Chapter 4
Meiofauna Meets Microbes—Chemosynthetic Symbioses

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Abstract Nutritional symbioses of meiofauna with chemosynthetic bacteria occur across the globe, from deep-sea vents and seeps to shallow water sediments. The bacteria provide nutrition to their hosts, and the hosts provide both habitat and the efficient bridging of long redox gradients. In this chapter, we summarize our current understanding of these intricate symbioses, identify knowledge gaps and point out future-oriented research directions in this expanding field. The peak species diversity of meiobenthic hosts of chemosynthetic bacteria is found in shallow water sediments towards the tropics, however in only a few higher taxa, including ciliates, platyhelminths, nematodes and oligochaetes. The degree of association ranges from ectosymbioses, subcuticular endosymbioses to intracellular endosymbioses. Independent of the association type, several modes of nutritional transfer have been documented, even a transfer of nutrients via outer membrane vesicles. The mode of symbiont transmission is independent of association type or nutrient transfer. It can be strictly vertical or a mixed mode depending on the host group, but largely remains unknown. The symbiotic life style has profound influences on morphology and functions in both partners. The mouth and several other key structures related to food uptake or excretion are reduced in members of all host phyla. Several bacterial partners exhibit a strongly modified cell biology with longitudinal division as an adaptation to secure contact with the host. The host immune system, responsible for establishment and maintenance of the symbiotic association, appears highly specific and except for the oligochaetes, allows only one microbial partner across the host phyla. The receptor and effector molecules that ensure the selective presence of the "right", and the effective defence against the "wrong", microbes appear convergent for both nematodes and oligochaetes. In both hosts, the symbionts appear integrated into the host defence. Diverse carbon and energy sources are exploited and the ability to use small organic molecules as carbon source puts the strict autotrophy of these symbiotic consortia in question. Mixotrophy and even heterotrophy are possible,

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and in addition, anaplerosis seems to play an important role in inorganic carbon acquisition. Among the symbionts, the Gammaproteobacterium *Ca.* Thiosymbion stands out with an extremely broad physiological spectrum that includes nitrogen fixation in some hosts. This flexibility has enabled it to associate with phylogenetically unrelated host groups and adopt all possible life styles, from ectosymbiont to intracellular endosymbiont. Frontiers and challenges of future research in this field include the still unresolved taxonomic diversity of these symbioses, their puzzling evolutionary dynamics, the lack of cultivable representatives, and the unknown scale of their global influence in permeable sediments, one of the largest global habitats.

4.1 Introducing a Special Relation

The living world runs on solar energy: photosynthesis is the dominating process that converts oxidized inorganic carbon into reduced organic carbon compounds as the building blocks of life. However, the greater part of the surface, and even more of the volume, of the biosphere is lightless. Life in the vast volume of water, sediment and crustal rock below the euphotic zone depends on the production by photosynthetic organisms inhabiting ecosystems that receive enough sunlight where a surplus build-up of organic matter can be sustained.

Whilst the existence of alternative ways to reduce inorganic carbon for production of organic matter has been acknowledged for some time, it was considered an insignificant part of global production. The discovery of abundant deep-sea hot vents where microbes utilized reduced compounds to incorporate inorganic carbon renewed the interest in such alternative ways of carbon fixation (see Chap. 2). The main pathway is the oxidation of reduced sulphur compounds, mainly hydrogen sulphide, as an energy-yielding process. Many of the microorganisms involved in this chemolithoautotrophic production live in symbiosis with animals. They provide a plentiful, stable and safe environment for their bacterial partners and in turn are nourished by the microbial production. The ecological and physiological processes that evolved in these symbioses seemed to be restricted to the lightless deep sea.

However, in the wake of the discoveries in the deep ocean, scientists turned their attention to shallow water ecosystems where reduced compounds, such as sulphide are abundant on sheltered sedimentary coasts and in subtidal shelf areas. These habitats revealed an astonishing diversity of animal-microbe symbioses comparable to those in the deep sea (Dubilier et al. 2008; Sogin et al. 2020). Surprisingly, both hosts and symbionts differed from those found in the deep. At deep water vents and seeps the hosts of thiotrophic symbioses are represented by macrofauna, whereas in shallow water, they belong, with the exception of bivalve molluscs, to the meiobenthos, including various taxa of e.g., Ciliata, Nematoda, and Annelida (Fig. 4.1).

Higher taxa that make up the chemosynthetic meiofauna are different from those in the deep sea. Of the four groups included in this chapter, amongst Platyhelminthes and Nematoda so far, no symbiotic representatives have been found around deep-sea hot vents and seeps, although symbiotic nematodes occur in bathyal habitats.

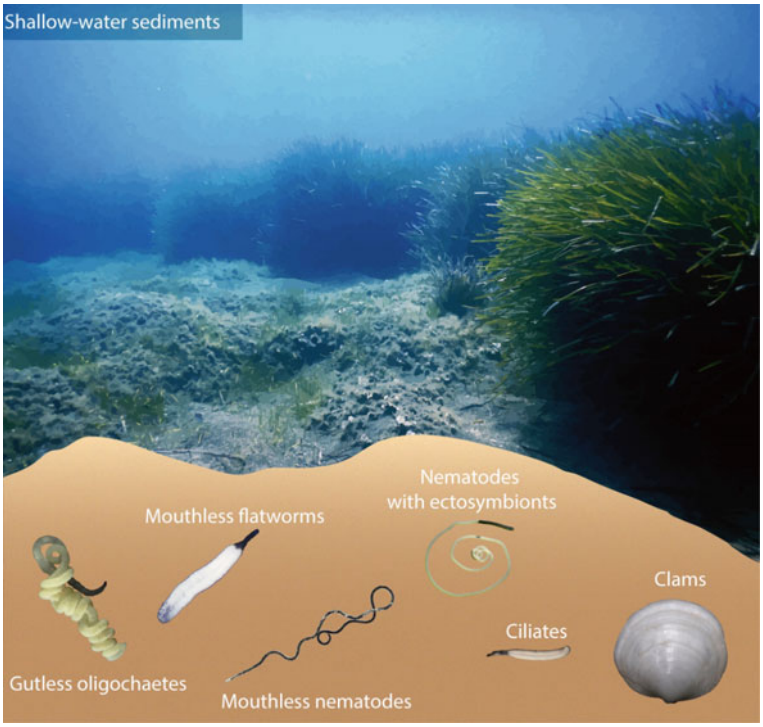

Fig. 4.1 Overview of chemosynthetic meiofauna. Today, a wide range of chemosynthetic organisms live in sediments surrounding seagrass meadows and coral reefs. Many of the taxa are small, like the gutless oligochaetes (*Olavius* and *Inanidrilus*), mouthless flatworms (*Paracatenula*) and nematodes (Stilbonematinae with ectosymbionts, mouthless *Astomonema* with endosymbionts), and single-celled ciliates (*Kentrophoros*). These habitats also support larger fauna, such as clams (lucinids and *Solemya*, latter not shown). Image credits: Seagrass meadow, Y. Sato; *Olavius algarvensis*, A. Gruhl; *Kentrophoros*, B. K. B. Seah; and Stilbonematid, U. Dirks. Modified after Sogin et al. (2020)

Symbiotic Ciliata and Annelida are represented by taxonomic groups that differ from those in deep water.

Complex animal life evolved with the Precambrian oxygenation of the oceans some 850–540 Ma ago, (see Chap. 1). The intimate relationship with bacteria that may have existed a billion years prior to the origin of the eukaryotic cell shaped the evolution of animal diversity to the present day (McFall-Ngai et al. 2013). The oldest chemosynthetic symbioses documented so far are the marine catenulid flatworms (*Paracatenula*) and lucinid mussels, with estimated ages of 500–400 million years (Gruber-Vodicka et al. 2011; Stanley 2014). The fossil lucinids were probably already associated with chemosynthetic symbionts as they are showing imprints of enlarged gills that, in the present relatives, house the symbionts (Stanley 2014). With the evolution of terrestrial vegetation and export into coastal sediments, available carbon and energy sources diversified. A second major organic input was provided by the evolution of seagrasses in the Late Cretaceous Tethys Sea (65–100 Ma). It

should be noted that chemosynthetic production in these shallow water systems is not independent of light and photosynthesis since the energy for inorganic carbon fixation is ultimately derived from bacterial decomposition of organic matter.

Numerous meiofauna organisms have developed mutualistic associations with microbiota enabling the colonization of new niches. This process resulted in the evolution of novel metabolisms and tissue adaptations. In these associations, microbial symbionts colonized meiofauna hosts via various routes. The fact that most multicellular organisms harbour a variety of microorganisms, the microbiome, is already textbook knowledge substantiated by a great number of studies. In meiofauna, however, such studies are rare for those organisms that are not in an obvious symbiotic relationship. A study on the microbiome in marine free-living nematodes (Schuelke et al. 2018) did not find correlations with either geographic location, habitat, feeding type, or phylogenetic position of the host, but on host species level identified putative pathogenic, parasitic, or symbiotic interactions.

4.1.1 Why Study Chemosynthetic Symbioses in Meiofauna?

There are both advantages and drawbacks when choosing meiofauna as the preferred research objects.

Advantages are:

(1) Easily accessible, most known chemosynthetic meiofauna live in shallow water where no costly equipment, ships, robots, submersibles, etc., are needed for sample collection.
(2) All stages of integration of the microbial symbiont into the holobiont are represented: ectosymbiosis, extracorporal to intracellular endosymbiosis.
(3) High diversity, all types and many species may be found together in a few handfuls of sediment, raising the question of niche partitioning/niche diversification.
(4) Sampling is possible without exposing the objects to excessive stress (temperature, pressure) as is the case in deep-sea sampling.
(5) Possibility of keeping the objects alive for extended periods under near-natural conditions.
(6) Ease of experimental manipulation (small size, no high-pressure chambers needed); possibility of work with high numbers of individuals increases statistical power of arising data sets.

Disadvantages are:

(7) Small size/low biomass, but genetic and biochemical techniques become increasingly sensitive.
(8) Identification is often difficult, tedious, requiring microscopical preparations; knowledge of specialists is necessary, risk of misidentification.

(9) Information on biology is scarce due to limitations in observing the objects under (near) natural conditions.

We discuss symbiont transmission and integration, cell biology of symbionts, the immunological basis of symbiont recognition and maintenance, and, finally, the physiology of the holobionts. We highlight recent advances in the study of meiofauna chemosynthetic symbioses. In a final section, we identify challenges in understanding the intricate relationships between eukaryotes and their prokaryotic partners and map frontiers for the advancement of science in meiofauna and general biology. For explanation of terms used see Box 4.1.

Box 4.1 Definitions

Autotroph—an organism capable of synthesizing its own food from inorganic substances using light or chemical energy.
Heterotroph—an organism feeding on sources of organic carbon.
Chemosynthesis—synthesis of organic compounds using energy derived from inorganic chemical reactions.
Chemolithoautotroph—a chemosynthetic organism that obtains energy from the oxidation of inorganic compounds and uses inorganic carbon as sole source of carbon.
Chemolithoheterothroph—a chemosynthetic organism that obtains energy from the oxidation of inorganic compounds and uses organic compounds as a source of carbon.
Ectosymbiont—a partner in a symbiotic relationship that lives on the surface of its host.
Endosymbiont—an organism that lives within the body or cells of another organism.
Holobiont—an assemblage of a (often eukaryotic) host and another (often prokaryotic) species living in or on it, together forming an ecological unit.
Morphospecies—a species whose taxonomic definition is based on morphological characters.

4.2 Ecological Settings

All microorganisms using energy sources alternative to light depend on chemical gradients between electron donors and acceptors. The quantitatively most important of these is the redox gradient from sulphide to sulphate, which provides the highest energetic yield. In sharp gradients over a few millimetres, non-symbiotic microorganisms dominate, some of them with a (limited) capability to move or with other adaptations (e.g., *Beggiatoa*, *Thioploca*, cable bacteria).

