ductive hypoblastic layer was artificially depolarized by dissociating and reaggregating the hypoblastic cells. This layer retained the capacity to induce axial mesodermal structures, that developed according to a weaker polarity stored in the epiblast (*10*).

Soluble growth factors have been shown to induce mesoderm formation in *Xenopus*. Slack *et al.* (*11*) reported that bFGF can induce the formation of non-axial mesoderm in ectodermal animal caps of *Xenopus* blastulae. Later, Smith (*12*) showed that XTC CM can induce animal cap cells to differentiate into patches of disorganized muscle and notochord. In the *Xenopus* assay system, a number of growth factors have now been identified which can induce mesoderm in animal caps (*13, 14*). In that system, factors from the FGF family can induce mesoderm mainly of the more ventral or nonaxial type. Factors of the TGF-β family can, to a certain extent, potentiate this effect and induce differentiation of more dorsal types of mesoderm (*14, 15*). In the amphibian system, there have been no reports of induction of fully organized mesodermal tissue structures by soluble factors. Secondary axes, however, have been obtained when animal caps, treated with soluble factors, are grafted into normal *Xenopus* blastulae (*16*). In such cases the axial structures arise as a secondary interaction with the induced grafted tissue, although the type of structures that develop depend on the factor previously applied to the graft (*16*).

Our experiments show that bFGF is not sufficient to induce axial structures in the chick, but do not rule out the possibility that it could be involved in the induction of nonaxial mesoderm. Recent experiments indicate that in the chick, both bFGF RNA and protein are already expressed in the epiblastic region, before stage XII (*17*). Direct experiments on induction of nonaxial mesoderm cannot be performed in the chick since by the time the epiblast and hypoblast tissues sort themselves out into two separate entities, nonaxial mesoderm has already been determined (*4, 8*).

We were unable to obtain induction of axial mesodermal structures by TGF-β1, TGF-β2 or a combination of both, with and without bFGF. These results, although in apparent contradiction to the *Xenopus* findings, suggest that other factors are probably required for the formation of axial structures. In *Xenopus*, it is becoming clear that the mesoderm-inducing activity found in XTC CM (*18*) (XTC MIF) is probably due to a factor related to but distinct from TGF-β2 (*15, 19*). We have no evidence whether the same factor is operating in the chick or more than one factor is necessary in the avian system. Boiling XTC CM does not remove the inducing activity, although we have not been able to enhance the activity by this treatment (see Table 1) as is the case in *Xenopus* (*12*).

Our results show that soluble factors applied in a nonpolar manner to a polarized competent tissue can act as true morphogens, since they can induce the formation of complex organized axial structures.

REFERENCES AND NOTES

1. H. Eyal-Giladi and S. Kochav, *Dev. Biol.* **49**, 321 (1976).
2. L. J. Vakaet, *J. Embryol. Exp. Morphol.* **10**, 38 (1962); G. J. Nicolet, *ibid.* **23**, 79 (1970).
3. C. H. Waddington, *Philos. Trans. R. Soc. London Ser. B* **211**, 179 (1932); Y. Azar and H. J. Eyal-Giladi, *J. Embryol. Exp. Morph.* **61**, 133 (1981).
4. Y. Azar and H. Eyal-Giladi, *ibid.* **52**, 79 (1979).
5. H. Eyal-Giladi, *Cell Differentiation* **14**, 245 (1984).
6. D. A. T. New, *J. Embryol. Exp. Morphol.* **3**, 326 (1955).
7. E. Mitrani and Y. Shimoni, *Development* **107**, 275 (1989).
8. E. Mitrani and H. Eyal-Giladi, *Differentiation* **21**, 51 (1982).
9. M. Putney, M. G. R. Verma, C. J. Leake, *Experientia* **29**, 466 (1973).
10. E. Mitrani and H. Eyal-Giladi, *Nature* **289**, 800 (1981).
11. J. M. W. Slack *et al.*, *ibid.* **326**, 197 (1987).
12. J. C. Smith, *Development* **99**, 3 (1987).
13. D. Kimelman and M. Kirschner, *Cell* **51**, 869 (1987).
14. D. L. Weeks and D. A. Melton, *ibid.*, p. 861; S. F. Godsave, H. V. Isaacs, J. M. W. Slack, *Development* **102**, 555 (1988).
15. J. C. Smith, *Development* **105**, 665 (1989).
16. J. Cooke, J. C. Smith, E. J. Smith, M. Yaqoob, *ibid.* **101**, 893 (1987); A. Ruiz i Altaba and D. A. Melton, *Nature* **341**, 33 (1989).
17. E. Mitrani *et al.*, *Development*, in press.
18. J. C. Smith, M. Yaqoob, K. Symes, *ibid.* **103**, 591 (1988).
19. F. Rosa *et al.*, *Science* **239**, 783 (1988).

