A Proteomic Snapshot of Life at a Vent

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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 lifestyle 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...
sulfur) 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 chemooautotrophic symbionts in these animals, confirming the nonphotosynthetic nutritional source of carbon and nitrogen. Mussels and clams had $\delta^{13}$C values of about $-30$ per mil ($\%$), in the range that was expected for carbon that is derived from chemooautotrophic bacteria. However, the $\delta^{13}$C value of Riftia was much higher ($\sim -15\%$) 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 chemooautotrophic 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 lifestyle. 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.

**References**


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**MOLECULAR BIOLOGY**

### Amplified Silencing

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