Both, highest diversity and numbers of chemosynthetic meiofauna, are found in sediments where the redox gradient stretches over several centimetres. Here, the association with a motile host appears to be a selective advantage for microorganisms despite the tribute they have to pay for the transportation service (Giere et al. 1991; Ott et al. 1991). In some of the sediments containing the most diverse symbiotic meiofauna, sulphide is not detectable in the field and appears only when sediment is kept under stagnant conditions in the laboratory. Here, obviously, production and removal of sulphide by both biotic and abiotic processes, such as percolation of oxic water, is in perfect balance.

Prime habitats for the groups included in this chapter (marine Catenulida, Stilbonematinae, Astomonematinae, and Phallodrilinae) are subtidal sediments. Here, the silt and clay fractions are sufficiently low to allow both interstitial metazoan life and percolation of water through subtidal pumping preventing stagnation and sulphide build-up. There are, though, a few reports of Stilbonematinae and Astomonematinae from deeper shelf water (Ansari et al. 2016; Ingole et al. 2010) or in continental slope canyons (Leduc 2013; Tchesunov et al. 2012).

Highest abundance and diversity are found in tropical to warm-temperate climates. Especially rich are back-reef sediments where locally produced sand often has a coarser grain size than the hydrodynamic situation would predict, while at the same time the organic fraction is high. There is some evidence that the sediment near and within seagrass beds supports a more diverse and abundant meiofauna with thiotrophic symbioses than bare sediments do. Seagrasses provide shallow water sediments with both fresh and decaying organic material, available to fuel chemosynthesis via remineralization. Decaying seagrass might e.g., be the source for CO and H_2, and by stabilizing the habitat it also enhances development of chemical gradients. Seagrass material incorporated into sediments increases the surface area of the redoxcline in a three-dimensional way, enhances development of micro-niches and, thus, fosters local diversity. Furthermore, seagrasses often have seasonal dynamics, adding temporal variation to the habitats of many chemosynthetic symbioses. In addition, the root system provides valuable protective habitats for meiofauna like annelids but also macrofauna like the lucinids. All these factors might have contributed to the high diversity of chemosynthetic hosts we encounter today that are often linked to seagrass stands, and to the underlying rampant radiations of chemosynthetic meiofauna in several host groups over the last 100 million years alongside the evolution of seagrasses.

So far, the marine Catenulida and Phallodrilinae have been found predominantly under tropical to warm temperate conditions. Stilbonematinae and Astomonematinae have also been recorded from cold temperate and even subpolar locations, albeit in much lower abundance and diversity than in warmer climates. Also, reports of Stilbonematinae and Astomonematinae in canyons originate from cold deep areas. *Kentrophoros* appears to be ubiquitous in sheltered sulfidic sediments.

All thiotrophic symbionts store large amounts of elemental sulphur and polyhydroxyalkanoates (PHA) in intracellular vesicles causing the hosts to appear bright white in incident light, and facilitating their detection in live samples under low magnification.

4.3 Introduction to the Organisms Included in this Chapter

The hosts in chemosymbiotic meiofauna belong to diverse and unrelated taxa. Here, we present them ordered from the lowest to the highest degree of functional intimacy with their microbial symbionts.

Stilbonematinae (Nematoda, Chromadorea, Desmodorida, Desmodoridae) are a taxon classified presently as a subfamily, comprising 12 genera with approximately 50 species, both numbers which tend to increase. The slender, cylindrical worms are 3 to almost 10 mm long and 30–50 μm in diameter. Except for two monotypic genera from bathyal canyons, all other known species have been reported from intertidal or shallow subtidal sands. A synapomorphic character is the possession of complex glandular sense organs (GSO) that play an important role in host-symbiont recognition and adhesion. Despite their close molecular relationship, they show a large morphological diversity with regard to the structure of the cuticle, the pharynx and especially the arrangement of the coat of ectosymbiotic sulphur-oxidizing Gammaproteobacteria. The symbionts belong to the *Candidatus* genus Thiosymbion and are host-species specific. Mucus-embedded bacteria are attached to the host cuticle and are, therefore, directly exposed to the environment. Worms migrate between oxic and sulfidic layers. For several host species, there is evidence (gut content, stable isotope ratio) that the bacteria constitute all or at least the bulk of the host nutrition.

Kentrophoros (Ciliata, Karyorelictea), a worm-shaped ciliate genus comprising 17 species, is characterized by the lack of an oral apparatus ("mouth"). The ribbon-shaped body has a dense coat of ectosymbiotic sulphur bacteria on one side. The symbiont-bearing surface is non-ciliated, while the other side is covered with somatic kineties. Folding of the symbiont-bearing body surface provides some separation from the environment. *Kentrophoros* consumes its symbionts by direct phagocytosis into digestive vacuoles. The symbiotic Gammaproteobacteria *Ca.* Kentron is chemolithoheterotrophic in contrast to the autotrophic microbial partners in most thiotrophic symbioses.

Gutless oligochaetes/clitellates (Annelida, Clitellata, Tubificidae, Phallodrilinae). This monophyletic taxon comprises over 100 described species worldwide. They are 100–200 μm in diameter and up to 4 cm long. They are found in tropical and subtropical soft sediments with redox gradients, e.g., in mangroves, coral reefs, or seagrass meadows, as well as in coastal upwelling zones. Both, digestive tract and excretory organs, are completely reduced. Between cuticle and epidermis, they contain a species-specific consortium of extracellular symbionts with a gammaproteobacterial sulphur oxidizer *Ca.* Thiosymbion as numerically dominant symbiont

phylotype in all but one species (exception *Inanidrilus exumae*; Bergin et al. 2018). Additional symbiont phylotypes can be other sulphur oxidizers, sulphate-reducing Deltaproteobacteria or Alphaproteobacteria. In at least one species (*Olavius algarvensis*; Dubilier et al. 2001), syntrophic sulphur cycling occurs between gamma- and deltaproteobacterial symbionts. Additionally, spirochaetes with unclear functional roles (heterotrophic, possibly parasitic) can occur. As in Stilbonematinae, the worms migrate between oxic and sulfidic layers (Giere and Langheld 1987). Transmission of bacteria from host to host is apparently vertically. There are indications that the majority of sulphur oxidation takes place under oxic conditions.

Astomonematinae (Nematoda, Chromadorea, Monhysterida, Siphonolaimidae). The subfamily comprises two genera, *Astomonema* and *Parastomonema.* The very slender worms lack a mouth and pharynx. The majority of the body is occupied by large endosymbiotic bacteria, which are located in either the lumen or the cells of a gut rudiment. Like in the Stilbonematinae and the gutless oligochaetes, the symbionts belong to *Ca.* Thiosymbion. Little is known about the ecology of the Astomonematinae. For the type species, *Astomonema jenneri*, an association with the tubes of sediment-dwelling Annelida has been reported.

Paracatenula is a genus of marine catenulid flatworms, lacking a mouth. Except for the anterior-most region (rostrum), the body is filled with a mass of symbiocytes (trophosome) that contain large Alphaproteobacteria packed with sulphur and polyhydroxybutyric acid (PHB) inclusions which constitute the primary energy storage for the holobiont. Transfer of nutrition from symbiont to host is via outer membrane vesicles. The mechanism of infection of new stem cells for trophosome growth is still unclear. Reproduction of the host is mainly by vegetative fission where symbiont transmission is vertical.

4.4 Symbiont Transmission and Physical Integration in Chemosymbiotic Meiofauna

Chemosynthetic symbioses vary widely in terms of quality, specificity, and integration (Dubilier et al. 2008; Sogin et al. 2020). In most cases, the symbionts are the primary source of energy and nutrients for the host. However, further symbiont functions can add to the host's benefit or even constitute the main "currency" in the association. Sulphur-oxidizing symbionts, for example, remove poisonous sulphide allowing their host to live in sulphide-rich habitats without own detoxification mechanisms. While some host taxa have very specific, single symbiont phylotypes, other associations can involve several partners. Chemosymbioses cover a wide range from very low to high stabilities over time, between individuals or between geographical locations. In this section, we explore the physical interaction between the symbiotic partners. Focussing on structural host adaptations, such as specific organs or cells for hosting symbionts, as well as symbiont transmission mechanisms, we compare the

different levels of host-symbiont integration and discuss their significance for both the interactions between the partners and the evolution of their symbioses.

One of the main characteristics of symbiosis is the degree of integration or physical connection between the partners. Commonly, ectosymbioses in which the symbionts are on the surface of the host are juxtaposed with endosymbioses in which symbionts reside within the host body. In the latter case, symbionts occur in specific host organs, tissues or compartments (e.g., body cavities). On a finer scale, the symbiont location can be either intra- or extracellular (e.g., between cells in a tissue or in acellular, fluid-filled compartments). Intracellular symbionts may occur in specialized cells, called bacteriocytes, where they either occur freely in the cytoplasm, or are enclosed by the cell membrane into vesicles or vacuoles, called symbiosomes. Intracellular symbionts can be restricted within their host cell to certain parts of the cytoplasm or associated with specific cell structures, compartments or organelles, such as cytoskeleton, ER, or mitochondria (Fig. 4.2).

Host and symbiont structures forming and mediating the physical interaction between the partners are collectively referred to as the host-symbiont interface. We expect the structure of the host-symbiont interface to both shape and be shaped by the quality and quantity of physiological interactions between the partners. Nutrient transfer, for example, depends on the number, structure, and function of barriers

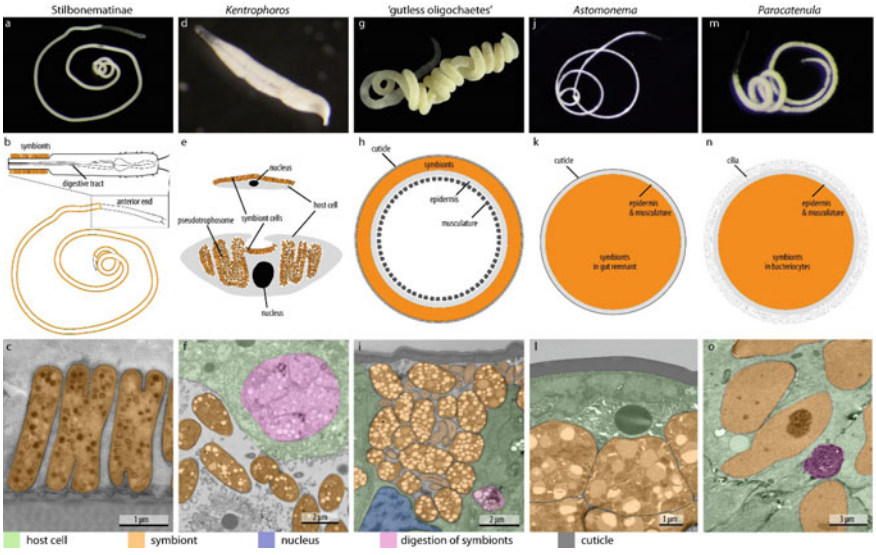

Fig. 4.2 Chemosymbiotic meiofauna and the integration of their symbionts into their body plans. Top row: low magnification micrographs of live holobionts. Middle row: schematic drawings indicating the location of the symbionts on or in the body of the host. Bottom row: False-coloured transmission electron microscopy of the symbionts on or in the host tissues. **a–c** Stilbonematinae; **d–f** *Kentrophoros*; **g–i** 'gutless oligochaetes'; **j–l** *Astomonema*; **m–o** *Paracatenula.* From Sogin et al. 2020, modified and supplemented. Photos courtesy of U. Dirks (Stilbonematid) and B. K. B. Seah (*Kentrophoros*)

(membranes, cell walls, tissue layers) that these substances have to pass on their way between the partners. In many cases, environmental substrates required by the symbionts have to either pass host structures, or be transported by the host, enabling the latter to exert control over the symbionts' access to these substances. Conversely, the location of the symbionts as well as the characteristics of their cell wall and membrane determine, for example, their visibility to the host immune defence.

