reduction (*7*). Concentrations of Cl^- and Na^+ differ from those in seawater by <2%. Although the steel liner eliminates direct inward penetration of the borehole casing by sediment pore waters, sedimentary processes were suspected to influence regional basement crustal fluids (*6*, *8*). Diffusion of SO_4^{2-} from basement to overlying sediments (*6*), for example, could partly explain the depletion of SO_4^{2-} in 1026B fluids without commensurate H_2S or particulate sulfur. Conversely, 1026B fluid ammonia concentration exceeds that potentially derivable through reduction of seawater NO_3^- alone, requiring additional sources of ammonia for crustal fluids such as degradation of organic matter imported from adjacent sediments (*8*) or with recharge seawater.

Mean microbial cell abundance (*9*) in 1026B fluid samples is 8.5×10^4 ($\pm 4.1 \times 10^4$) cells ml^{-1}, likely an underestimate due to the extensive exopolymers (*10*) that obscured cells and contributed to background fluorescence. Nearby ambient bottom seawater samples have ~8.7×10^4 ($\pm 1.9 \times 10^4$) cells ml^{-1}, indicating that cell concentration in basaltic crust-derived water is subequal to that of bottom seawater. Cells in 1026B fluids exhibit diverse morphologies ranging from coccoidal to short rods, crescents, and very long rods or chains (5 to 20 μm in length). Transmission electron microscopy reveals distinct prokaryotic cells including large extended rods (>5 μm). Such large free-living prokaryotic cells are rare in deep-sea "steady state" hydrothermal plumes or in background deep water, although similar cell size and morphological heterogeneity have been observed in MOR vent fluids (*11*) and in plumes associated with recent volcanic activity (*12*).

Small-subunit rRNA genes cloned from microorganisms collected on BioColumn filters (*13*) revealed a diverse collection of Archaea and Bacteria (Fig. 2). The most abundant class of clones is most closely related to *Ammonifex degensii* (91% similarity), a chemolithoautotrophic organism that produces ammonia as a product of energy-yielding metabolism according to the reaction $4H_2 + NO_3^- + 2H^+ \rightarrow NH_4^+ + 3H_2O$ (*14*). Although the relationship between this group of clones and *A. degensii* is not particularly close, they do share a common evolutionary history to the exclusion of any other known taxa (Fig. 2). We hypothesize that the presence of microorganisms capable of reducing nitrate to ammonia could partially explain the abundance of ammonia in exiting borehole waters; microorganisms represented by this group of clones appear to be the most likely candidates for carrying out such a process in this environment. Nitrogen fixation is another potential source of ammonia in crustal fluids. The growing list of authenticated Archaea and Bacteria nitrogen-fixers includes thermophiles and species of the low G+C Gram-positive genus *Desulfotomaculum* (*15*), with the latter closely related to clones isolated from 1026B.

Bacterial clones related to sulfate reducers of the δ-Proteobacteria and *Desulfotomaculum*, as well as archaeal clones related to the thermophilic sulfate-reducing genus *Archaeoglobus* (Fig. 2), are also common and could help explain the decreased sulfate concentration of exiting waters. Other bacterial clones are affiliated with the Thermotogae, which are predominantly thermophilic, fermentative heterotrophs. A crenarchaeal clade related to uncultured organisms was also found; its closest relatives are genes recovered from microorganisms found in a Yellowstone hot spring (*16*).

