Arginine Metabolism in the Deep Sea Tube Worm Riftia pachyptila and Its Bacterial Endosymbiont*

Zoran Minic‡ and Guy Hervé¶

From the Laboratoire de Biochimie des Signaux Régulateurs Cellulaires et Moléculaires, UMR 7631, Centre National de la Recherche Scientifique, Université Pierre et Marie Curie, 96 Boulevard Raspail, F-75006 Paris, France

The present study describes the distribution and properties of enzymes involved in arginine metabolism in Riftia pachyptila, a tubeworm living around deep sea hydrothermal vents and known to be engaged in a highly specific symbiotic association with a bacterium. The results obtained show that the arginine biosynthetic enzymes, carbamyl phosphate synthetase, ornithine transcarbamylase, and argininosuccinate synthetase are present in all of the tissues of the worm and in the bacteria. Thus, Riftia and its bacterial endosymbiont can assimilate nitrogen and carbon via this arginine biosynthetic pathway. The kinetic properties of ornithine transcarbamylase strongly suggest that neither Riftia nor the bacterium possess the catabolic form of this enzyme belonging to the arginine deiminase pathway, the absence of this pathway being confirmed by the lack of arginine deiminase activity. Arginine decarboxylase and ornithine decarboxylase are involved in the biosynthesis of polyamines such as putrescine and agmatine. These activities are present in the trophosome, the symbiont-harboring tissue, and are higher in the isolated bacteria than in the trophosome, indicating that these enzymes are of bacterial origin. This finding indicates that Riftia is dependent on its bacterial endosymbiont for the biosynthesis of polyamines that are important for its metabolism and physiology. These results emphasize a particular organization of the arginine metabolism and the exchanges of metabolites between the two partners of this symbiosis.

Deep sea hydrothermal vents display a set of unique environmental characteristics, including high pressure, steep temperature gradients, and high concentrations of toxic elements such as sulfides and heavy metals. These hydrothermal vents are very dynamic, with rapidly changing physical and chemical parameters. However, in the area around these springs, dense communities of animals are found (1–3). Some of them are marine invertebrates that survive through symbiosis with chemosynthetic bacteria. One of the most studied species living at deep sea hydrothermal vents along the East Pacific Rise and the Galapagos Rift is Riftia pachyptila (4, 5), a giant tubeworm whose anatomical organization is shown in Fig. 1. To support its large size and high growth rate, the nutritional needs of Riftia are satisfied by an endosymbiotic relation with a chemolithoautotrophic sulfide-oxidizing bacteria located intracellularly in a specialized organ, the trophosome (6–8). The trophosome is located within the large sac (coelome) made by the body wall and terminated by the oiphosome and is bathed by the coelomic fluid (1). It represents about 15% of the weight of the worm (9). The bacterial volume represents between 15 and 35% of the total volume of the trophosome (10). Since the bacterium is a sulfide-oxidizing autotroph (8, 11, 12), the worm must take up sulfide, ammonia, oxygen, and carbon dioxide from the medium and supply these products to the symbiont. They are taken up from the environmental water in the large plume (Fig. 1), a highly vascularized organ that has a large surface area and brings the hemolymph very close to the surrounding water (13, 14). Most of the metabolite exchanges between the trophosome and the environment are mediated via the vascular system. The hemolymph and the coelomic fluid both contain abundant extracellular hemoglobin, which transport not only oxygen but also sulfide to the bacteria in the trophosome and possibly other metabolites to the different tissues of the worm (9, 13).

The assimilation and respiration of the metabolites such as CO₂, O₂, H₂S, NH₃, NO₃ are mainly carried out in the bacteria, which, in return, provides organic compounds to the worm (15–17). In these processes, the bacteria produces metabolic energy from the oxidation of H₂S (18).