Finally, animal-microbe symbioses show a wide variety of transmission strategies (Bright and Bulgheresi 2010; Russell 2019). In order to achieve continuity of the symbiotic association over time, the hosts have to either pass on the symbionts directly or evolve other mechanisms that ensure the reliable establishment of a consistent symbiont community after an aposymbiotic life cycle stage.

4.4.1 Host-Symbiont Interfaces and Transmission in the Different Taxa

All members of the nematode subfamily **Stilbonematinae** carry dense coats of coccoid, rod-shaped or filamentous *Ca.* Thiosymbion bacteria on their cuticles (Bayer et al. 2009; Ott et al. 2004; Scharhauser et al. 2020). However, the connection of the symbionts to the host cuticle differs between species. In species of *Stilbonema* and *Leptonemella,* the bacterial coat is multi-layered and the bacteria are embedded in a mucous matrix, the exact composition and origin of which is unclear. It could be both parts of the cuticle and a secretion of the bacterial cells. Monolayered coats are found, for example, in the genera *Laxus*, *Catanema,* and *Robbea.* Within these, the symbionts are typically rod-shaped and attach with one end to the cuticle. A mucous matrix has been shown in some cases. More complex coats of filamentous bacteria are present in the genera *Eubostrichus* and *Adelphus.* Here, the symbionts are attached with either one or both ends and often arrange in regular spiral patterns along the host body. Stilbonematinae have fully functional intestinal tracts and are suspected to feed on their symbionts. The mode of transmission of ectosymbionts in Stilbonematinae has not been demonstrated directly. However, high consistency of host species and associated symbiont phylotypes as well as congruence between symbiont and host phylogenies make a vertical transmission likely (Zimmermann et al. 2016). In Stilbonematinae, stability of the symbiont population does not only have to be achieved across generations, but also across life-cycle stages, as the cuticle is shed four times in the regular moults. The mechanisms of 'inter-' and 'intra-generational' transmission could differ, for example feeding on exuviae after moult versus egg-smearing.

In the ciliate ***Kentrophoros,*** the gammaproteobacterial *Ca.* Kentron symbionts densely cover the dorsal surface of the body. The currently 17 distinguished morphospecies differ in the degree of involution of the dorsal surface, amongst other characters. In species with flat or slightly rolled-up dorsal surfaces, the ectosymbiont coat is monolayered. In some species, the involuted dorsal surface forms pouches packed

with symbionts (Seah et al. 2020, 2017). The enclosed space, termed "pseudotrophosome", however, still communicates with the outside via a small pore or slit. Attached symbionts appear to be connected to the host cell membrane (called pellicle in ciliates) and proximally embedded in a mucous matrix (Foissner 1995). *Kentrophoros* has reduced its cytostome (the cellular feeding apparatus) and symbionts are digested by phagocytosis via the entire symbiont-covered surface. Symbiont transmission is not documented, but reproduction of the hosts seems to happen mainly by fission, during which also the symbiont population would simply be distributed to the daughter cells, thus resulting in vertical transmission. Partial incongruence between symbiont and host phylogenies, however, indicates at least occasional horizontal transmission or host-switching (Seah et al. 2017). Thus, we have to assume a mixed mode of transmission. Whether horizontal transmission happens directly between individuals, for example during conjugation, or mainly as environmental uptake is currently unknown.

Gutless oligochaetes harbour their symbiotic bacteria in spaces between the epidermis and the cuticle. The cuticle is secreted by epidermal cells and is connected to protrusions of their apical surfaces via spot-like hemidesmosomes. This 'symbiont space' is formed by connected invaginations and surface extrusions of the epidermal cells. Regularly repeated constrictions of the symbiont space are visible as annuli, a type of secondary segmentation that occurs in a regular pattern of around seven annuli per segment. The composition of the symbiotic consortium differs between body regions. Whereas in the postgenital (trunk) region all symbiont phylotypes are present and intermixed, the symbiont space in the pregenital region (tip) is much narrower and only contains the smaller morphotype symbionts, excluding *Ca*. Thiosymbion. There is evidence that symbionts are regularly digested by phagocytosis (Giere and Langheld 1987). All gutless oligochaetes reproduce exclusively sexually. Despite a high potential for regeneration, fragmentation of worms never results in proliferation. Individuals can regenerate the post-genital trunk region, but tip regeneration only happens in cases where the prostomium or first segment was amputated. As typical clitellates, gutless oligochaetes are hermaphrodites and self-fertilization does not seem to play a role. Sperm transferred during copulation is stored in spermathecae and used to fertilize oocytes during or directly after oviposition. During the reproductive season, gutless oligochaetes develop prominent structures, so-called "genital pads". These are formed by ventrally located epidermal swellings of the genital segments that are filled with abundant symbionts. In most species, the genital pads surround or adjoin the female genital opening so that they rupture and release their contents onto the egg surface. In newly deposited eggs, the symbionts are located in the fluid-filled space of the cocoon, surrounding the embryo. During embryogenesis, they get incorporated into the epidermis once the cuticle forms (for details of symbiont transfer in *Inanidrilus leukodermatus* see Krieger 2000).

In the siphonolaimid nematode genus ***Astomonema***, the buccal region and intestinal tract are reduced and the body (behind the head region) is filled with endosymbiotic bacteria. In those species studied in detail by electron microscopy, different situations have been described. In *A. jenneri* two morphotypes of bacteria

occur, one smaller and one larger type. The symbionts reside intracellularly in cells interpreted as gut rudiment (Ott et al. 1982). Contrarily, in *A. southwardorum* the cells of the single symbiont morphotype are surrounded by a layer of eukaryotic cells interpreted as gut lining (Giere et al. 1995). The modality of nutrient transfer is unresolved as no evidence for phagocytotic digestion has been found. In both *A. southwardorum* and *A. jenneri,* the intestinal cells appear amorphous with very electron-lucent cytoplasm and few organelles. The mode of symbiont transmission is not known for any *Astomonema* species.

The catenulid flatworm ***Paracatenula*** houses intracellular *Ca.* Riegeria symbionts in specialized bacteriocytes in the trunk region of the body, also termed 'trophosome' (Dirks et al. 2011; Ott et al. 1982). The body wall consists of epidermal cells, musculature and neoblasts; large bacteriocytes fill almost the entire inner lumen of the worms (Gruber-Vodicka et al. 2011; Leisch et al. 2011). Each bacteriocyte, which, in turn, is surrounded by a vacuolar membrane, contains numerous symbionts. Interestingly, bacteriocytes themselves do not divide, but are formed, like all differentiated cells in platyhelminths, from dividing pluripotent stem cells, so-called neoblasts. *Ca.* Riegeria symbionts divide within the bacteriocytes, but how the newly formed bacteriocytes are infected is not known. In terms of nutrient transfer, digestion of entire symbionts by the bacteriocytes via phagocytosis seems to play a minor role: phagolysosomal structures in bacteriocytes are very rare compared to other nutritional symbioses in which transfer via phagocytosis is the major pathway (Jäckle et al. 2019). There is also no evidence for transporter-mediated exchange of nutrients. Instead, nutrients are likely transferred via outer membrane vesicles (OMVs) which are abundantly found in the vacuolar spaces that surround the symbionts. Reproduction of the holobionts in *Paracatenula* happens mostly by asexual fission; sexual reproduction has never been documented (Dirks et al. 2012). Fragmentation in the trophosome region results in division of the bacteriocyte population to the daughter animals. Highly congruent co-diversification patterns support strict vertical transmission (Gruber-Vodicka et al. 2011).

4.5 Structure and Function of Host—Symbiont Interfaces

In chemosymbiotic meiofauna, we see a wide range of host-symbiont interfaces. In ectosymbioses, the symbionts are firmly attached to the cuticle (in the case of nematodes) or cell membrane (in the case of ciliates). Based on the ultrastructure, it seems reasonable that this contact is mediated by both partners, i.e., by secretion of glycocalyx by the host, mucus that could come from both partners, and specific cell polarity and surface structures by the symbiont. The symbionts have direct and unrestricted access to environmental substrates from the sediment pore water. Thus, host control of symbiont proliferation can only happen via host behavioural adaptions, harvesting of symbionts, or immunological interaction. Conversely, symbiont secretion products will hardly be efficiently taken up by the host, limiting nutrient transfer pathways in these systems to intra- or extracellular symbiont digestion. In the extracellular endosymbioses, represented here by the gutless oligochaetes and *Astomonema southwardorum*, direct uptake of substrates is still possible, but symbionts are in a slightly more restricted compartment (subcuticular space and gut) where the chemical composition may differ from the surrounding pore water. Intracellular symbionts in *Paracatenula* experience a much higher level of host control. For example, considering that bacteriocytes do not divide, symbiont cell proliferation needs to be restricted. Substrate provisioning happens via the bacteriocyte cytoplasm and is, thus, potentially highly regulated by the host. Conversely, nutrient transfer has been shown to happen via exchange of OMVs, a process that is putatively controlled by the symbionts and not the host (Jäckle et al. 2019).

4.5.1 Symbiosis as a One-Way Street?

So, do the differences in the extent of host-symbiont integration represent adaptations to specific biological conditions or can they be interpreted as stages in an evolutionary series of increasingly higher integration and dependency, culminating in an organelle-like role of the symbionts? Naturally, in each symbiotic system, the partners have co-evolved based on their biological properties and environmental conditions. Thus, a specific degree of integration may be an optimal, evolutionary stable strategy. For example, a certain openness for horizontally acquired symbionts may not indicate an evolutionary young association, but can be an adaptation to unstable conditions or enable the animal to easily move into new habitats and take advantage of locally well-adapted pools of potential symbionts (Russell 2019). Hosts often evolve mechanisms to control symbionts by separating them or confining them to certain cellular or body compartments, a concept termed compartmentalization (Chomicki et al. 2020b). This may help the host to control symbiont reproduction, prevent infection, "punish" or "reward" symbionts based on their performance. However, some mechanistic explanations suggest that a pathway to higher integration and dependence may be a common phenomenon in mutualistic symbioses (Bennett and Moran 2015). For

example, hosts may get locked into an association at some point by having adopted so many changes and losses that they cannot easily revert back. On the symbiont side, reduction of effective population size by strict vertical transmission can lead to genome reduction and accumulation of deleterious mutations, leading to reduced performance outside the host (Fisher et al. 2017).

In the known chemosynthetic meiofauna taxa, the phylogenetic positions of the hosts provide clear evidence that these associations have evolved multiple times independently. However, each taxon (i.e. gutless oligochaetes, Stilbonematinae, Astomonematinae, *Kentrophoros, Paracatenula*) is a well-defined monophylum including only symbiotic species within its higher taxon of non-symbiotic relatives. This shows that in meiofaunal chemosymbioses the hosts, once the association is firmly established, hardly ever revert to a non-symbiotic lifestyle. Also, signs for adaptive radiations are seen in some of the chemosymbiotic meiofaunal taxa, suggesting a strong selective advantage of these symbiotic associations (Seah et al. 2017). Dependence is not always symmetrical between hosts and symbionts. This is shown, for example, in Stilbonematinae and gutless oligochaetes, whose gammaproteobacterial symbionts have repeatedly switched between major host lineages (Zimmermann et al. 2016). An interesting question is whether chemosymbioses are particularly prone to strong dependence phenomena, which might have further implications. For example, highly dependent mutualists are suspected to be less adaptable towards new and fluctuating environmental conditions (Chomicki et al. 2020a), a possible explanation for the rarity or lack of chemosynthetic taxa in cold-temperate or limnic habitats.