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Stable Carbon Isotopic Evidence for Carbon Limitation in Hydrothermal Vent Vestimentiferans

C. R. FISHER, M. C. KENNICUTT II, J. M. BROOKS

Stable carbon isotope composition ($\delta^{13}C$ values) can be used to evaluate an animal's source of nutritional carbon. Most animals with chemoautotrophic endosymbionts have quite negative tissue $\delta^{13}C$ values due to discrimination against ^{13}C associated with chemoautotrophic assimilation of inorganic carbon. However, the $\delta^{13}C$ values of hydrothermal vent (HTV) vestimentiferans are significantly higher than the values reported for non-HTV vestimentiferans or other invertebrates with chemoautotrophic endosymbionts. Tissue $\delta^{13}C$ values of two species of HTV vestimentiferans increase with increasing size of the animals. This relation supports the hypothesis that the relatively high $\delta^{13}C$ values are the result of inorganic carbon limitation during carbon fixation. A more favorable relation between gas exchange and carbon fixation in the smaller individuals is expected, due to differences in the geometric scaling of gas-exchange surfaces and trophosome volume.

THE STABLE C ISOTOPE CONTENT OF HTV animals was one of the first indications that nonphotosynthetic food sources were utilized by these communities (*1*). Measurements have now been made of over 30 symbioses between marine invertebrates and chemoautotrophic or methanotrophic bacteria (*2*). The basis for the utility of stable C isotopes in investigations of these symbioses is twofold. First, chemoautotrophic bacteria discriminate significantly (~25 per mil) against ^{13}C during the incorporation of inorganic C into organic compounds (*3*), and this partitioning results in organic compounds that have more negative $\delta^{13}C$ values than those originating from photosynthetically fixed C. Second, methanotrophs discriminate only slightly during incorporation of methane C into cellular components, and methane in the marine environment is highly enriched in ^{12}C ($\delta^{13}C = -35$ to -90 per mil) (*4*). As a result, tissues from most invertebrates with chemoautotrophic endosymbionts have $\delta^{13}C$ values that range from -23 to -50 per mil and invertebrates with confirmed methanotrophic symbionts have values that range from -40 to -76 per mil, whereas $\delta^{13}C$ values of other marine organisms normally range from -12 to -22 per mil (*2*).

Unlike $\delta^{13}C$ values from other animals

C. R. Fisher, Marine Science Institute and Department of Biological Sciences, University of California, Santa Barbara, CA 93106.
M. C. Kennicutt II and J. M. Brooks, Geochemical and Environmental Research Group, 10 South Graham Road, Texas A&M University, College Station, TX 77845.

with chemoautotrophic endosymbionts, the $\delta^{13}C$ values of HTV vestimentiferan tissues do not reflect the expected discrimination against ^{13}C (Table 1). Vestimentiferans lack a mouth, gut, and anus but contain an internal organ, the trophosome, which is estimated to contain between 15 and 35% symbiotic bacteria by volume (*5*). Physiological, morphological, and enzymatic evidence indicates that the symbionts of vestimentiferans are chemoautotrophic sulfide-oxidizing bacteria, which most likely provide the bulk of the organic C requirements for the intact symbiosis (*5, 6*). Reported $\delta^{13}C$ values for the tissues of five species of non-HTV vestimentiferans fall into the expected range for animals with chemoautotrophic symbionts (Table 1). The reasons for the discrepancy between HTV and non-HTV vestimentiferans remain uncertain.