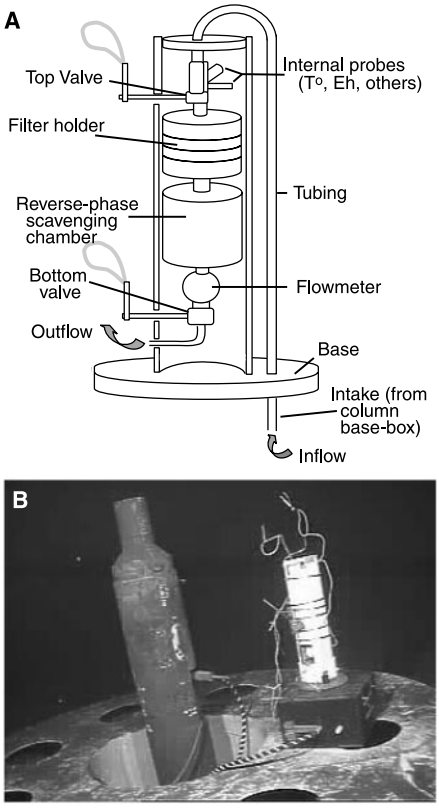

Fig. 1. The BioColumn device channels crustal fluids discharged at the CORK spigot past temperature and flow sensors, particle filters (>0.4 μm), and an organic compound scavenging column. BioColumn deployment durations varied from days to 1 year, with flow rates of ~4.5 liters min^{-1}. Filters were partitioned according to analysis (e.g., 1-inch disks were cut for quantitative particulate organic carbon analysis). (**A**) Schematic drawing of representative second-generation BioColumn; "Intake" plugs into base box are shown at lower right of (B). (**B**) Photo of data logger and platform of ODP Hole 1026B CORK. BioColumn is plugged into entry port on top of base-box resting on right side of platform. Striped tubing connects base to hole through the spigot (on data logger) and 0.5-inch-diameter titanium pipe.

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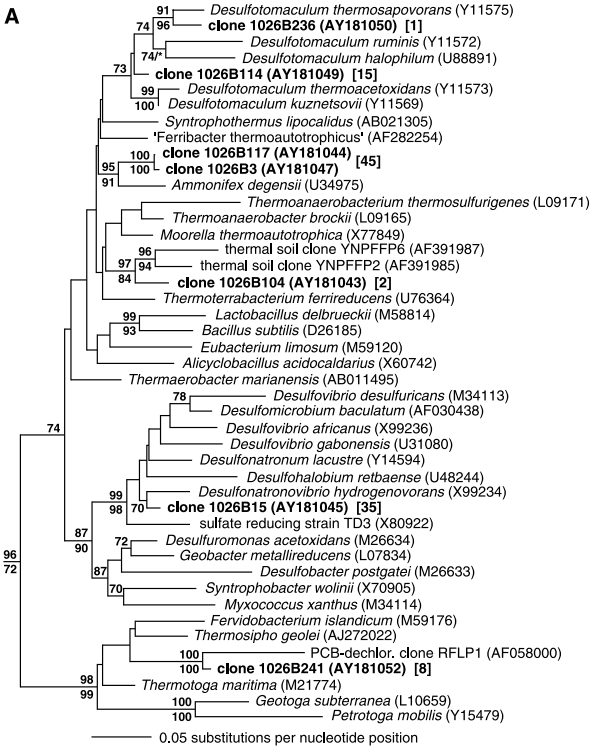

Fig. 2. Phylogenetic trees showing positions of major (**A**) Bacteria and (**B**) Archaea small-subunit rRNA gene sequence groups recovered from borehole 1026B fluids (*13*). Bootstrap proportions >70% that supported the branching order are shown above (evolutionary distance) and below (parsimony) their respective nodes. In cases where the bootstrap proportions are side by side, values derived from distance analyses are listed first. An asterisk indicates a value <70%. GenBank accession numbers are listed in parentheses, and values in brackets refer to the number of times particular bacterial and archaeal clone phylotypes were recovered in a library of 112 analyzed clones. The scale bar corresponds to 0.05 substitutions per nucleotide position.

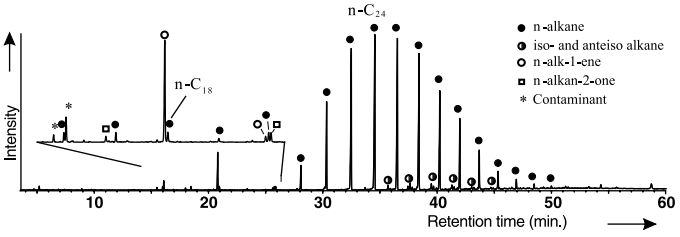

Fig. 3. Reconstructed ion chromatogram of the acetone fraction (*13*) recovered from 4 g (dry weight) of the custom-made *n*-C_{18} reverse-phase silica deposited at ODP site 1026, deployed on 14 October 1997 and recovered 9 July 1998. Contaminants are isomers of isophorone diisocyanate, a common contaminant derived from resin and metal coating. Aliphatic compounds with <18 carbon atoms are probably derived from the alteration of the *n*-C_{18} reverse-phase silica.

Table 1. Chemistry of fluid samples collected from 1026B borehole fluids and near-bottom seawater. EF, enrichment factor with respect to seawater; SD, standard deviation; NA, not applicable. Precolumn fluids were collected at the base box (Fig. 1B) by a submersible-operated titanium sampler for inorganic chemical analyses. Fluid alkalinity was analyzed by titration. Major ions (Cl^-, SO_4^{2-}, Na^+, K^+, Mg^{2+}, and Ca^{2+}) were analyzed by ion chromatography; SiO_2, NH_4^+, and H_2S by colorimetry; and Li^+ and Sr^{2+} by atomic absorption spectroscopy (*6*).