4.6 Symbiotic Associations Are a Window into Environmental Bacterial Cell Biology

Symbiotic associations between animals and bacteria face the challenge to coordinate the rapid cell cycle of the bacteria within the cell- and the-life cycle of the eukaryote. Ectosymbionts for example need to ensure that their bacterial offspring stays in contact with the animal host, to continue the symbiotic association. Endosymbionts on the other hand need to be able to cope with the host's immune system and strike the balance of growing without "overrunning" the host, and in the case of chemosynthetic symbioses grow enough to satisfy the metabolic demand of the host. To understand these symbiotic associations, one really needs to understand the bacterial cell biology. Research in this field has shown that sophisticated systems are in place to ensure that bacteria keep their shape and can propagate it to their offspring over generations. Despite the misleading simplicity, multiple molecular systems interact with each other to ensure a coordinated cell cycle. In rod-shaped bacteria like *Escherichia coli*, the cell cycle has two morphologically distinct phases. Initially, the rod-shaped cell elongates, along the whole length of the cell. Key to this is the protein MreB, a homologue of the eukaryotic actin protein. MreB binds to the cytoplasmic face

of the inner membrane and coordinates cell elongation. Its binding behaviour lets MreB "sense" the local curvature of the cell wall and ultimately maintains the rod shape (reviewed in Shi et al. 2018). The cell elongation is followed by the actual septation process. This centres around the bacterial tubulin homologue FtsZ. FtsZ, a GTPase, is the first protein to localize to the future division site where it self-polymerizes into a ring-like structure termed the Z-ring. It recruits approximately 30 more proteins into a macromolecular complex called the divisome, which organizes the cell wall constriction, peptidoglycan synthesis and overall formation of the two new poles, until the two daughter cells are separated (reviewed in McQuillen and Xiao 2020). Research in the last decades has highlighted the complexity of this whole process, but most research was limited to a handful of cultivable model organisms. Symbiotic associations with a low diversity, however, are ideal to gain insights into the cell cycle of uncultivable environmental bacteria.

Among the chemosynthetic associations, nematodes of the sub-family **Stilbonematinae** are the ideal model to study bacterial cell division. The association is highly specific, and each worm species carries a monoculture of a single symbiont on its cuticle (Fig. 4.3) The symbionts are still in contact with the environment and therefore need to cope with both the symbiotic and the free-living aspect simultaneously, and, finally, one can easily remove the symbiont monoculture from its host for experimentation. The symbionts do rely on the host for transport through their habitat and have therefore evolved strategies to ensure that the contact with the host is transmitted to the offspring upon cell division.

The nematode *Eubostrichus fertilis* carries one of the most complex but also aesthetically appealing bacterial coats (Fig. 4.3a, b). Under the microscope, the worm has a rope-like appearance, which is due to the symbionts on its cuticle. The bacterium is crescent-shaped and attaches with both cell poles to the worm's cuticle. However, the bacteria span one order of magnitude in length, ranging from 4 to 45 μm in length. The shortest bacteria are attached closest to the worm and layered on top

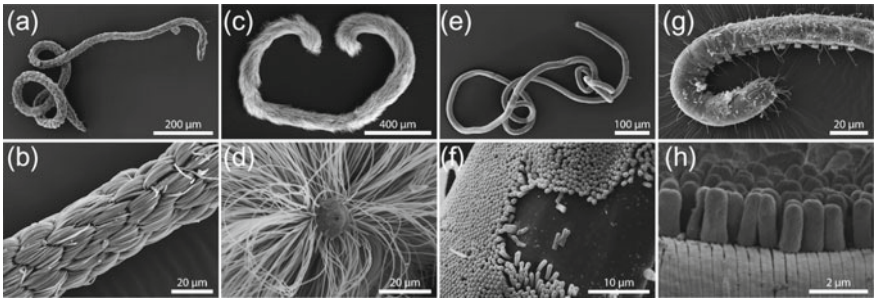

Fig. 4.3 Symbiotic nematodes of the subfamily Stilbonematinae and their ectosymbionts. Overview (**a**) and detail (**b**) of the crescent-shaped bacteria that cover *Eubostrichus fertilis*. The long thin filaments covering *Eubostrichus dianeae* give it a furry appearance (**c**,**d**). The nematode *Laxus oneistus* (**e**) is covered by a monolayer of rod-shaped bacteria (**f**). While the head of *Robbea hypermnestra* (**g**) is symbiont free, the rest of its body is covered with rod-shaped bacteria, arranged in a picket-fence-like manner (**h**)

are longer and longer bacteria (Ott et al. 2014; Pende et al. 2014). Typically, the individual cells of a bacterial population deviate very little from its size-optimum (e.g., *E. coli* approximately 1–4% in length), as the surface-to-volume ratio governs most bacterial processes (reviewed in Young 2010). It is therefore surprising to see such a huge range of cell length within a single *Eubostrichus fertilis* symbiont population. Immunofluorescence marking of the FtsZ protein showed that this population structure is actively maintained, as all cells from 4 to 45 μm length formed Z-rings and underwent cell division (Pende et al. 2014). One explanation for the stark differences in size might be the arrangement itself. The symbiont cells are stacked on top of each other and require reduced sulphur compounds and oxygen from the environment to fuel their metabolism. The topmost cells might simply have better access to these than the bottom ones, therefore growing faster and with this nutrient gradient established, the complex 3D structure is perpetuated further.

The closely related nematode *Eubostrichus dianeae* is similarly covered by long filamentous bacteria, however, they only attach with one pole to the cuticle and grow even longer, up to 120 μm in length (Fig. 4.3c, d). Despite their large cell size, these bacteria are dividing by binary fission (Pende et al. 2014). While bacterial gigantism has been observed in multiple endosymbionts, like nodulating root bacteria, insect symbionts or bacteria inhabiting the surgeonfish gut, these are often under strong host control. Here, cell division is inhibited, resulting in large, polyploid bacteria (Bulgheresi 2016; de Velde et al. 2010; Login et al. 2011; Mendell et al. 2008). As the symbiont still actively divides in a FtsZ-based manner, this makes it not only the longest non-septate bacterial cells that undergo binary fission, but also highlights how the positioning system for the Z-ring can function even in bacteria of extreme length, to reliably find mid-cell. One of the open questions here is how the apically formed daughter cell gets in contact with the host's surface, as, after division, this is far away from the host's cuticle.

The most studied symbiont is that of the nematode *Laxus oneistus* (Fig. 4.3e, f). Based on electron microscopy, Polz et al. (1992) pointed out that the rod-shaped bacteria colonizing this nematode attach with one pole to the host's cuticle where they are arranged like a picket fence. Moreover, they seem to split along their longitudinal axis, instead of transversal like typical rod-shaped bacteria (e.g., *E. coli*). Using a combination of morphometric analyses, transmission electron microscopy and immunofluorescent labelling, Leisch et al. (2012) showed that this symbiont, *Ca.* Thiosymbion oneisti, grows in width instead of length, and the division is mediated by the Z-ring forming at mid-cell, along the length axis.

The arrangement and division mode of the symbiont *Ca.* Thiosymbion hypermnestrae of the co-occurring nematode *Robbea hypermnestra* do look identical at first glance but differ in an important detail (Fig. 4.3g, h). At the basal pole of the symbiont, which attaches to the host cuticle, a patch of FtsZ localizes and initiates cell division earlier than the apical pole, resulting in an asynchronous division. Only later on in the division process, a full Z-ring is formed and cell division will terminate in the upper third of the bacterial cell length (Leisch et al. 2017). Using D-amino acids which are incorporated into the bacterial peptidoglycan layer, and which can be fluorescently labelled, together with immunofluorescent detection of

MreB, Pende et al. (2018) started to dissect the growth mechanisms of these two symbiont species. They showed that MreB is required for septal growth, which starts at the poles, a region typically thought to be inert in model rod-shaped bacteria, and furthermore that growth of new cell wall is mainly in the region of the new septum (Pende et al. 2018). This is in stark contrast to textbook knowledge of model rod-shaped organisms where MreB-based cell elongation occurs along the length of the cell, independently of the FtsZ-driven septal growth. This re-orientation of the division plane not only highlights the flexibility of prokaryotic protein machineries, but it allows both daughter cells to remain in contact with the nematode host throughout the whole division process.

The ectosymbionts of the ciliate ***Kentrophoros*** also show an extraordinary reproduction mode. They are rod-shaped bacteria which attach with one pole to the host. Based on morphological observations, their longitudinal cell division initiates at the distal pole and proceeds unilaterally towards the basal pole (Fenchel and Finlay 1989).

Few insights are available from endosymbionts. For both the symbionts of the mouthless nematode *Astomonema* and the mouthless flatworm *Paracatenula***,** no data are available on growth rates, division strategy or host control. In the case of *Astomonema,* the symbionts are clearly understudied, with the main published work focussing on the phylogenetic identity, their position within the host or the host anatomy (Giere et al. 1995; Musat et al. 2007; Ott et al. 1982; Tchesunov et al. 2012). None of the *Paracatenula* species analysed with electron microscopy showed clear signs of dividing cells (Jäckle et al. 2019; Leisch et al. 2011). However, as the symbiont seems to rely on outer membrane vesicle secretion to supply the host with nutrients, this could be a symbiotic system in which bacterial cell division is under tight host control.

The symbionts of **gutless oligochaetes** seem to be fairly "unconstrained" compared to other endosymbionts. Representing a complex consortium with up to five bacterial types, that occupy the extracellular space between epidermis and cuticle, these endosymbionts show no strictly ordered arrangement like the symbionts of the Stilbonematinae. Not being within cells or cellular compartments, they are exposed to different local micro-niches with varying nutrient supply and bacterial-bacterial interactions. For the main symbiont of the gutless oligochaete *Olavius crassitunicatus,* longitudinal division has been documented, based on transmission electron microscopy (Giere and Krieger 2001).

Methodological improvements in fluorescent imaging, ranging from super-resolution to novel dyes and stains, have rapidly accelerated our understanding of bacterial cell biology and have highlighted the complexity of the processes that control bacterial growth and division. Whilst most of these studies stem from a handful of cultivable model organisms, symbiotic associations have proven ideal to gain broader insights into the cell cycle of uncultivable and environmental bacteria. Most importantly, research on these symbiotic bacteria allows us to evaluate which of the findings that originated from bacterial model organisms are applicable more broadly. The range of biological solutions to the deceptively simple question "How to divide one bacterium into two?" is wide.

4.7 Should I Stay or Should I Go? How Chemosynthetic Bacteria Are Chosen by Their Meiofauna Hosts

Although immunology has so far focussed on pathogenic microbes and on laboratory-reared animals, much can be learned by studying how immune systems cope with beneficial microbes in their natural habitats. In this section, we discuss and compare immune components and mechanisms that likely allow meiofauna to engage in successful relationships with chemosynthetic bacteria (Fig. 4.4). At present, host transcriptomics and proteomics have only been performed for the nematode *L. oneistus* (Bulgheresi 2011; Paredes et al. (2022) and for the oligochaete *O. algarvensis* (Wippler et al. 2016; L. König and Y. Sato, unpublished). This section therefore only reviews the immune systems of these two symbiotic meiofauna worms. Comparing their repertoires to those of the model nematode *Caenorhabditis elegans* and the marine annelid *Capitella capitata*, respectively, allows us to identify putative symbiosis-specific components. Finally, we review immunoreceptors and immune effectors, both of which represent host immunity components that directly interact with microbes. Immune signalling pathways, on the other hand, will not be covered here, because the core set of invertebrate immune signalling pathway components is present in both *L. oneistus* and *O. algarvensis* (Bulgheresi 2011; Paredes et al. (2022); L. König and Y. Sato, unpublished).