Anomalously high $\delta^{13}C$ values have also been reported for a variety of other HTV animals (*7, 8*). Although two of the species of polychaetes harbor extracellular symbionts on their body surfaces, some of which may be chemoautotrophic, the nutritional relation between the external symbionts and the polychaetes is uncertain, and available evidence indicates minimal exchange of organic C (*9*). Unlike vestimentiferans, which because of their endosymbionts may be considered primary producers in HTV communities (*2, 5, 6*), all of these other animals are consumers, and their $\delta^{13}C$ values are therefore a reflection of their food source: either vestimentiferans or a hypothetical bacterial food source with a $\delta^{13}C$ near −11 per mil (*8*).

In order to investigate variation in $\delta^{13}C$ values between species of vestimentiferans, within a species of vestimentiferan, and between tissues within an individual, we sampled animals at an HTV site at 13°N on the East Pacific Rise (*10*) and at a cold hydrocarbon seep site in the Gulf of Mexico (*11*). Tissue from *Riftia pachyptila* and two other species of vestimentiferans from the HTV site were analyzed (*12*), and their tissue $\delta^{13}C$ values were all in the same range (Table 1). These data indicate that the causes of the high $\delta^{13}C$ values in *R. pachyptila* are not species-specific but are rather a function of a combination of factors inherent to vestimentiferans and their HTV environment. In the larger individuals of *R. pachyptila* and *Tevnia jerichonana* a variety of tissues and portions of the tubes were sampled. The average variation among tissues within any animal was 1.3 per mil (n = 12, SD = 0.95 per mil), and the largest variation was 3.1 per mil. The most positive value obtained was −8.8 per mil for the gonads of a male *T. jerichonana*; its other tissues averaged −10.6 per mil. The most negative value was −19.3 per mil for the tube of the smallest *R. pachyptila* sampled. There was no consistent difference between the trophosome and symbiont-free tissues, or between other living tissues of the worms. Values for the tubes, however, were generally more negative (up to −5.3 per mil, average = −1.9 per mil, n = 20, SD = 1.5 per mil) than those for the animal tissues. Four *T. jerichonana* tubes were subsampled, and the base was always more negative than the anterior end (average difference = −0.75 per mil, SD = 0.2 per mil).

Rau (*1*) postulated that the heavy $\delta^{13}C$ values of *R. pachyptila* tissues might be a result of C limitation. Depletion of substrate (CO_2) at the site of fixation would cause a mass balance shift toward the substrate $\delta^{13}C$ value. Current understanding of the *R. pachyptila* symbiosis supports this possibility. These vestimentiferans grow quickly in the warm, energy-rich hydrothermal waters (*13*). The trophosome, which comprises about 16% of the worm's wet weight, is densely packed (up to 10^{10} cells per gram) with relatively large symbionts (from 2 to 6 μm in diameter) (*5*). This tissue has a high chemoautotrophic capacity, and the intracellular symbionts must rely on the host circulatory system to meet their demand for inorganic C.