Parameter	Bottom seawater		1026B fluids		EF
	Average	(SD)	Average	(SD)	
Temperature (°C)	1.7		55		NA
pH	7.66	(0.04)	7.63	(0.09)	NA
Alkalinity (meq liter^{-1})	2.38		0.53	(0.14)	0.22
NH_4^+ (μmol kg^{-1})	0.86	(0.40)	122	(18)	142
H_2S (mmol kg^{-1})	<0.0003		<0.0005		NA
SiO_2 (mmol kg^{-1})	0.191	(0.002)	1.13	(0.28)	5.91
Cl^- (mmol kg^{-1})	540.3	(2.2)	552.1	(2.5)	1.0
SO_4^{2-} (mmol kg^{-1})	27.9	(0.1)	17.7	(0.3)	0.64
Li^+ (μmol kg^{-1})	26.0	(0.4)	14.7	(0.7)	0.6
Na^+ (mmol kg^{-1})	469.0	(1.6)	467.5	(1.3)	1.0
K^+ (mmol kg^{-1})	10.22	(0.07)	6.53	(0.08)	0.65
Mg^{2+} (mmol kg^{-1})	53.92	(0.45)	2.74	(0.82)	0.07
Ca^{2+} (mmol kg^{-1})	10.33	(0.08)	55.8	(0.7)	5.4
Sr^{2+} (μmol kg^{-1})	91.1	(1.1)	109.5	(2.4)	1.2

The fluids from borehole 1026B harbor a diverse assemblage of phylogenetic groups that are often most closely affiliated with cultured thermophiles, many of which are known for their metabolic capacity to reduce sulfate or nitrate. Although speculative, this suggests that microbial processes with these electron acceptors may play an important role in this region of the ocean crust. However, H_2S concentration is very low or absent in 1026B fluids (Table 1), requiring other mechanisms for the storage or oxidation of reduced sulfur. A reservoir of sulfide may form within the basaltic crust; secondary sulfides do occur in 1026B crust as minor or trace fine-grained crystals along surfaces of fractures, veinlets, and vesicles (*17*). Concomitantly, reduced S may be exported as organic sulfur, as observed in diffuse discharge fluids from Axial Volcano, JFR (*18*). A third, intriguing possibility is microbial sulfide oxidation coupled to nitrate reduction. Such a process, which reduces nitrate to ammonia to oxidize H_2S (*19*), appears capable of maintaining low or undetectable H_2S despite high sulfate reduction rates (*20*).

There is also uncertainty about the electron sources being used by these organisms for metabolism. Organic acids or aliphatic hydrocarbons released from the overlying sediments or organic matter dissolved in seawater could serve as electron donors. Alternatively, H_2 production, as well as organic compound synthesis via abiotic reactions between seawater and basalts, are thermodynamically favored in the ~50° to 250°C range (*21*) and could also

fuel this deep subsurface biosphere. Preliminary results (*13*) indicate that aliphatic compounds are present in the escaping fluids (Fig. 3): long-chain *n*-alkanes (C_{20} to C_{33}), composed of monomethylalkanes dominated by 2-methyl (iso) and 3-methyl (anteiso) alkanes but with trace amounts of centrally branched isomers up to 9-methylalkanes. The uniqueness of the carbon distribution and invariant stable-carbon isotopic composition of the individual *n*-alkanes [average $\delta^{13}C_{alk}(C_{20}\text{-}C_{29}) = -30.1 \pm 0.2$ versus Vienna Pee Dee Belemnite standard, $n = 8$] (see supporting online text) seem compatible with abiotic synthesis of aliphatic compounds within the crust, although the source of carbon for *n*-alkane synthesis remains unclear. It can be speculated that the absence of short-chain *n*-alkanes ($<C_{20}$) (Fig. 3) results from preferential use of shorter homologs by heterotrophic organisms within the crust. The δ-proteobacteria strain TD3 was shown to consume *n*-alkanes in the C_6 to C_{16} range under strictly anaerobic conditions (*22*).