4.7.1 Immune Receptors

For microbes to associate with their hosts, microbial signals must first be detected by immunoreceptors. These recognize microbial molecules that are essential for microbes, but are absent in multicellular eukaryotes, such as the cell surface molecules lipopolysaccharide (LPS), peptidoglycan, or flagellin. They can also recognize bacteria-derived molecules, such as signal peptides or short-chain fatty acids. Immune receptors include Toll-like receptors (TLRs), G-protein coupled receptors (GPCRs), peptidoglycan-binding receptor proteins (PGRPs) and C-type lectin receptors (CTLRs). In contrast to the former two classes, PGRPs and CTLRs can also directly control the growth of bacteria and may therefore be considered both, immune receptors and effectors. They can activate immune pathways that lead to bacterial death and, at the same time, they can directly agglutinate and immobilize bacteria (as in the case of CTLRs) or kill bacteria by, for example, hydrolysing their peptidoglycan (as in the case of PGRPs).

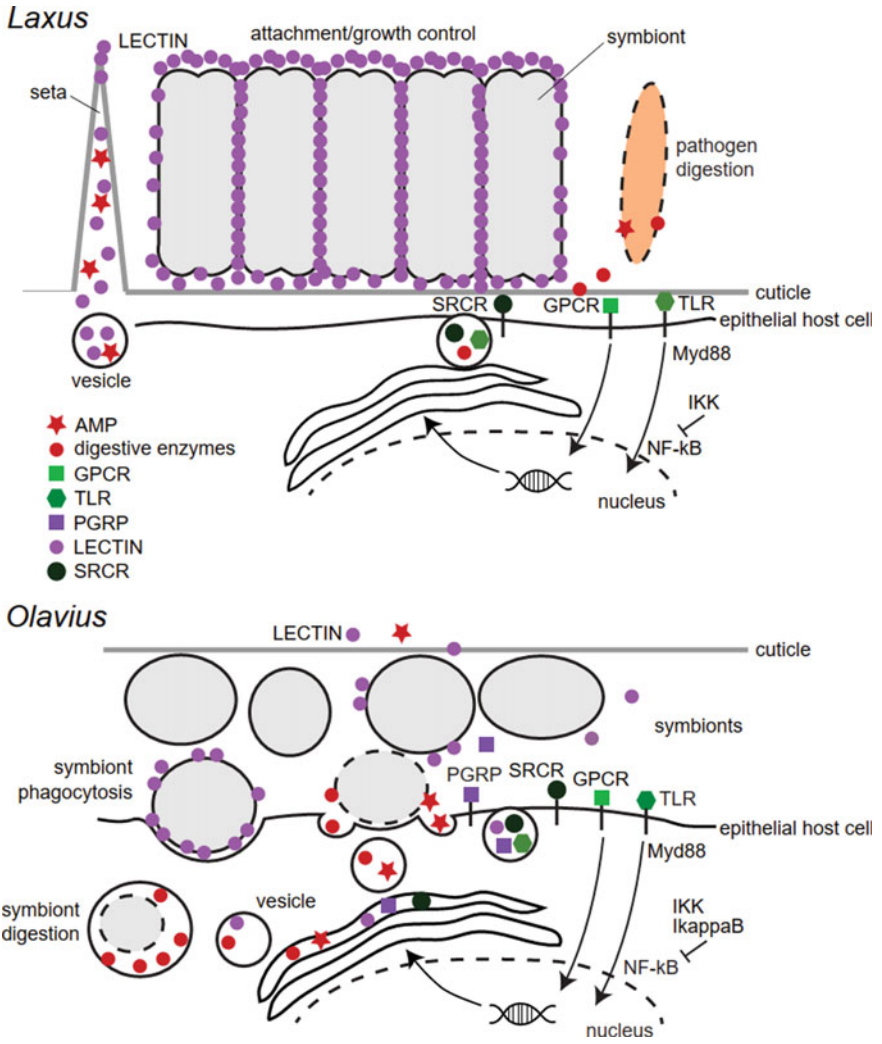

Fig. 4.4 Schematic overview of immune system components present in *Laxus oneistus* and *Olavius algarvensis* transcriptomes. The immune systems of chemosynthetic meiofauna are adapted to maintain their bacterial symbionts (grey) while controlling their symbionts' growth and defending against pathogens. AMP, antimicrobial peptide; ER, endoplasmic reticulum; GPCR, G-protein coupled receptor; LECTIN, C-type lectin receptor; PGRP, peptidoglycan-binding receptor protein; SRCR, scavenger receptor-like cysteine-rich protein; TLR, Toll-like receptor

4.7.2 Toll-Like Receptors (TLRs)

Although functional evidence of the role of TLRs in immunity is only available for model organisms, bacteria are known to modulate the expression of genes encoding

TLR pathway components even in the most basal metazoans, which suggests that microbial recognition is the ancestral function of TLRs.

Canonical, bona fide TLRs are transmembrane receptors with several extracellular leucine-rich repeat (LRR) motifs and an intracellular Toll/interleukin-1 receptor (TIR) domain. The extracellular LRR motifs of TLRs can bind a wide range of microbe-derived signals, but also endogenous ligands derived from damaged cells such as fibronectin (Yu et al. 2010). TLR stimulation ultimately causes the transcription factor NF-kB to enter the nucleus and to switch on the expression of inflammatory antimicrobial peptides (AMPs) or cytokines. In addition to NF-kB signalling, TLR receptors can activate mitogen-activated protein kinase (MAPK) and interferon regulatory factor signalling cascades (Akira et al. 2006; Kawai and Akira 2010).

Initially identified as essential in fruit fly early development (Anderson and Jiirgens 1985), the Toll signalling was later found to protect adult flies from bacterial and fungal pathogens. Curiously, although one Toll homolog (Tol-1) was identified in *C. elegans*, this nematode lacks key proteins of the canonical TLR-signalling cascade including the NF-kB transcription factor (Pujol et al. 2001). Moreover, rather than being required to kill pathogens, *C. elegans tol-1* is necessary for the development of chemosensory neurons that nematodes need to sense and avoid pathogens (Brandt and Ringstad 2015; Pradel et al. 2007).

One bona fide TLR was found to be expressed in *L. oneistus* and two were found in *O. algarvensis* (L. König and Y. Sato, unpublished). In addition to complete TLRs, *L. oneistus* and *O. algarvensis*, encode for a similar number of TIR-only or LRR-only-containing proteins, reported to be related to TLR proteins (Brennan and Gilmore 2018). Therefore, besides their TLRs, it is possible that both symbiotic worms use LRR-containing proteins in combination with other signalling components to interact with microbes.

In neither *L. oneistus* nor *O. algarvensis*, we observed the expansion of the TLR family reported, for example, for humans and for the polychaete *Capitella capitata* (10 TLRs have been identified in our genomes and we confirmed the presence of eight *C. capitata* TLRs out of the 105 previously reported), or the impressive explosion observed in some invertebrates such as sea urchins (Davidson et al. 2008; L. König, unpublished). However, all the key components of the Toll signalling pathway were identified in both *L. oneistus* and *O. algarvensis*, indicating that this pathway is active and may mediate successful host-microbe negotiations (see Wippler et al. 2016).

Concerning what is downstream of the Toll receptors, *L. oneistus* seems to bear a more ancient version of the Toll pathway in comparison to the gutless oligochaete, but a far more complete one when compared to the model nematode *C. elegans*. Most strikingly and in stark contrast to all other nematodes, which notoriously do lack a NF-kB transcription factor, *L. oneistus* encodes one. The presence of a NF-kB1-like protein in the symbiotic nematode suggests that triggering of the Toll pathway might result in the expression of immune effectors, as observed in fruit flies and humans. Whether the presence of this key immune transcription factor enables *Laxus* to wear its symbiont coat awaits to be proven.

4.7.3 G Protein-Coupled Receptors (GPCRs)

GPCRs are central for the perception of external stimuli and the transduction of the signal to the cytoplasm and, therefore, vital for connecting organisms with their environments. GPCRs are characterized by a conserved signature motif consisting of seven transmembrane (TM) spanning helix domains. Upon ligand binding, a conformational change activates the cytoplasmic C-terminal domain, which, in turn, through coupling to heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins), starts the intracellular signalling cascade (de Mendoza et al. 2014; Dierking and Pita 2020). Although there is evidence of the involvement of GPCRs in the immunity of model invertebrates and although, in *C. elegans*, GPCRs present a potential link between the nervous system and immunity, it is as yet unclear if they directly respond to microbes or to microbe-triggered endogenous ligands.

The family of GPCRs represents the largest receptor family in animals. Vertebrate genomes may contain over 1300 GPCRs, whereas in invertebrates, numbers vary unpredictably: from the hundreds of GPCRs found in *Drosophila melanogaster* and sponges to over a thousand in *C. elegans* (Dierking and Pita 2020). As for *L. oneistus* and *O. algarvensis*, 238 and 118 GPCRs were predicted, respectively. In both worms, the largest group of GPCRs are the rhodopsin receptor-like class A GPCRs. Within this GPCR class, both organisms have a relatively high number of FMRFamide receptors (59 in *Laxus* and 20 in *Olavius).* Interestingly, FMRFamide-like receptors have been functionally linked to alterations in microbial pathogen susceptibility in *C. elegans*. In contrast to *Olavius*, *Laxus* also displays an expanded repertoire of neuropeptide Y receptors (NPYR). NPY is found at all levels of the mammalian brain-gut axis and it may control the impact of the gut microbiota on inflammatory processes, pain, brain function and behaviour (Holzer and Farzi 2014). Although the impact of neuropeptides on the gut microbiota-brain interaction awaits elucidation, it is possible that biologically active peptides will emerge as neural and endocrine messengers in orchestrating animal-microbe interactions. Why should FMRFamide-like and Y receptors be more represented in *Laxus* than in gutless oligochaetes? As mentioned in Sec. 4.3 of this chapter, the nematode GSOs are composed of both gland and neuronal cells. Local neuronal regulation of the glandular component of the GSOs might, therefore, allow localized secretion of immune effectors.

4.7.4 Peptidoglycan Receptors (PGRPs)

PGRPs are key innate immunity components known to be involved in many animal-bacteria symbioses, where they mediate symbiont tolerance, control symbiont proliferation or regulate symbiosis establishment and maintenance (Dierking and Pita 2020; Dziarski and Gupta 2018; Royet et al. 2011). PGRP overexpression was observed in the trophosomes of hydrothermal vent tube worms and mussels, but

their function within these deep-sea symbioses remains unknown (Bettencourt et al. 2014).

Transmembrane PGRPs that carry intracellular domains often induce an antimicrobial response by activating immune pathways such as the Toll pathway. However, some PGRP receptors bind peptidoglycan without passing on an intracellular signal which results in down-regulation of immunity. Similar to transmembrane PGRPs, secreted PGRPs can induce an antimicrobial response by indirectly activating immune pathways or acting as bacterial growth inhibitors or antimicrobials themselves (Lu et al. 2006). Notably, if they possess amidase activity, they can also dampen the host immune response by cleaving PG into non-immunogenic fragments.

Although six PGRPs were originally identified in *Olavius* (Wippler et al. 2016), a subsequent round of sequencing, assembly and annotation could only identify three (L. König and Y. Sato, unpublished). One corresponds to OalgPGRP2 (Wippler et al. 2016); it contains a signal peptide, an amidase catalytic site and it is homologous to the symbiont PGRP2 of the squid *Euprymna scolopes*. As for the other two PGRPs, they do have amidase catalytic domains, but their N-terminal PGRP domains are incomplete and transmembrane domains are absent. Because the existence of a signal peptide cannot be ruled out, they can either act as intracellular or secreted amidases. All in all, given that all three confirmed *Olavius* PGRPs could function as amidases, they might contribute to symbiont tolerance by digesting immunogenic peptidoglycan fragments, which are released as a by-product of bacterial growth (Wippler et al. 2016). Moreover, *Olavius* PGRPs may also play a role in symbiont population control and host nutrition by contributing to symbiont digestion. Intriguingly, two of the three recently confirmed *Olavius* PGRPs are diaminopimelic acid (DAP)-specific, i.e., they may specifically target the peptidoglycan of Gram-negative bacteria including, for example, *Ca.* Thiosymbion (Schleifer and Kandler 1972; Swaminathan et al. 2006).