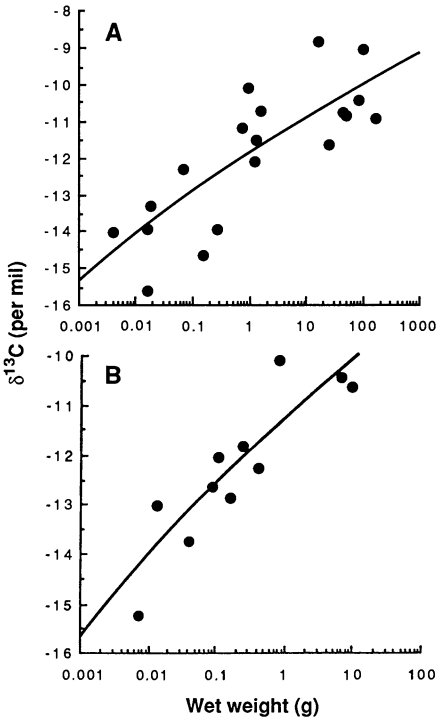

Fig. 1. Relation between wet weight and stable carbon isotope content ($\delta^{13}C$) of soft tissues in two species of HTV vestimentiferans. (**A**) *Riftia pachyptila*. Allometric equation for the line shown is $-\delta^{13}C$ (per mil) = 11.837 wet weight$^{-0.0374}$, SE of the exponent is 0.007. (**B**) *Tevnia jerichonana*. Allometric equation for the line shown is $-\delta^{13}C$ (per mil) = 11.263 wet weight$^{-0.0477}$, SE of the exponent is 0.008.

We sampled a size series of two species of HTV vestimentiferans (*R. pachyptila* and *T. jerichonana*) at 13°N to test this hypothesis. We postulated that if the symbionts in adults are C-limited, then this limitation should be reduced in the smallest individuals, which, because of the geometric scaling of the structures involved, have a more favorable relation between gas exchange and transport capacities on the one hand, and tissue mass of the CO_2-consuming, symbiont-packed trophosome on the other (*14*). The data (Fig. 1) show that discrimination against ^{13}C was most pronounced in the smallest individuals of each species that were sampled. Variations in the $\delta^{13}C$ value of the ΣCO_2 in HTV waters is not a significant factor in this study for two reasons. First, the maximum variation in the $\delta^{13}C$ value of end-member ΣCO_2 over the temperature range to which the vestimentiferans are exposed is less than 0.2 per mil (*15*). Second, in the case of *T. jerichonana* (Fig. 1B), the

Table 1. The $\delta^{13}C$ values of vestimentiferan soft tissues; n is the number of individuals. CS, cold seep; EPR, East Pacific Rise; FE, Florida escarpment; G, Galapagos Rift; GB, Guymas Basin; GR, Gorda Ridge; GM, Gulf of Mexico; HTV, hydrothermal vent; JF, Juan de Fuca; JSZ, Japan subduction zone; OSZ, Oregon subduction zone; TS, this study.

Species	Habitat	$\delta^{13}C$ (per mil)	n	References
Riftia pachyptila	HTV:Gr, EPR	−9.0 to −15.6	50	(*1, 6*), TS
*Riftia pachyptila**	HTV:GB	−14	1	(*20*)
Tevnia jerichonana	HTV:EPR	−8.8 to −15.2	12	TS
Oasisia alvinae	HTV:EPR	−10.9	1	(*8*)
Unidentified sp.†	HTV:EPR	−11.0 to −11.1	2	TS
Unidentified sp.†	HTV:G	−10.9	1	TS
Ridgia spp.‡	HTV:JF, GR	−10.7 to −16.8	4	(*20*)
Unidentified escarpid	CS:GM	−24.5 to −41.0	10	(*11, 18*), TS
Lamellibrachia sp.	CS:GM	−21.1 to −43.2	36	(*11, 18*), TS
L. barhami	CS:OSZ	−31.9	1	(*21*)
L. sp.	CS:JSZ	−25.8	1	(*22*)
Escarpia laminata	CS:FE	−42 to −47	7	(*23, 24*)

*Originally thought to be *Riftia pachyptila*; Jones now considers *Riftia* sp. from Guymas Basin to be a separate species (*25*). †These undescribed species are not the same (*25*). ‡Taxonomy and distribution of this genus are currently under study (*25*).

two largest individuals were attached at their bases and the smaller individuals were attached to the tubes of the two larger individuals, and therefore all individuals were exposed to virtually identical environments and ΣCO_2 pools.