The most distinctive advantage of collecting fluid samples through a "faucet" at the top of an overpressured borehole CORK is the unlimited access to high–flow-rate crustal fluids (*23*). The greatest disadvantage of this early-generation sampling arrangement is that the fluids are collected from the top of the 295-m borehole, rather than near its bottom where crustal fluids seep into the borehole. Consequently, the crustal fluids must flow through the length of the hole. The extent to which the fluids are altered by interaction with casing cement or the steel liner is unknown. The surface of the steel liner could support a biofilm community (*10*, *24*) that could contribute to the cellular biomass and molecular diversity observed in the 1026B fluid and BioColumn samples. Regardless, the chemical and physical character of the crustal fluids exiting the 1026B hole is conducive to microbial growth. Thus, our data support the hypothesis that life in the ocean crust is not limited to high-flow hydrothermal systems within new ocean crust, but also extends to low-flow older crust of the midocean ridge flanks.

References and Notes

1. E. E. Davis, K. Becker, T. Pettigrew, B. Carson, B. R. MacDonald, *Proc. Ocean Drill. Program Initial Rep.* **139**, 43 (1992).
2. E. E. Davis, K. Becker, *Earth Planet. Sci. Lett.* **172**, 141 (1999).
3. E. E. Davis *et al.*, *Proc. Ocean Drill. Program Initial Rep.* **168**, 470 (1997).
4. A tidal periodicity (*2*, *5*) in the fluid discharge rate (~4.4 to 5.4 liters min^{-1}) was measured by the BioColumn differential-pressure flow meter during a 3-day deployment in summer 1997. The 1026B borehole pressure (>27,100 kPa) exceeds seafloor pressure (<27,045 kPa) and creates substantial outward leakage of crustal fluids from the CORK seal at the top of the borehole (*2*), resulting in a total (combined leakage and open valve) fluid discharge rate at the seafloor of ~16 liters min^{-1}.
5. K. Becker, E. E. Davis, A. T. Fisher, *Eos* **80**, F931 (1999).
6. H. Elderfield, C. G. Wheat, M. J. Mottl, C. Monnin, B. Spiro, *Earth Planet. Sci. Lett.* **172**, 151 (1999).
7. D. B. Butterfield, B. K. Nelson, C. G. Wheat, M. J. Mottl, K. K. Roe, *Geochim. Cosmochim. Acta* **65**, 4141 (2001).
8. C. G. Wheat, M. J. Mottl, *Geochim. Cosmochim. Acta* **64**, 629 (2000).
9. K. G. Porter, Y. S. Feig, *Limnol. Oceanogr.* **25**, 943 (1980).
10. The presence of exopolymers and large cells suggests a possible biofilm origin for some of the microorganisms filtered from 1026B fluids. The combusted glass filters trapped cells that were freely suspended in the fluids, many of which presumably originated from subsurface communities dominated by biofilms (*24*). There is likely a high surface (crust) to volume ratio for crustal fluid passages; cells must be in close contact with surfaces and thin biofilms are likely within the crust. Additionally, biofilms likely formed on combusted glass filter during the 10-day in situ sampling interval (for biomass and phylogenetic studies).
11. D. M. Karl, C. O. Wirsen, H. W. Jannasch, *Science* **207**, 1345 (1980).
12. J. P. Cowen *et al.*, *Geophys. Res. Lett.* **26**, 3637 (1999).
13. Materials and methods are available as supporting material on *Science* Online.
14. R. Huber *et al.*, *Syst. Appl. Microbiol.* **19**, 40 (1996).
15. B. Warwick, D. R. Musgrave, in *Studies in Plant Science*, vol. 1, *Biology and Biochemistry of Nitrogen Fixation*, M. J. Dilworth, A. R. Glenn, Eds. (Elsevier, Amsterdam, 1991), pp. 162–186.
16. S. M. Barns, C. F. Delwiche, J. D. Palmer, N. R. Pace, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9188 (1996).
17. P. Marescotti, D. A. Vanko, R. Cabella, *Proc. Ocean Drill. Program Sci. Results*, A. Fisher, E. E. Davis, C. Escutia, Eds., vol. 168 (Ocean Drilling Program, College Station, TX, 2000).
18. F. Kenig, unpublished data.
19. S. Otte *et al.*, *Appl. Environ. Microbiol.* **65**, 3148 (1999).
20. T. G. Ferdelman *et al.*, *Geochim. Cosmochim. Acta* **61**, 3065 (1997).
21. E. Shock, *Origins Life Evol. Biosphere* **22**, 67 (1992).
22. P. Rueter *et al.*, *Nature* **372**, 455 (1994).
23. Once the CORK valve is connected to a collection box, the fluids can be routinely accessed by submersible without additional drilling or specialized collection equipment.
24. J. W. Costerton *et al.*, *J. Bacteriol.* **176**, 2137 (1994).

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