Previous observations indicated that the supply of inorganic carbon and nitrogen from the environment must be a limiting factor for the growth of Riftia. In the case of carbon, the external concentration of CO₂ varies around 5 mM, whereas it was emphasized that the worm metabolism implies a much higher demand for inorganic carbon (19, 20). In the case of nitrogen, the concentrations of nitrate and ammonia around the worm are 15–40 μM and 0.1–0.3 μM, respectively, concentrations that are also considered as limiting for the development of the worm (21). Consequently, the concentration of inorganic carbon and nitrogen must be limiting inside Riftia (19, 20), a situation that suggests the presence of very efficient mechanisms for carbon and nitrogen assimilation. Analyses with radiolabeled carbon dioxide confirmed that the symbiotic bacteria is the primary up-taker of inorganic carbon (22). Although the bacterial symbiont was shown to be able to reduce nitrate to nitrite (17, 23, 24), the mechanisms by which the worm assimilates and metabolizes nitrogen from its inorganic sources are much less well documented. However, ammonia assimilatory enzymes such as glutamine synthetase and glutamate dehydrogenase were found to be present in both the bacterial symbiont and the host (17, 23).

It was previously reported that the carbamoylphosphate syn-
thetase specific of the pyrimidine pathway (CPSase-P) is absent in all of the worm tissues and is present only in the bacteria (17). In contrast, the carbamylphosphate synthetase specific of the arginine biosynthetic pathway (CPSase-A) was found in all of the tissues of the worm (25). This enzyme catalyzes the production of carbamoylphosphate from \( \text{NH}_3 \), bicarbonate, and ATP.

\[
\text{NH}_3 + \text{HCO}_3^- + 2\text{ATP} \rightarrow \text{Carbamylphosphate} + 2\text{ADP}
\]

**Reaction 1**

Thus, this enzyme can participate in the assimilation of inorganic carbon and nitrogen. Consequently, we investigated the distribution in *Riftia* of enzymes involved in the arginine biosynthetic pathway as well as some enzymes involved in the metabolism of this amino acid.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Sodium \([^{14}\text{C}]\)bicarbonate (55 mCi/mmol), \( \text{L-[^{1-14}\text{C}]ornithine} \) (56 mCi/mmol), and \( \text{L-[^{1-14}\text{C}]arginine} \) (55 mCi/mmol) were purchased from Sigma. \( \text{L-[^{U-14}\text{C}]ornithine} \) (262 mCi/mmol) was purchased from PerkinElmer Life Sciences.

**Source and Storage of Riftia Pachyptila Samples**—Samples of the deep sea tube worm were collected in the East Pacific volcanic range at a depth of 2600 m. To avoid interference with the subsequent enzyme tests, the specimens were immediately bled and dissected on board, and each isolated organ was frozen in liquid nitrogen as previously described (25).

**Purification of the Bacterial Symbiont**—After collecting and bleeding the animal, the bacterial symbiont was immediately purified by the method proposed by Distel and Felbeck (26) under conditions previously described (25).

**Cell-free Extracts from Each Organ of R. pachyptila**—Extracts from the organs were freshly prepared before the enzyme assays. Frozen tissue (2–2 g) was suspended in 6 ml of ice-cold extraction buffer (30 mm Tris-HCl, pH 7.8, 10 mm \( \text{NaCl} \), 10 mm \( \text{KCl} \), 1 mm \( \text{dithiothreitol} \), 5% (v/v) glycerol, 30% (v/v) ethylene glycol, and 4 \( \mu \text{M} \) sodium cacodylate), and the following protease inhibitors were added: 30 \( \mu \text{g/ml} \) phenylmethylsulfonyl fluoride, 0.3 mg/ml EDTA, 0.7 \( \mu \text{g/ml} \) peptatin A, and 0.5 \( \mu \text{g/ml} \) leupeptin. The mixture was homogenized in a Potter homogenizer with a Teflon pestle. The homogenate was further disrupted by sonication three times for 60 s each with a Bionikon III sonicator at 20 kilocycles/s. The homogenate was then centrifuged at 10,000 \( \times \text{g} \) for 20 min and dialyzed against extraction buffer, and the resulting supernatant was used for enzyme assays.