Although *C. capitata* has a similar number of PGRPs, namely four, these are absent from all nematodes including *L. oneistus*. Therefore, if PGRPs are likely involved in mediating the *Olavius* symbiosis, they do not seem to be universally required by meiofauna to establish chemosynthetic symbioses.

4.7.5 C-Type Lectin Domain-Containing Proteins (CTLD-Containing Proteins)

The C-type lectin-like domain family contains secreted, as well as transmembrane proteins that differ regarding their tertiary structures, but all share primary and secondary structural homology in their carbohydrate recognition domain (Cummings and McEver 2009). The first described members of this family indeed bound carbohydrates in a calcium-dependent (C-type) manner, and were thus true lectins. However, the carbohydrate recognition domain was subsequently identified also in proteins

that did not bind carbohydrates, but other ligands such as proteins and lipids, and also did not require calcium for binding. The term C-type lectin-like domain (CTLD) was thus introduced to reflect the structural similarity to the CRD of bona fide C-type lectins without implying common function. CTLD genes occur in all multicellular eukaryotes and they may constitute more or less expanded and diverse gene families: the human genome contains 100 CTLD genes, the *C. elegans* genome 283 and *D. melanogaster* 56 CTLD genes. Based on their transcriptomes, *L. oneistus* encodes for 117 CTLD-containing proteins, 42 of which are predicted to be secreted and *O. algarvensis* for 49, 11 of which may be secreted (Wippler et al. 2016; L. König and Y. Sato, unpublished). Although nothing is known about CTLD-containing protein localization and function in *Olavius*, in the case of the Stilbonematinae *L. oneistus* and *Stilbonema majum*, we showed recombinant Mermaid CTLs to mediate symbiont aggregation and host-symbiont attachment. Furthermore, *L. oneistus* and *S. majum* Mermaids exclusively localized to symbiont-coated regions of the two nematodes and different isoforms bound the two respective symbionts more or less efficiently (Bulgheresi et al. 2011; Bulgheresi et al. 2006). Although our localization and functional studies suggested that Mermaid CTLs may be involved in the recruitment of specific symbionts by *L. oneistus* and *S. majum*, their transcripts were hardly detectable in our adult nematode transcriptomes. One possibility to explain this apparent under-representation of *mermaid* transcripts in adult nematodes is that Mermaid CTLs expression is limited to hatching and moulting (Paredes et al. 2022) stages. Transcriptomics of all nematode developmental stages will tell us whether Mermaids are exclusively expressed when the symbiosis must be established (during hatching) or re-established (during moulting).

4.7.6 Effector Molecules

Given that antimicrobial peptides (AMPs) are generally not conserved, it is not surprising that most species-specific AMPs identified in model invertebrates are absent from both *L. oneistus* and *O. algarvensis*. However, non-species-specific antimicrobial peptides such as saposin-like proteins were expressed in both worms. Additionally, *L. oneistus* encoded for thaumatin-like (*C. elegans*) and macin-like (*Hydra*) putative AMPs.

Concerning lysozymes, *O. algarvensis* only encodes for an invertebrate-type one, whereas *L. oneistus* almost exclusively encoded for lysozyme-like proteins, namely 12, nine of which are secreted. Given that invertebrate-type lysozymes were upregulated upon bacterial infection in *C. elegans*, how could Thiosymbion withstand host lysozymes? Given that Thiosymbion does not appear to encode for lysozyme inhibitors, it might modify its peptidoglycan to make it invulnerable to enzymatic digestion. Intriguingly, *Ca.* T. oneisti peptidoglycan displays a high degree of O-acetylation and cross-linking of its glycan strands (Wang et al. 2021). However, future studies need to clarify whether these two modifications enable the symbiont to escape host lysozyme-mediated lysis.

Bactericidal permeability-increasing proteins (BPIs) are AMPs that are found in vertebrates and invertebrates and play a crucial role in the innate immune response against Gram-negative bacteria (Chen et al. 2017). Indeed, by binding their LPS, they may literally perforate bacterial membranes. While most research focussed on mammalian BPIs, just a handful of studies have been carried out in invertebrate ones. For example, in the squid *Euprymna scolopes* BPI was expressed in the symbiotic (light) organ and showed bactericidal effects against its symbiont *Vibrio fischeri*. This suggests that the squid expresses BPIs to control the size of the symbiont population (Chen et al. 2017). A total of 16 bona fide BPIs were found to be expressed by *L. oneistus*, but a single one was identified in *O. algarvensis* (König and Sato, unpublished). *Laxus* BPIs are likely secreted from the GSOs onto the nematode cuticle throughout the nematode anteroposterior axis (Bauer 2012). Particularly in the symbiotic region of the cuticle, BPIs co-localized with and embedded in *Ca.* T. oneisti. Obviously, this symbiont is not harmed by these broadband antibiotics, however, more studies are necessary to prove that the *Laxus* BPIs contribute to symbiosis specificity, i.e., that they select out environmental, non-symbiotic bacteria.

4.7.7 Environmental Regulation of Host Immunity

Because immune systems have traditionally been studied in the laboratory, we do not know much about how environmental, abiotic factors affect vertebrate and invertebrate immunity. The transcriptional response of *L. oneistus* to the presence of oxygen has been recently analysed by comparative transcriptomics. Transcripts of innate immune molecules, likely involved in *Ca.* T. oneisti attachment (e.g., CTLD-containing proteins) were more abundant in the absence of oxygen (Paredes et al. 2022), where this ectosymbiont was observed to proliferate more (Paredes et al. 2021). It is therefore conceivable that the nematode expresses more CTLs to retain and/or control a proliferating symbiont. Additionally, overexpression of lectins in anoxia could favour symbiosis establishment in deep sand. Conversely, transcripts encoding for the Toll receptor, an antifungal protein (e.g., endochitinase-B) and two BPIs were more abundant in the presence of oxygen. This could be explained by the fact that we expect microbial pathogens to be more abundant in oxygenated than in anoxic environments.

All in all, the *Laxus* immune system appears to be optimized to resist to potentially deleterious microbes where they most abound (superficial, oxic sand) and to recruit its symbiont *Ca.* T. oneisti where it thrives (deep, reduced sand).

4.7.8 Conclusions

- The ectosymbiotic nematode *L. oneistus* and the endosymbiotic gutless oligochaete *O. algarvensis* engage similar classes of receptors to interact with

microbes, the important exception is the PGRPs which are completely absent from nematodes (Fig. 4.4).

- Both worms may use very diverse immune receptors and effectors (e.g., GCPR, CTLD-containing proteins, lysozymes) to achieve highly specific symbioses.
- A mix of symbiont-induced suppression of host immunity and secretion of growth-inhibiting immune effectors (e.g., CTLD-containing proteins, lysozymes) could mediate symbiont population control (Fig. 4.4); additionally, the gutless *Olavius* appears to directly digest its symbiotic partners.
- In *L. oneistus*, symbiont restriction to specific regions of the cuticle could be mediated by neuronal regulation of the epidermal immune system as suggested by the expansion of genes encoding for neuropeptides (e.g., NPY) and neuropeptide receptors (e.g., NPYR).
- The bacterial skin may be regarded as part of the nematode and oligochaete immune system in the sense that symbiont antimicrobials and/or secretion systems likely repel deleterious or non-beneficial environmental microbes.
- Recent transcriptional studies on *L. oneistus* suggest an exquisite sensitivity of its innate immunity to environmental changes. Indeed, abiotic factors such as oxygen may greatly affect both its capacity to withstand pathogens and to establish microbial symbioses.

4.8 New Insights from the Physiology of Chemosynthetic Symbionts in Meiofauna

4.8.1 Carbon and Energy Sources

The discovery of bacterial sulphide (H_2S) oxidation in a mouthless animal host sparked the characterization of chemosynthetic symbioses at deep-sea hydrothermal vents. Soon after, not only sulphide oxidation but also the oxidation of other reduced sulphur species such as thiosulfate was detected in many environments from the deep-sea to shallow water habitats, coupled with the reduction of a suitable electron acceptor such as oxygen or nitrate. In the initial concept of chemosynthesis framed more than four decades ago, the symbionts were interpreted as nutritional symbionts that provide two innovations to the metabolic spectrum of their eukaryote hosts: 1) the ability to use chemolithotrophic energy sources and 2) the ability to build biomass from one-carbon (C1) carbon sources (see Fig. 4.5 for convergent features in meiofaunal symbionts). It was quickly accepted that, in addition to Carbon Dioxide (CO_2), which defines autotrophic metabolism, also methane (CH_4) might be a C1 carbon source in chemosymbiosis, which, strictly speaking, renders these symbionts chemo-organo-heterotrophs. Methane, as single energy and carbon source, plays a major role at deep-sea sites, but has, however, not been shown to play a role in shallow water habitats and in meiofaunal hosts. Another leap forward in our understanding of the diversity of energy sources was the discovery of hydrogen use in deep-sea mussels that also prompted the discovery of hydrogen use in shallow water symbioses (Kleiner

et al. 2015; Petersen et al. 2011). An additional energy source, and the only substrate that, so far, has been only shown in shallow water hosts and not in deep-sea habitats is carbon monoxide (CO), an energy-rich but toxic compound that likely is ubiquitous in decaying seagrass materials around the globe (Kleiner et al. 2015). In addition to these energy sources based on the oxidation of reduced inorganic or C1 organic compounds such as sulphide and methane, a diverse range of more complex organic substrates have been shown to fuel chemosynthetic symbioses in deep-sea environments. In oil-rich sediments of the Gulf of Mexico, chemosynthetic mussel hosts, for example, draw a substantial amount of carbon and energy from short-chain alkanes such as propane or butane (Rubin-Blum et al. 2017). The impact of these hydrocarbons as energy and/or carbon sources is a current frontier in chemosynthesis research and has received quite some attention, as it connects chemosynthetic symbioses and bioremediation, for example, in oil-contaminated shallow water habitats.

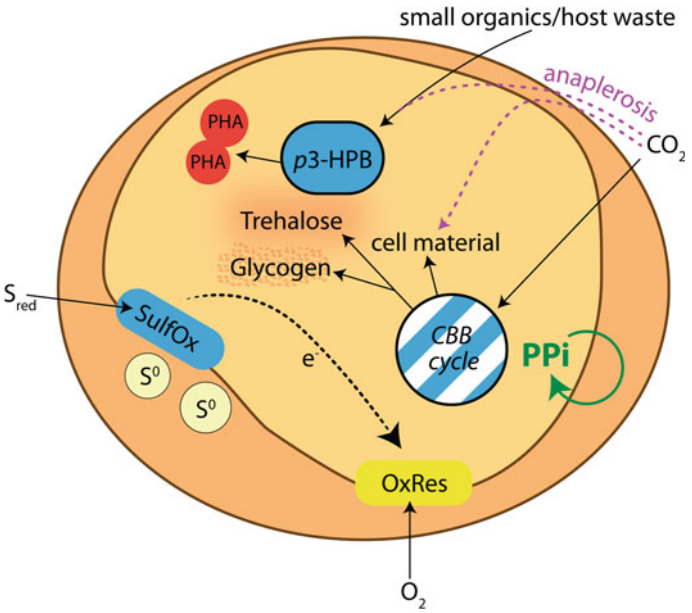

Fig. 4.5 Convergence in major parts of the metabolism characterizes shallow water chemosynthetic symbionts. Depicted are central metabolic features present in all thiotrophic symbionts of meiofaunal hosts. Anaplerosis indicates several carboxylation reactions that lead to a significant top-up to the total carbon budget that is very cheap compared to the same amount of carbon fixed via the Calvin-Benson-Bassham (CBB) cycle. S_{red}, reduced sulphur species; SulfOx, oxidation of reduced sulphur compounds; PHA, polyhydroxyalcanoates; PPi, Pyrophosphate; p3-HPB, partial 3-hydroxypropionate bicycle; CBB cycle, Calvin-Benson-Bassham cycle

4.8.2 Autotrophs, Mixotrophs or Heterotrophs?

These complex carbon and energy sources point to a conundrum in chemosynthesis research: Where to place the consortia in the spectrum from autotrophy to heterotrophy? In the symbioses that are clearly within an autotrophic framework, symbiont carbon fixation via Calvin-Benson-Bassham cycle (CBB) is the main carbon source. CBB has in fact been shown in most symbiont groups from both the deep sea and shallow waters, apart from the methane and alkane oxidizers. Other pathways for carbon fixation such as the reverse tricarboxylic acid cycle (rTCA) pathway have been documented as well, and some deep-sea symbionts are apparently able to use more than one carbon fixation pathway (Hinzke et al. 2021; Kleiner et al. 2012; Rubin-Blum et al. 2017). In shallow-water meiofaunal hosts, carbon fixation via the CBB dominates carbon source (Fig. 4.5).

