MICROBIOLOGY

A Proteomic Snapshot of Life at a Vent

Survival of a polychaete worm in a deep sea hydrothermal vent depends on complex metabolic interactions with symbiotic bacteria.

Charles R. Fisher and Peter Girguis

In the late 1970s, scientists discovered rich communities of animals living around deep-sea hydrothermal vents in the eastern Pacific. Since then, hundreds of other chemoautotrophic-based communities and animals have been discovered. What were once thought of as biological oddities are now confirmed to be abundant in all the world's oceans. What the sites all have in common is an abundance of reduced chemicals (such as sulfide or methane) at the sea floor. Many of the sites, including mineral-rich hydrothermal vents and the oil- and gas-rich hydrocarbon seeps, are increasingly affected by anthropogenic activities. To assess the effects of these activities, we must better understand the lifestyles of the creatures that thrive in this extreme habitat. The use of powerful molecular tools, such as the approach described by Markert *et al*. on page 247 of this issue, will advance our understanding of the abundant chemoautotrophic symbioses of the deep sea.

One of the most prominent members of the hydrothermal vent community is the tubeworm *Riftia pachyptila*, a siboglinid polychaete that has become the unofficial poster child for hydrothermal vents. *Riftia* has no mouth, gut, or anus and cannot feed by normal means. Instead, *Riftia* depends on intracellular chemoautotrophic symbionts—which fill a large internal organ called the trophosome—for nutrition. The symbionts are γ-proteobacteria, which are functionally analogous to plant chloroplasts in that they generate organic carbon as a food source for their worm host (using sulfide as an electron donor and oxygen as an electron acceptor). Key to understanding the biology of *Riftia* and other chemoautotrophic symbioses is an understanding of the biology of their symbionts. However, no chemoautotrophic symbiont has ever been cultured in a laboratory, and this has long hampered our ability to study their metabolism. Markert *et al*. use a proteomic approach to examine protein expression in *Riftia* symbionts and gain new insights into their biochemistry and metabolism (*1*).

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Hydrothermal vents are dynamic and potentially dangerous environments. Temperatures can range from near freezing to more than 300°C over centimeters. Within animal communities, temperatures can vary over more than 40°C within seconds. Hydrothermal vents are also quite ephemeral, with local sources of hydrothermal flow often lasting only a few years. Consequently, the habitat fluctuates, and vent fauna must balance exposure to the hot and potentially toxic vent fluid with the need to obtain nutrition either directly from the fluid or from microbes living in the fluid. *Riftia* is supremely adapted for its symbiotic life style in this environment. They live with their highly vascularized gill-like plumes exposed to vent fluid and have circulating hemoglobins that bind to both oxygen and sulfide reversibly and with high affinity and capacity (*2*, *3*). This allows *Riftia* to take up and store large amounts of these chemoautotrophic substrates, transport them through its tissues with no harmful effects, and provide its symbionts with a bountiful supply of both (*4*). In return, the symbionts are extremely efficient and productive, fixing carbon at high rates to support the host's growth (*5*). This suite of adaptations enables *Riftia* to be very fast growing and quite fecund, while reliant on its symbionts for nutrition.

These and earlier studies of *Riftia* focused on characterizing its major biochemical, physiological, and ecological attributes, such as hemoglobin properties, oxygen uptake rates, and habitat characteristics. More recent studies have grown in both breadth and depth, investigating the expression of genes, quantifying the metabolic interactions between host and symbiont, and describing the ecological dynamics of *Riftia* aggregations. Markert *et al.* have now used the power of genomic analyses coupled with high-throughput protein profiling to obtain a snapshot of the proteins (or proteome) expressed by the *Riftia* symbiont. Their results illustrate the degree to which *Riftia* symbionts are poised for high rates of chemoautotrophic carbon fixation powered by sulfide oxidation. For example, Markert *et al*. find that 12% of the total cytosolic proteome of these symbionts consists of three proteins involved in coupling energy production to sulfide oxidation. This is a marked departure from fast-growing, free-living heterotrophic bacteria that instead expend a considerable fraction of their energy synthesizing amino acids for their own cell division and growth (*6*). The prominence of these three proteins underscores the central role of the symbionts: to provide nutrition to the association by harnessing energy from sulfide.

The mechanism of inorganic carbon uptake and transport by the host, and fixation by the symbiont, has been the subject of much inquiry and debate. The first indication that hydrothermal vent fauna obtain their nutrition from chemoautotrophic sources (not from photosynthetically derived products but from primary production powered by hydrogen

The life aquatic. *Riftia pachyptila* tubeworms at a hydrothermal vent field located near 21°N on the East Pacific Rise.