**Carbamyl Phosphate Synthetase Assay**—The activity of CPSase was determined by the radioactive method (27) under the standard conditions described previously by Simon et al. (25).

**Ornithine Transcarbamylase Assay**—The activity was measured by the radioactive test (28) using \( \text{L-[^{U-14}\text{C}]ornithine} \). The reaction was performed in 50 mm Tris-HCl (pH 8.0), 5 mm \( \text{L-ornithine} \), 10 mm carbamyl phosphate at 37 °C for 20 min.

**Argininosuccinate Synthetase Assay**—Enzyme activity for this reaction was determined at 37 °C using a coupled-enzymes assay, monitoring the decrease in absorbance at 340 nm that accompanied the conversion of NADH to NAD+ (29). The reaction mixture contained 100 mm Tris-HCl, pH 7.5, 1 mm ATP, 5 mm \( \text{MgCl}_2 \), 2 mm KCl, 20 mm phosphoenolpyruvate, 5 mm \( \text{L-citrulline} \), 0.1 mm NADH, 2 units inorganic pyrophosphatase (Sigma), 2 units of lactic dehydrogenase (Sigma), 2 units of pyruvate kinase (Sigma), and 2 units of adenylate kinase (Sigma), and 50–100 \( \mu \text{l} \) of the extract (0.5–1.5 mg of protein). The reaction was initiated by the addition of 5 mm \( \text{L-aspartate} \). The final volume was adjusted to 1 ml with distilled water. Control cuvettes contained 1 ml of the same reaction mixture without the addition of \( \text{L-aspartate} \).

**Arginine Deiminase Assay**—Arginine deiminase activity was assayed by coupling the production of ammonia with the glutamate dehydrogenase reaction (30). The rate of NADH disappearance was measured at 25 °C in 1 ml containing 10 mm \( \text{o-ketoglutarate} \), 0.2 mm NADH, 50 mm TES buffer, pH 7.2, 0.1% Triton X-100, 1 mg of glutamate dehydrogenase (Sigma, type II), and extract of protein (0.5–1.5 mg of protein). The addition of arginine (the final substrate concentration was 0.6 mm) initiated the reaction. Initial velocities were calculated from recording at 340 nm.

**Ornithine Decarboxylase Assay**—The ornithine decarboxylase activity was determined by an adaptation of the method of Clark (31). The reaction was carried out at 37 °C for 1 h in a scintillation vial containing an Eppendorf tube with 1 ml of hyamine hydroxide (ICN) for the absorption of \( ^{14}\text{CO}_2 \) released from \( \text{L-[^{1-14}\text{C}]ornithine} \). The reaction mixture (1 ml) contained 100 mm sodium phosphate, pH 6.5, 0.2 mm pyridoxal phosphate, 2.5 mm \( \text{L-dithiothreitol} \), 2 mm \( \text{L-[^{1-14}\text{C}]ornithine} \). The reaction was initiated by the addition of the cell-free protein extract. A control reaction was performed in the absence of protein extract. The reaction was stopped by the addition of 0.4 ml of 0.5 M \( \text{H}_2\text{SO}_4 \), and the release of \( ^{14}\text{CO}_2 \) was measured as described by Fox (32).

**Arginine Decarboxylase Assay**—The activity of arginine decarboxylase was measured as for ornithine decarboxylase by an adaptation of the method of Graham et al. (33). The standard reaction mixture contained 100 mm sodium phosphate, pH 6.5, 0.2 mm pyridoxal phosphate, 1 mm \( \text{L-dithiothreitol} \), 0.5 mm EDTA, 1 mm \( \text{L-[^{1-14}\text{C}]arginine} \). The reaction was initiated by the addition of the protein extract, and the mixture was incubated at 37 °C for 1 h.