In addition to CBB-based autotrophy, many chemosynthetic symbionts, particularly in shallow water environments, can also make use of small organic molecules as substrates (Fig. 4.5). These compounds are, for example, propionate or acetate, and symbionts also express transporters for their specific uptake (Jäckle et al. 2019; Kleiner et al. 2012; Paredes et al. 2021). Unrelated symbionts in *Paracatenula* and gutless oligochaetes, for example, express an incomplete 3-hydroxypropionate bicycle (3-HPB) that can be used for the heterotrophic assimilation of these small organic acids (Fig. 4.5; Jäckle et al. 2019; Kleiner et al. 2012; Paredes et al. 2021). These substrates can be connected to a role of the symbionts in host waste recycling. This is a logical conclusion, given that important and well-researched host groups such as the gutless oligochaetes lack a gut, and excretory organs. Recent data from stable isotope analyses of the chemosynthetic consortia on one hand and the habitat's biochemistry on the other hand, however, suggest that such "small organic substrates" can also come from the environment. The fermentation of the substrates could be performed by the hosts, by environmental organisms, or by the symbionts themselves. A major source for these external substrates is, for example, seagrass. Recent observations show that many seagrasses massively export metabolites into sediments, both directly as simple sugars or indirectly via the slow decay of the dead plant biomass that has been accumulated in a peat-like fashion (Sogin et al. 2019).

The use and importance of such heterotrophic resources in chemosynthesis were long overlooked. This comes as no surprise, given the fact that their use might be buried in the complexity of the overall metabolism of the symbionts present in a given host. Only very recent technical innovations, that allow to track stable isotope data for different members of chemosynthetic communities, have shown that some members of these communities clearly have a non-autotrophic signature for their carbon source (Kleiner et al. 2018). The data also shows that this additional carbon source appears to have a strong effect on the overall carbon budget in the gutless oligochaete *O. algarvensis*, as the host signature can only be explained by an even mix of both types of carbon uptake (Kleiner et al. 2018). In extreme cases, these symbiont groups likely provide a substantial part of their holobionts' carbon budget and would effectively put this chemosynthetic holobiont on the heterotrophic part of the spectrum, despite a large autotrophic potential in their symbionts. The most extreme

case of heterotrophy in chemosymbioses was discovered in the meiofaunal ciliate *Kentrophoros* that showed that their *Ca.* Kentron symbionts have no pathway for autotrophic carbon fixation, but rather express an array of importers of small organic substrates including small C3 and C4 organic acids as well as sugars that fuel a completely heterotrophic metabolism (Seah et al. 2019). Initially thought to be a protist-only phenomenon, the striking observation that a Kentron symbiont has apparently replaced the Thiosymbion (Gamma1) symbiont in a gutless oligochaete species from the Caribbean also points to the importance of such chemoorganoheterotrophic lifestyles in meiofaunal animal hosts.

4.8.3 Anaplerosis as a New Force to Reckon with in Chemosymbiosis

The observation that the *Ca.* Kentron symbionts lack an autotrophic pathway for carbon fixation came as a particular surprise, as experiments that were already conducted in the early days of chemosynthetic symbiosis research in Kentron; *Kentrophoros* symbioses showed strong signals of carbon fixation in physiological experiments (Fenchel and Finlay 1989). Both the genomic and expression data from *Ca.* Kentron, but also from *Ca.* Thiosymbion and *Ca.* Riegeria, the alphaproteobacterial symbiont in *Paracatenula* flatworms, showed that a process called "anaplerosis" could explain the conflicting results between sensitive tracer experiments based on using radioactive CO_2 and the recent metabolic reconstructions (Jäckle et al. 2019; Paredes et al. 2021; Seah et al. 2019). Anaplerosis is the replenishment of intermediates for the TCA cycle. These pathways that fuel the TCA all involve carboxylation steps and therefore the fixation of carbon from CO_2. Anaplerosis is a ubiquitous process and is, for example, also taking place in human mitochondria. In most animal hosts, the anaplerotic additions to the total carbon pool are minor and make up less than one percent of the total carbon uptake into the system via heterotrophic nutrition. Both in the *Ca.* Kentron symbionts that lack an autotrophic carbon fixation pathway, but also in the *Ca.* Riegeria symbionts that massively express CBB-based autotrophy, several such carboxylation steps fuel the central carbon metabolism (Jäckle et al. 2019; Seah et al. 2019). When constantly supplied by a high flux of turned over substrate, pathways such as the "incomplete 3-HBP pathway" the Ethyl-Malonyl-CoA pathway can add substantial amounts of carbon to the total carbon budget. In *Kentrophoros*, this apparently reaches such high levels that the positive signal from radiotracer-based analytics can be mistaken for signatures of a chemoautotrophic lifestyle (Fenchel and Finlay 1989; Seah et al. 2019).

The recent expansion of available genomic resources for symbionts has revealed that anaplerosis is widespread in the metabolism of chemosynthetic symbionts. Anaplerotic pathways and carboxylation steps have been detected in all meiofaunal systems investigated (see Fig. 4.5; *Kentrophoros*, *Paracatenula*, gutless oligochaetes,

and Stilbonematinae). The advantage of a massive integration of anaplerotic carboxylation into the symbiont's carbon metabolism and the host-symbiont carbon cycling could be the low energy demand per mol carbon fixed. It is a highly efficient supplement to the already accumulated carbon in the system, be it from auto- or heterotrophy or from host waste recycling. These insights are very similar to what has been suggested for efficient free-living heterotrophs that make most of light in the coastal ocean (see e.g., review by Moran and Miller 2007). They point to a much larger role of anaplerosis on carbon budgets across marine habitats that is starting to get more and more attention (Braun et al. 2021).

4.8.4 A Call for Precise and Detailed Physiological Data

In symbionts with versatile genomes, which can use complex organic substrates, the type of metabolic input, be it autotrophic or heterotrophic, cannot be determined by genomic analyses alone. This recent insight in chemosynthetic research is a prime example for the need of e.g., community-resolved stable isotope analyses that can differentiate and resolve e.g., carbon sources that are the two deltaproteobacterial symbionts in *O. algarvensis*. Both have a very similar genomic potential, but one effectively contributes as a net heterotroph and one as a clear autotroph (Kleiner et al. 2018). Particularly in the sediments around seagrasses that are rich in sugars and other plant materials (Sogin et al. 2019), such analyses must be considered imperative if any conclusion on the overall status of the holobiont and the contributions of any given symbiont is drawn.

4.8.5 Nitrogen Sources

Animals have high demands of nitrogen. Therefore, it was a revelation of recent meio- and macro-faunal chemosynthetic symbiosis research to see that symbionts from shallow water sediments are capable of fixing N_2 even within the tissue of their hosts (Petersen et al. 2017; Paredes et al. 2021). While this is essential in nitrogen-limited environments, the major nitrogen source for most symbionts still appears to be ammonium. The mode of nitrogen fixation is not stably retained throughout symbionts, not even within a single symbiotic genus such as *Ca.* Thiosymbion where only some members can fix nitrogen. The host supply with nitrogen and with amino acids are tightly coupled processes. All chemoautotrophic symbionts are fully self-reliant on amino acid production and can provide their hosts with all essential and non-essential amino acids. This complete potential for de-novo production of amino acids is in stark contrast to the nutritional symbioses present in terrestrial systems, e.g., in insects. Selection for a fully autonomous metabolism in the bacterial symbionts seems to prevent integration of amino acid synthesis and metabolism into the host's metabolism. However, for most chemosynthetic symbioses details of amino acid supply remain unresolved.

4.8.6 Biomass Transfer and Storage

Typically, chemosynthetic symbionts are food items for their hosts, and as such form the natural stocks to 'harvest' and consume. The standing stock of symbionts also forms the storage reservoirs that hosts can draw on when environmental resources are limited and symbiont populations are not growing. Most symbioses, including the gutless oligochaetes, some Stilbonematinae and *Kentrophoros,* digest their symbionts at high rates. They share this with their deep-sea counterparts such as giant tube worms or *Bathymodiolus* mussels. Digestions can happen in the gut as in Stilbonematinae, or through phagocytosis and lysosomal digestions such as in gutless oligochaetes and *Kentrophoros.* In contrast to this, the *Paracatenula* symbiosis has developed a different way to transfer large amounts of biomass from the symbionts to the host. The bacterial symbionts massively secrete OMVs which the host takes up via phagocytosis. Unlike the crop harvest model typical for most chemosynthetic symbioses, the *Paracatenula* symbiosis rather functions like a battery-and-current system, where the symbionts are a rechargeable storage unit that can supply a current of OMVs for nutrition. Hence, the symbionts become only very rarely digested, they rather develop massive and versatile storage inclusions comparable to fat cells and other specialized storage cell types in metazoans (Jäckle et al. 2019). A similar pattern likely applies to *Astomonema* nematodes that also have very large symbionts, but of a lineage of the gammaproteobacterial *Ca.* Thiosymbion that is specific to this host genus. The *Astomonema* Thiosymbion are much larger than the host cells and the symbionts are completely filled with storage vesicles (Fig. 4.2). Similar to *Paracatenula*, the *Astomonema* symbiont populations show few signs of symbiont digestion in electron microscopy data, suggesting a convergent role for these symbiont lineages from different bacterial phyla as nutritional and storage symbionts (Leisch pers. comm.; Giere et al. 1995; Ott et al. 1982).

4.8.7 The Role of the Hosts

While in these symbioses many details are known about the metabolic role of the bacterial partners, the hosts' input in carbon uptake and total carbon and energy budget remains as yet far less resolved. Meiofaunal animals have long been suggested to take up dissolved organic substrates. This is especially important and needs to be considered in those representatives with an open and soft body surface or epidermis such as ciliates, flatworms, or annelids. Nematodes, on the other hand, with their dense and multi-layered cuticle, are relatively unlikely to live of dissolved organic matter, particularly those groups lacking a gut. Proteomic approaches that capture expression and also generate host and symbiont-specific stable isotope data are promising tools to explore the host role.

4.8.8 *Ca. Thiosymbion—The Archetypical Chemosynthetic Symbiont in Meiofaunal Hosts*

While the autotrophic symbionts *Ca.* Riegeria in flatworms and the heterotrophic *Ca.* Kentron symbionts in *Kentrophoros* represent two extremes of a broad spectrum ranging from pure autotrophy to pure heterotrophy, the entire metabolic spectrum is largely covered by *Ca.* Thiosymbion, one of the most successful and archetypical chemosynthetic symbionts. Associated with three unrelated host groups and more than a hundred host species (see Table 4.1 and Fig. 4.2; Musat et al. 2007; Scharhauser et al. 2020; Zimmermann et al. 2016), *Ca.* Thiosymbion:

- can use both nitrate and oxygen as electron acceptors,
- utilizes a wide range of carbon sources,
- uses anaplerosis to top up carbon,
- can fix nitrogen,
- has multiple options to store carbon and energy (Kleiner et al. 2018, 2012; Paredes et al. 2021),
- can flexibly employ all of the metabolic pathways mentioned above in the typical oxic to anoxic gradients, and, at least when associated with *Laxus oneistus*, appears to prefer anoxic conditions (Paredes et al. 2021).