Interpretation of the $\delta^{13}C$ values of vestimentiferans from cold seep communities is much more complex than for HTV vestimentiferans. The animal tissue $\delta^{13}C$ values of the cold seep vestimentiferans are more negative and fall broadly within the range expected from animals with chemoautotrophic endosymbionts (Table 1). However, the values may not be a result of the same processes that produce these values in other chemoautotrophic symbioses. The variation in the $\delta^{13}C$ values of the two species of hydrocarbon seep vestimentiferans is also greater than in the HTV vestimentiferans (Table 1) or in other animals with chemoautotrophic symbionts. The differences in the $\delta^{13}C$ values of cold seep and HTV vestimentiferans, as well as the range of $\delta^{13}C$ values of cold seep vestimentiferans, could be a result of a number of causes. (i) The specific environmental requirements of the seep vestimentiferans are not known, and, unlike HTV vestimentiferans (*16*), seep vestimentiferans may be able to survive under a relatively wide range of conditions. Exposure to significantly different levels of sulfide in different seep microhabitats could have a profound effect on the symbionts' capacity for C fixation and therefore on the demand for inorganic C. (ii) Several observations indicate that the hydrocarbon seep vestimentiferans are long-lived. Animals have been collected with tubes encrusted with colonies of deep sea sponges and octocorals that are estimated to be more than 50 years old, and individuals are routinely collected that had portions of their tubes more than a meter deep in the sediments. The cumulative effects of recycling respiratory CO_2 in a chemoautotrophic symbiosis are unknown but could be quite pronounced in long-lived, slow-growing, species. (iii) Burial of the tubes by sediment also confounds determination of the $\delta^{13}C$ of the inorganic C the animals are utilizing, because the animals may be able to take up CO_2 directly across their tubes. The $\delta^{13}C$ values and the concentration of ΣCO_2 in interstitial waters vary over a wide range in cold seep environments because of microbial oxidation of isotopically light methane and organic C (*17*). Reported ΣCO_2 concentrations and $\delta^{13}C$ values range from 1.3 to 10.8 m*M* and −10.1 to −45.2 per mil, respectively, in interstitial waters from the Gulf of Mexico hydrocarbon seep site, and from 3.2 to 16.8 m*M* and −13.1 to −34.8 per mil, respectively, in interstitial waters of sediments from the Oregon subduction zone (*17*). The $\Delta^{14}C$ values of some cold seep vestimentiferans indicate that they are incorporating "dead" CO_2 derived from microbial oxidation of fossil C, that is, methane or oil (*18, 19*).

The relation between the size of the HTV vestimentiferans and tissue $\delta^{13}C$ values is direct evidence of the animals' role in discrimination against ^{13}C and provides strong support for the theory of C limitation in adult HTV vestimentiferans. However, a variety of factors can affect the $\delta^{13}C$ values of vestimentiferans, as well as other animals with chemoautotrophic symbionts. Several of these factors are intrinsically difficult, if not impossible, to quantify (such as the $\delta^{13}C$ values of the ΣCO_2 to which animals in the soft sediment are exposed and the degree of C limitation within the symbiont's vacuole inside the host cell). The extreme variation found in $\delta^{13}C$ values of hydrocarbon seep vestimentiferans attests to the cumulative effects of the variable intrinsic and extrinsic factors, even within a single species.

REFERENCES AND NOTES

1. G. H. Rau and J. I. Hedges, *Science* **203**, 648 (1979); G. H. Rau, *ibid.* **213**, 338 (1981); *Bull. Biol. Soc. Wash.* **6**, 243 (1985); all values are reported as

$$\delta^{13}C = \frac{^{13}C/^{12}C_{sample}}{^{13}C/^{12}C_{PDB} - 1} \times 10^3 \text{ (per mil)}$$