**Size Exclusion Chromatography**—Soluble protein extract (10 mg/200 \( \mu \text{l} \) ) was injected into a column of Superdex 200 precalibrated with the following molecular mass markers: thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), ovalbumin (44 kDa), and cytochrome c (12.4 kDa). Equilibration and elution were performed with 25 mm Tris-HCl, pH 7.8, and 0.1 mm NaCl. One-milliliter fractions were collected and assayed for OTCase activity.

**Anion Exchange Chromatography**—A cell-free extract (10 mg/500 \( \mu \text{l} \) ) was prepared as described above, was loaded on a DEAE-Sephrose anion exchange column (1.5 × 4 cm; Sigma). Proteins were then eluted with 25 mm Tris-HCl, pH 7.8, first alone and then in a 0.0–0.5 M NaCl discontinuous gradient. One-milliliter fractions were collected and assayed for OTCase activity. Fractions from each peak of OTCase activity were pooled and used for the kinetic determinations.

**Protein Measurements**—Protein concentrations were determined by the method of Lowry et al. (34) using bovine serum albumin dissolved in extraction buffer as the standard.
Arginine Catabolism via the Catabolic OTCase of the Arginine Deiminase Pathway

There are two types of OTCases, which participate in either the anabolism or the catabolism of arginine. The anabolic OTCase belongs to the biosynthetic arginine pathway and catalyzes citrulline formation from ornithine (35). A number of prokaryotes possess also a catabolic OTCase, which belongs to the arginine-deiminase catabolic pathway, leading to the aerobic degradation of arginine to produce NH₃, CO₂, and ATP (30, 35–37). In this pathway, OTCase catalyzes the transformation of citrulline to ornithine. In view of the above remark concerning the limiting supply of NH₃ and CO₂ to Riftia from the environment, this arginine catabolic pathway could constitute an interesting source of these inorganic metabolites. Consequently, the characteristics of the OTCase activities present in the bacteria-containing trophosome and, as a control, in the vestimentum extracts were examined.

Chromatographic Analysis of OTCase from the Trophosome and Vestimentum Extracts

For the estimation of OTCase molecular weights and to test for the presence of putative different enzyme forms, the cell-free extracts of the trophosomal and vestimentum tissues were analyzed by size exclusion chromatography on a Superdex 200 column. The results obtained are given in Fig. 2. In both the vestimentum and the trophosome tissues, a single peak of activity is observed (Fig. 2A). The calibration of the column indicates molecular masses of 170 ± 10 and 95 ± 10 kDa for the OTCases from vestimentum and trophosome, respectively (Fig. 2B). A survey of previously characterized anabolic OTCases showed that most of these enzymes have low molecular weights, ranging from 110 to 150 kDa (38). Catabolic enzymes, with some rare exceptions, display much higher molecular weights. Thus, the result obtained in the case of the trophosomal OTCase suggested already that this enzyme is anabolic.

In order to precisely determine the nature of this OTCase activity, the trophosome extract was analyzed by DEAE-Sepharose ion exchange chromatography. The result obtained is presented in Fig. 3, which shows clearly the separation of two
different forms of this enzyme. The presence of these two enzymatic activities raises several questions. They could simply correspond to the anabolic OTCases from the worm and the bacteria. Alternatively, one of these activities could correspond to a catabolic form of this enzyme. In order to test these possibilities, some kinetic parameters of these two OTCase forms were further characterized.