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sulfide) came from analyses of their stable carbon (^{13}C) and nitrogen (^{15}N) isotope contents (*7*, *8*). The amount of these isotopes detected in dominant fauna (tube worms, mussels, and clams) did not reflect normal deep-sea carbon and nitrogen. Later studies demonstrated the presence of intracellular chemoautotrophic symbionts in these animals, confirming the nonphotosynthetic nutritional source of carbon and nitrogen. Mussels and clams had δ^3C values of about –30 per mil (‰), in the range that was expected for carbon that is derived from chemoautotrophic bacteria. However, the $\delta^{13}C$ value of *Riftia* was much higher (~ –15‰) and consequently more difficult to understand. A variety of explanations have been put forward to explain these isotope values, but none has proven completely satisfactory (*9*–*11*).

Markert *et al*. find high amounts of enzymes involved in the reductive tricarboxylic acid cycle in extracts of the *Riftia* symbiont and suggest that this is an important pathway of carbon fixation by the symbiont. In addition to the implications for more energy-efficient carbon fixation, this finding may help explain the anomalously high carbon isotope values that have puzzled researchers for decades.

Far less than 1% of the microbes present in nature have been successfully cultured in the laboratory. No chemoautotrophic symbiont has yet been cultured, and it is possible that many never will be. Not only is the milieu of a living host difficult to imitate in vitro, but in some cases, the exchange and integration of host and symbiont genes may have yielded a symbiont more analogous to an organelle than to a free-living microbe. In such instances, genomic and proteomic approaches provide valuable information on the symbiont's metabolic capabilities and evolutionary history. Quantitative proteomics has the additional value of allowing one to use protein expression levels as a metric for studying the importance of metabolic pathways used by these symbiotic microbes in situ.

Many questions remain about these enigmatic animals and their rather extreme life styles. *Riftia*'s trophosome, which is packed with billions of bacteria per gram of tissue, is intertwined with the animal's gonads. Considering the rarity of active hydrothermal vents on the sea floor, and the improbability of larvae finding a suitable home, it is likely that a high percentage of the nutritional input from the symbionts goes directly to reproduction. How is this accomplished and coordinated? Furthermore, transmission of the symbionts between generations is not direct because the larvae are aposymbiotic and newly settled tube worms must acquire their symbionts anew each generation from an apparently free-living pool. How is the metabolism of the free-living stage different from that of the symbiotic stage? Once contact with a host is made, how do the symbionts contribute to successful establishment of the symbiosis? Molecular approaches like that of Markert *et al*. may help answer such questions about life and relationships in this remote and inhospitable environment.

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MOLECULAR BIOLOGY

Amplified Silencing

Small RNA molecules that silence gene expression are amplified by different mechanisms in nematodes and plants.

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Ten years ago, we knew nothing about how double-stranded RNA blocks gene expression through the silencing of targeted RNA. We now have a good understanding of this process, and current interest is turning to variations on the basic mechanism. Recent studies involving plants and the nematode *Caenorhabditis elegans* continue this trend, including those reported in this issue by Pak and Fire on page 241 (*1*) and Sijen *et al.* on page 244 (*2*). Two other papers by Axtell *et al.* (*3*) and Ruby *et al.* (*4*) are also relevant. These studies deal with the amplification of silencing-related RNA and explain how strong, persistent silencing can be initiated with small amounts of "initiator" double-stranded RNA. The amplification process has implications for application of RNA interference to control gene expression in biotechnology and for understanding the effects of silencing RNAs on cell function and organism development.

Specifically, these new studies investigate how the target of silencing can spread (or transit) within a single strand of RNA. The initiator of transitivity is a double-stranded RNA that is first processed by Dicer, a ribonuclease III–like enzyme, into short interfering RNA (siRNA) or a related type of RNA referred to as microRNA (miRNA). These 21- to 25-nucleotide single-stranded RNAs are the primary silencing RNAs in the transitive process. A primary silencing RNA binds to a ribonuclease H–like protein of the Argonaute class. The resulting Argonaute ribonucleoprotein can target long RNA molecules by Watson-Crick base pairing. The targeted RNA then becomes a source of secondary siRNAs. Transitivity occurs when the secondary siRNAs correspond to regions adjacent to the target sites of the primary silencing RNA.

RNA-directed RNA polymerases (RdRPs) produce secondary siRNA, and the new results indicate that they catalyze two different mechanisms of silencing amplification. One mechanism is characterized by Axtell *et al*. (*3*), who investigated endogenous secondary siRNAs in plants. They show that efficient secondary siRNA production occurs if a single-stranded RNA has two target sites for the Argonaute ribonucleoprotein. Optimal secondary siRNA production occurs when the targeted RNA is cleaved by Argonaute. Cleaved RNA then recruits RdRP, which generates double-stranded RNA. Dicer then produces transitive secondary siRNAs (see the figure).

Another biogenesis mechanism of secondary siRNAs has, so far, only been described in *C. elegans*. The discovery of this distinct mechanism by Sijen *et al*., Pak and Fire, and Ruby *et al*. follows from the observation that a type of siRNA is underrepresented in

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