where PDB is a standard.
2. C. R. Fisher, *Rev. Aquat. Sci.*, in press.
3. E. G. Ruby, H. W. Jannasch, W. G. Deuser, *Appl. Environ. Microbiol.* **53**, 1940 (1987).
4. M. P. Silverman and V. I. Oyama, *Anal. Chem.* **40**, 1833 (1968); V. M. Ovsyanidov, V. S. Lebedev, V. M. Bogdanova, G. A. Mogilevski, *Mikrobiologiya* **42**, 589 (1973); B. Bernard, J. M. Brooks, W. M. Sackett, *Earth Planet Sci. Lett.* **31**, 48 (1976); *Proc. Offshore Tech. Conf. 2934* (1977), p. 435.
5. J. J. Childress, A. J. Arp, C. R. Fisher, *Mar. Biol.* **83**, 109 (1984); M. L. Jones, *Bull. Biol. Soc. Wash.* 6, 117 (1985); M. A. Powell and G. N. Somero, *Biol. Bull. (Wood's Hole, MA)* **171**, 274 (1986); S. Belkin, D. C. Nelson, H. W. Jannasch, *ibid.* **170**, 110 (1986); H. Felbeck and J. J. Childress, *Oceanol. Acta* **SP8**, 131 (1988).
6. C. R. Fisher *et al.*, *Deep Sea Res.* **35**, 1745 (1988).
7. D. Desbruyères, F. Gaill, L. Laubier, D. Prieur, G. H. Rau, *Mar. Biol.* **75**, 201 (1983).
8. C. L. Van Dover and B. Fry, *ibid.* **102**, 257 (1989).
9. D. Desbruyères, R. Gaill, L. Laubier, Y. Fouquet, *Bull. Biol. Soc. Wash.* **6**, 103 (1985); A. M. Alayse-Danet, D. Desbruyères, F. Gaill, *Symbiosis* **4**, 51 (1987).
10. A. Fustec *et al.*, *Biol. Oceanogr.* **4**, 121 (1987).
11. M. C. Kennicutt II *et al.*, *Nature* **317**, 351 (1985); I. R. MacDonald *et al.*, *Mar. Biol.* **101**, 235 (1989).
12. Freshly collected animals were removed from their tubes and weighed on a motion-compensated shipboard balance system [J. J. Childress and T. J. Mickel, *Deep Sea Res.* **27A**, 965 (1980)] and then frozen whole in the case of the smallest animals or dissected with subsamples of tissue frozen in liquid N_2. Tissue samples were prepared in a Craig type combustion system with CO_2 determination on a Finnigan MAT 251 isotope ratio mass spectrometer, and values are reported as $\delta^{13}C$ (per mil) relative to the PeeDee belemnite standard.
13. M. Roux *et al.*, *C. R. Acad. Sci. Ser. C* **308**, 121 (1989).
14. R. H. Peters, *The Ecological Implications of Body Size* (Cambridge Univ. Press, Cambridge, 1983); K. Schmidt-Nielsen, *Animal Physiology: Adaptation and Environment* (Cambridge Univ. Press, Cambridge, 1983).
15. This estimate is based on a $\delta^{13}C$ of ambient bottom water ΣCO_2 of −0.2 per mil and a $\delta^{13}C$ of ΣCO_2 in the 350°C end-member hydrothermal fluid of −4.3 per mil; P. Kroopnick, W. G. Deuser, H. Craig, *J. Geophys. Res.* **75**, 7668 (1969); J. A. Welhan, thesis, University of California, San Diego (1981).
16. R. R. Hessler and W. M. Smithy, Jr., in *Hydrothermal Processes at Seafloor Spreading Centers*, P. A. Rona *et al.*, Eds. (Plenum, New York, 1983), pp. 735–770; R. R. Hessler *et al.*, *Deep Sea Res.* **35**, 1681 (1988).
17. E. Suess and M. J. Whiticar, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **71**, 119 (1989); M. C. Kennicutt II, J. M. Brooks, R. A. Burke, Jr., *Proc. Offshore Tech. Conf. 5952* (1989), p. 649.
18. J. M. Brooks *et al.*, *Science* **238**, 1138 (1987).
19. C. K. Paull *et al.*, *Nature* **342**, 166 (1989).
20. C. L. Van Dover and B. Fry, unpublished data.
21. L. D. Kulm *et al.*, *Science* **231**, 561 (1986).
22. T. Saino and S. Ohta, *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **71**, 169 (1989).
23. C. K. Paull, A. J. T. Jull, L. J. Toolin, T. Linick, *Nature* **317**, 709 (1985).
24. C. B. Cary, B. Fry, H. Felbeck, R. D. Vetter, *Mar. Biol.* **100**, 411 (1989).
25. M. L. Jones, personal communication.

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