**Substrate Saturation Curves**—The OTCase substrate saturation curve for carbamyl phosphate was determined in the presence of a saturating concentration of ornithine, and its substrate saturation curve for ornithine was established in the presence of a saturating concentration of carbamyl phosphate. The results obtained are shown in Fig. 4, and the corresponding kinetic parameters are summarized in Table II. Due to the fact that some OTCases can be differentiated on the basis of some apparent substrate cooperativity (25, 39, 40), these parameters were calculated on the basis of the Hill equation. These analyses give $S_{0.5}$ values of 1.1 ± 0.1 mM for ornithine, in the cases of both peak I and II, whereas the Hill coefficient is 1.6 ± 0.2. Thus, on this basis, the peak I and peak II activities do not differ. These low values of Hill coefficient and the values of $S_{0.5}$ are similar to those found in the case of anabolic OTCases from other sources (41–44) but also for some catabolic OTCases (36, 45). As far as the carbamyl phosphate saturation curves are concerned, $S_{0.5}$ and Hill coefficients are identical for the two peaks of activity (Table II). Literature shows that catabolic OTCases display significantly higher values of $S_{0.5}$ and Hill coefficients than anabolic OTCases (46–48). For instance, the $S_{0.5}$ of the catabolic OTCase from *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* are 38 and 4 mM, respectively, and their Hill coefficients are 5.7 ± 0.1 and 4.0 ± 0.2, respectively (49–51). Again, the values obtained for the trophosomal OTCase forms, $S_{0.5}$ of 2.18 ± 0.10 and 2.04 ± 0.11 mM and Hill coefficients of 1.4 ± 0.1 and 1.2 ± 0.1, suggest the anabolic nature of these enzymes.

**Influence of Various Metabolites**—Previous studies of catabolic OTCases showed that these enzymes bear distinct sites for substrates, activators, and inhibitors (46, 49, 51, 52). In order to further characterize the type of OTCases present in the trophosome, their activities were measured in the presence of various compounds shown to have inhibitory or activatory effects on these catabolic enzymes. Phosphate is an activator of the catabolic OTCase but not of the anabolic OTCase (46, 49). All catabolic OTCase forms tested previously were shown to be inhibited by agmatine and putrescine, whereas anabolic OTCases are insensitive to these compounds (46, 49). In addition, catabolic OTCases are activated by ATP (46, 49). In contrast, norvaline is known as a strong competitive inhibitor of both anabolic and catabolic OTCases (52) and was used here as a control. The results obtained are shown in Table III, where it can be seen that none of these compounds has a regulatory influence on the OTCase activities of peak I and II, with the exception of norvaline, which is an inhibitor of both activities. These results are also indicative of the anabolic nature of these two OTCase activities.

**Lack of Arginine Deiminase Activity**

In order to confirm the lack of the arginine deiminase pathway, the activity of arginine deiminase was tested in dialyzed cell-free extracts of different tissues of *Riftia* (trophosome, ves-
Arginine Metabolism in R. pachyptila

The CPSase activity was measured using the pooled fractions of peak I and peak II, respectively, coming from DEAE-Sepharose chromatography of the trophosome extract (Fig. 3) under the standard conditions.

| Effector | Concentration | Relative activity |
|----------|---------------|------------------|
|          |               | Peak I | Peak II |
| Phosphate | 5             | 93    | 99    |
| Phosphate | 10            | 85    | 91    |
| ATP       | 5             | 100   | 99    |
| ATP       | 10            | 101   | 93    |
| Agmatine  | 5             | 98    | 103   |
| Agmatine  | 10            | 107   | 99    |
| Putrescine| 5             | 101   | 97    |
| Putrescine| 10            | 108   | 98    |
| Norvaline | 0.5           | 33    | 42    |
| Norvaline | 1             | 14    | 24    |
| Norvaline | 2             | 5     | 12    |

The CPSase activity was measured using the pooled fractions of peak I and peak II, respectively, coming from DEAE-Sepharose chromatography of the trophosome extract (Fig. 3) under the standard conditions.

**Arginine Catabolism via the Arginine and Ornithine Decarboxylases**

Although *R. pachyptila* and its endosymbiont appear not to possess the enzymes of the arginine deiminase pathway, there exist several other routes for the catabolism of this amino acid. Among them, arginine decarboxylase (ADase) and ornithine decarboxylase (ODase) can play an important role leading to the synthesis of putrescine, precursor of polyamines. Besides their important physiological role, polyamines can be degraded and constitute an alternative source of inorganic carbon and nitrogen (51, 53). Consequently, the existence and distribution of ADase and ODase were investigated in *Riftia* and its bacterial endosymbiont. The results obtained are shown in Table IV. It appears that ADase and ODase are present only in the trophosome, the symbiont-harboring tissue, and in the isolated bacteria. The specific activities of these enzymes are higher in the isolated bacteria than in the trophosome. These results indicate unambiguously that these enzymes are present only in the bacteria.

**Temperature Dependence of the CPSase and OTCase Activities**

*R. pachyptila* is an organism that lives in an environment characterized by important temperature variations. In order to test whether CPSase and OTCase from this organism are adapted to the transient increases of temperature of their environment, the dependence of their activities on temperature was examined. Due to the instability of CPSase, its temperature dependence was determined using dialyzed cell-free extracts of trophosome and vestimentum, whereas the effect of this parameter on OTCase activity was tested after 5 times purification (350 nmol/min/mg protein) by chromatography on a Superdex 200 column. The results of these experiments are shown in Fig. 5.

The CPSase temperature/activity profile is significantly different in the trophosome and in the vestimentum. This is most probably due to the fact that the vestimentum contains only the CPSase-A, whereas the trophosome contains both CPSase-A and CPSase-P. The apparent optimum temperature for OTCase is 45 °C in both the vestimentum and the trophosome extracts. These results indicated that *Riftia* CPSase and OTCase do not show a particular adaptation to high temperature, contrary to what is observed in some microorganisms living in the same environment (54, 55).

**DISCUSSION**

In this study, we have investigated the distribution and properties of some enzymes involved in the biosynthesis and catabolism of arginine in *R. pachyptila*. Fig. 6 shows the scheme that summarizes the results of this investigation and emphasizes the particular organization of arginine metabolism in *R. pachyptila* and its endosymbiont.

The first three enzymes involved in the arginine biosynthetic pathway (CPSase, OTCase, and argininosuccinate synthetase) are present in both the host and the bacteria. The CPSase (ammonia-dependent) that uses ATP to catalyze the conversion of the inorganic molecules HCO3 and NH3 into carbamyl phosphate initiates the arginine biosynthesis. The existence of the enzymatic equipment for this biosynthesis in all the tissues of *Riftia* indicates that these tissues might assimilate inorganic nitrogen and carbon through this process. It also suggests that arginine is a nonessential amino acid for *Riftia*. In this way, although the symbiont is the primary site of carbon and nitrogen fixation, the host tissues participate in this process.

Carbon dioxide is the form of inorganic carbon that is fixed by the bacterial enzyme Rubisco, as the first step in the autotrophic assimilation (7, 8, 12, 56). Dissolved carbon dioxide is in equilibrium with bicarbonate through the activity of carbonic anhydrase. In *R. pachyptila*, this enzyme is probably of host origin, since it shows similarity with enzymes from mammalian sources (57, 58). Bicarbonate is then further assimilated by CPSase.

Ammonia, the other inorganic substrate of CPSase, can be supplied either directly from the medium or from the reduction of nitrate into nitrite by nitrate reductase (59) and further reduction to ammonia (17, 23, 60). Previous study has indicated that both the host and the bacteria might assimilate inorganic nitrogen by glutamine synthetase and glutamate dehydrogenase (23). Our results indicate that part of the inorganic nitrogen in form of ammonia can also be assimilated by both the host and the bacteria trough the arginine biosynthetic pathway. This unusual presence of the enzymes of this pathway in all of the tissues of *R. pachyptila* might contribute to its adaptation to the hydrothermal vent extreme environment.

The arginine deiminase pathway could have been of interest in the frame of the symbiosis. The final products of this pathway are CO2, NH3, and ATP, metabolites that are of prime importance for *Riftia*. However, the absence of catabolic OTCase and arginine deiminase indicates that neither the worm nor the bacteria possesses this catabolic pathway.

In contrast, it was shown that arginase and urease are present in all of the tissues of *Riftia*, including the trophosome (60). Accordingly, one observes high concentrations of ornithine and urea and a low concentration of arginine. Arginine can also
be catabolized through the arginine succinyl pathway, which leads to the production of NH₃, CO₂, glutamate, and succinate. This last metabolite, then, enters the citric acid cycle (61).

A basic metabolic utilization of arginine and of its derivate ornithine is the synthesis of polyamines through the production of agmatine and putrescine by ADase and ODase. In all living organisms, including viruses, polyamines play key roles in the biosynthesis and structure of nucleic acids and are reported to be involved in many biological processes such as membrane stability, growth, and development (62). Our study shows that ADase and ODase are present only in the bacterial endosymbiont. The absence of these enzymes that initiate the biosynthesis of polyamines in the host tissues strongly suggests that Riftia is dependent on the bacteria for this pathway. The bacterial production of agmatine and putrescine in the trophosome would be followed by transport of these polyamines to the other tissues of the worm. Agmatine, putrescine, and polyamine transport systems were described in many organisms (63–65).

The degradation of these polyamines can result in an additional source of carbon and nitrogen for the worm (66, 67). As reported above the worm does not possess any ODase or ADase activity. This absence has been reported also in the case of human and animal filarial worm parasites, Dirofilaria immitis, Brugia patei, and Litomosoides (68). In a similar way, we previously reported that in Riftia the first three enzymes of the pyrimidine nucleotide biosynthetic pathway are present only in the bacteria but not in the worm (17, 69). The absence of these enzymes is also characteristic of protozoan parasites such as Giardia lamblia, Trichomonas vaginalis, and Tritrichomonas fetus. Thus, it appears that Riftia has developed a metabolism for the biosynthesis of pyrimidines and polyamines that is reminiscent of what is observed in some parasites, suggesting some similarity in the adaptation of metabolic pathways in symbiosis and parasitism.

Acknowledgments—We are indebted to the skillful and enthusiastic crews of the oceanographic ship Atlante and of the submarine Nautile of Institut Français de Recherche et d’Exploitation des Mers.
REFERENCES

1. Gaill, F. (1993) FASEB J. 7, 558–565
2. Tunnell, V. (1991) Oceanogr. Mar. Biol. Ann. Rev. 29, 319–407
3. Tunnell, V. (1992) Am. Sci. 80, 334–349
4. Jones, M. L. (1981) Proc. Biol. Soc. Wash. 94, 1295–1313
5. Childress, J. J., and Fisher, C. R. (1992) Oceanogr. Mar. Biol. 30, 109–173
6. Cavanaugh, C. M., Gardiner, S. L., Jons, M. L., Jannasch, H. W., and Waterbury, J. B. (1981) Science 213, 340–342
7. Felbeck, H., Somero, G. N., and Childress, J. J. (1981) Nature 293, 291–293
8. Felbeck, H. (1981) Science 213, 336–338
9. Childress, J. J., Arp, A. J., and Fisher, C. R. (1984) Mar. Biol. (Berl.) 83, 109–124
10. Powell, M. A., and Somero, G. N. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 2250–2254
11. Lee, S., Shen, W. H., Miller, A. W., and Kus, L. C. (1990) J. Mol. Biol. 211, 255–269
12. Stalon, V., Leprince, C., and Wiame, J. M. (1977) Eur. J. Biochem. 74, 319–327
13. Joseph, R. L., Baldwin, E., and Watts, D. C. (1963) Biochem. J. 70, 409–416
14. Eisenstein, E., Osborne, J. C., Jr., Chaiken, I. M., and Hensley, P. (1984) Proc. Natl. Acad. Sci. USA 81, 109–112
15. Arp, A. J., and Childress, J. J. (1981) J. Biol. Chem. 256, 13540–13547
16. Felbeck, H., and Childress, J. J. (1988) Microbiol. Rev. 52, 7–59
17. Minic, Z., Cunin, R., Glansdorff, N., and Wiame, J. M. (1986) J. Bacteriol. 165, 5–16
18. Nelson, D. C., and Fisher, C. R. (1995) in The Microbiology of Deep-sea Hydrothermal Vents (Karl, D. M., ed) pp. 125–167, CRC Press, Inc., Boca Raton, FL
19. Childress, J. J., Lee, R. W., Sanders, N. K., Felbeck, H., Oros, D., Toulmond, A., Desbruyeres, D., Kennicutt, M. C., III, and Brooks, J. M. (1995) Nature 362, 147–149
20. Scott, K. M., Fisher, C. R., Vodenichar, J. S., Nix, E. R., and Minnich, E. (1994) Antonie Leeuwenhoek 76, 617–638
21. Lee, R. W., and Childress, J. J. (1996) Biol. Bull. 190, 367–372
22. Bright, M., Keckeis, H., and Fisher, C. R. (2000) Bull. Biol. Soc. Wash. 6, 289–300
23. Arp, A. J., and Childress, J. J. (1981) Science 213, 342–344
24. Hentschel, U., and Felbeck, H. (1993) J. Bacteriol. 171, 274–290
25. Simon, V., Purcarea, C., Sun, K., Joseph, J., Frebourg, G., Lechaire, J. P., Gail, F., and Herve, G. (2001) J. Bacteriol. 173, 2977–2978
26. Nelson, D. C., and Fisher, C. R. (1995) in The Microbiology of Deep-sea Hydrothermal Vents (Karl, D. M., ed) pp. 125–167, CRC Press, Inc., Boca Raton, FL
27. Robin, J. P., Penverne, B., and Herve, G. (1989) Eur. J. Biochem. 183, 519–528
28. Abadell, A. T. H., Kennedy, E. H., and Nainan, O. (1977) J. Bacteriol. 129, 1387–1396
29. Rycharsky, O., Kodowaki, H., and Ratner, S. (1977) J. Biol. Chem. 252, 5267–5280
30. Weckmann, J. L., and Fahney, D. E. (1977) J. Biol. Chem. 252, 2615–2620
31. Clark, J. L. (1976) Anal. Biochem. 74, 329–336
32. Fox, R. M. (1971) Anal. Biochem. 41, 578–589
33. Graham, D. E., Xu, H., and White, R. H. (2002) J. Biol. Chem. 277, 23500–23507
34. Lowery, O. H., Roebragh, N., Fonn, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275
35. Cunin, R., Glansdorff, N., Pierard, A., and Stalon, V. (1986) Microbiol. Rev. 50, 314–352
36. Stalon, V., Ramos, F., Pierard, A., and Wiame, J. M. (1987) Biochem. Biophys. Acta 919, 91–97
37. Vander Wauren, C., Pierard, A., Kley-Raymann, M., and Haas, D. (1984) J. Bacteriol. 160, 928–934
38. Legrain, C., Stalon, V., Noller, J. P., Mercenier, A., Simon, J. P., Broman, K., and Wiame, J. M. (1977) Eur. J. Biochem. 80, 401–409
39. Kus, L. C., Lipsomb, W. N., and Kantrowitz, E. R. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2250–2254
40. Lee, S., Shen, W., Miller, A. W., and Kus, L. C. (1990) J. Mol. Biol. 211, 255–269
41. Stalon, V., Leprince, C., and Wiame, J. M. (1977) Eur. J. Biochem. 74, 319–327
42. Joseph, R. L., Baldwin, E., and Watts, D. C. (1963) Biochem. J. 70, 409–416
43. Eisenstein, E., Osborne, J. C., Jr., Chaiken, I. M., and Hensley, P. (1984) Proc. Natl. Acad. Sci. USA 81, 109–112