4.9 Intricate Symbiotic Relationships—Present Frontiers, Emerging Challenges, and Future Research

The study of chemosynthetic symbioses in meiofauna has produced an appreciable number of fundamental insights into topics of general relevance in cell biology, immunology and physiology. Nevertheless, many questions still remain unanswered, opening new horizons for research and posing challenges for methodology. Below, we outline some of these, pertaining to the distribution of chemosynthetic symbioses amongst meiofauna groups, the pathways that led to the intimate symbioses that we observe today, the interactions between partners, the mechanisms for acquisition and maintenance of symbionts, the physiology behind the partnerships and, lastly, the role of chemosynthetic symbioses in their ecosystem.

- Up to now, chemosynthetic symbioses have been found **in a few meiofauna groups** only, (karyorelictid ciliates, catenulid platyhelminthes, nematoda, and oligochaetes). Are there more to be discovered? Currently, it is unclear if abundant and well-studied taxa such as Gnathostomulida, Gastrotricha, Kinorhyncha or the diverse interstitial crustacea have symbiotic representatives, although many of those co-occur with symbiotic species and live in environments favourable for chemosynthetic bacteria. Similarly, which traits enable the most successful symbiotic bacterium, *Ca.* Thiosymbion, to colonize most diverse hosts and adopt all lifestyles from ectosymbiont to intracellular endosymbiont? Comparative

Table 4.1 Host-symbiont interfaces and transmission in the different taxa

Taxon	Symbiont(s) (main type)	Type of association (single vs multiple phylotypes per host species etc.)	Symbiont location	Exchange of substances	Transmission mode
Kentrophoros	Gammaproteobacterial SOX (Ca. Kentron)	Single	Extracellular, on cell membrane	Phagocytosis, digestion	Vertical, by division
Paracatenula	Alphaproteobacterial SOX (Ca. Riegeria)	Single	Intracellular, in mesodermal? Bacteriocytes	OMV secretion, some digestion	Vertical, mainly by asexual fission
Stilbonematinae	Gammaproteobacterial SOX (Ca. Thiosymbion)	Single	Extracellular, on the cuticle	Digestion, in some species	Likely mixed
Astomonema	Gammaproteobacterial SOX (Ca. Thiosymbion)	Mostly single	Intracellular, in gut rudiment Extracellular, in gut lumen	Phagocytosis, digestion	Not known
Gutless oligochaetes	Gammaproteobacterial SOX (Ca. Thiosymbion, except *Inanidrilus exumae*)	Main symbiont phylotype and additional "secondary" symbionts (Gamma-, Alpha-, Deltaproteobacteria)	Extracellular, below cuticle	Phagocytosis, digestion by epidermis cells	Mostly vertical

SOX = sulfur-oxidizing
OMV = outer membrane vesicle

approaches could allow us to identify traits that either foster or prohibit symbiotic interactions in either symbiotic partner.

- What were **the evolutionary starting points and pathways that led to the establishment** of the symbioses? Are the symbiotic bacteria survivors from an ancestral microbial menu of the hosts, as seems probable in *Astomonema*? Are they the descendants of pathogens that the host succeeded to keep in check and finding an agreement with the "attacker", as may be the case in Vestimentifera and *Paracatenula*? Have the microbes just "hitched a ride" on the moving host that has proven to be beneficial to both partners as in *Kentrophoros* or the Stilbonematinae? Understanding the evolutionary background of many clades likely enables us to generalize the dynamics of host microbe associations along the mutualist to parasite spectrum (see Box 4.2).

Box 4.2 How and why did such symbioses evolve?

Expand food sources—Many animals feed on bacteria, and it is likely that the consumption of chemosynthetic bacteria by animals was a major driver for the first encounter of the two partners.
Expand symbiont habitat—Space is highly limited in highly productive environments, and a bacterium that colonizes animal epithelia or cuticles conquers large new habitats.
Expand host habitat—Sulphide detoxification by chemosynthetic symbionts might help to expand the range of the animal host. This effect likely is limited by the quick diffusion of sulphide into animal tissue and only very thick coats might mitigate sulphide stress for a significant period.
Expand symbiont access to resources—Oxygen and sulphide is the optimal red/ox couple, but are spatially separated. An animal host can easily traverse the gradient and provide access to both oxygen and sulphide much more efficiently than if the symbiont was on its own.
Provide buffering capacity—The symbionts can use host carbon and nitrogen waste as substrates, which makes them more independent from environmental conditions.
Provide shelter—Free-living bacterial populations are under pressure for exploitation, both from viruses as well as bacterial and animal predation. Intracellular endosymbionts are fully sheltered from many of these attacks, and even ectosymbionts are much more sheltered, for example, via biofilm formation, physical barriers such as invaginations or chemical barriers such as an extracellular matrix.

- None of the symbiotic meiofauna species has been cultivated over several sexual generations or for an extended period of time yet. This is a major challenge for methodology. Efforts must continue to overcome this shortcoming and thus pave

the way to **creating model organisms**, that can be physiologically and genetically manipulated. The recent successes with cultivation approaches for both *Paracatenula* flatworms and gutless oligochaetes (Gruber-Vodicka and Gruhl, pers. comm) are promising and might open new avenues, for example, in immunology and experimental physiology to gain a mechanistic understanding of meiofaunal animals that live in obligate symbiosis.

- In order to fully understand interactions between symbiotic partners, we need a **holistic approach**, combining high-resolution structural data with gene expression and chemical information. In this respect, the small size of meiofaunal organisms is both a challenge and a blessing. Nucleotide, protein and metabolite extraction as well as detection and sequencing methods are more difficult and prone to systematic error the smaller the amount of starting material is. However, low-input library protocols and sensitive sequencing methods are constantly improving towards detection of low-abundance transcripts and assembly of genomes from single cells. Chemical imaging approaches like EDX (Energy Dispersive X-Ray), Raman, SIMS (Secondary-Ion Mass Spectrometry) techniques and especially MALDI-MSI (Matrix-Assisted Laser Desorption Ionization-Mass Spectrometry Imaging) allow quantitative label-free imaging of elements or biomolecules. Here the challenges lie in the balance between spatial resolution and analytical range and in the necessary combination with structural imaging to provide the morphological framework. On the structural side, small organisms are much easier to image in full size than larger organisms. Modern 3D techniques like FIB-SEM (Focused Ion Beam-Scanning Electron Microscopy) allow acquisition of volumetric data sets at ultrastructural level of detail. New light microscopy techniques such as lattice light sheet microscopy make acquisition of near-isotropic 3D data near the diffraction limit possible, thus in the range of bacterial cells. Subcellular imaging can be achieved with new structured illumination or other super-resolution techniques. These are currently highly innovative fields and it is important to follow this progress and its potential for the study of meiofaunal organisms.
- Throughout their lives, meiofauna animals, just like us, need to **communicate with microbes,** and to decide whether to escape, destroy or cooperate. The study of meiofauna immune systems revealed that at least some of the underlying molecules are also at work in vertebrates, including humans. This was highlighted by the discovery of the Mermaid lectins in *L. oneistus*. The carbohydrate recognition domain of this family of proteins is structurally and functionally so similar to the human immunoreceptor DC-SIGN that it can compete with it and block pathogen uptake and transmission by human cells. The potential for discovering, for example, new AMPs by studying symbiotic meiofauna is vast, as it is that of understanding the role of neuropeptide signalling in immunity. Not before we succeed in cultivating and **genetically manipulating** symbiotic meiofauna, will it become possible to understand which receptors, pathways and effectors are responsible for symbiosis establishment and maintenance of highly specific symbioses.

- Can these **autonomous and efficient bio-factories inform synthetic biology**? Nutritional symbioses in insects, where symbionts provide a limited set of metabolic functions exhibit streamlining of the symbiont genomes where genomes lose most genes and only retain the very few metabolic functions necessary for the hosts. This drastic reduction can lead to a point of decay that was observed in many insect symbioses, but such deleterious reduction of the genome is rare in chemosynthetic symbionts. In fact, only two host groups, the deep sea Vesicomyidae clams and the *Paracatenula* flatworms, show pronounced symbiont genome streamlining compared to the free-living prokaryotic relatives. However, in both cases the symbiont genomes remain autonomous for carbon metabolism, amino acid and vitamin synthesis and the two symbiont groups are able to satisfy the full nutritional needs of their animal hosts with a common share of approximately 700 genes. The genomes of chemosynthetic symbionts are smaller than those of most free-living bacteria with highly streamlined genomes, and at the same time are tailored to serve as nutrition. Maybe one day we can learn from these symbionts how to efficiently provide nutrition for animal livestock or humans from recycled waste and at the same time detoxify problematic side products such as sulphide?
- What is the **influence of chemosynthetic symbioses in meiofauna on the conditions in the interstitial environment**? Is there an effect on flux rates in biogeochemical cycles, especially the sulphur or nitrogen cycle? In many cases, the density of symbiotic meiofauna is probably too low to leave a signature. In tropical back-reef sediments, however, chemosynthetic meiofauna can drastically outnumber non-symbiotic interstitial organisms and their role in processes which are largely controlled by abiotic physical and chemical forces in other sediments, is still unknown. Human-induced global change such as eutrophication, rising temperature and CO_2 concentrations are expected to result in expansion of sulfidic, hypoxic and oxygen minimum zones in marine habitats (see Chap. 7). How do chemosynthetic symbioses respond and adapt to these changing conditions? Experimental physiological and ecological approaches may help to assess adaptability and resilience in symbiotic systems.
- For most symbiotic meiofauna, little is known about their **reproductive biology**. However, most of them share traits like internal fertilization, direct development, and low number of offspring. With a lack of planktonic dispersal stages, the full life cycle is effectively locked into the sediment. This raises the questions of how their populations are structured and the effective range of dispersal in both space and time.
- In a symbiosis context, it is a completely **open frontier how population structure** and **dispersal** are linked to the acquired pool of symbionts. As many symbiotic meiofauna systems show a degree of horizontal symbiont uptake, answering these questions will help to identify the key traits that select for a successful association. The comparison between different hosts should allow to differentiate between host- and symbiont-driven selection.

References

Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. Cell 124:783–801. https://doi.org/10.1016/j.cell.2006.02.015

Anderson V, Jiirgens G (1985) Establishment of dorsal-ventral polarity in the *drosophila* embryo: genetic studies on the role of the *toll* gene product. Cell 42:779–789. https://doi.org/10.1016/0092-8674(85)90274-0

Ansari KGMT, Lyla PS, Ajmal Khan S (2016) New distributional records of free-living marine nematodes from Indian waters II. Monhysterids. Indian J Geo Mar Sci 45:342–351. http://nopr.niscair.res.in/handle/123456789/35063

Bauer L (2012) Molekulare charakterisierung und lokalisationsmuster zweier putativer bakterien-permeabilisierender proteine (bpi) eines marinen symbiotischen nematoden. MSc Thesis University of Vienna. https://doi.org/10.25365/thesis.21863

Bayer C, Heindl NR, Rinke C, Lücker S, Ott JA, Bulgheresi S (2009) Molecular characterization of the symbionts associated with marine nematodes of the genus *Robbea*. Environ Microbiol Rep 1:136–144. https://doi.org/10.1111/j.1758-2229.2009.00019.x

Bennett GM, Moran NA (2015) Heritable symbiosis: the advantages and perils of an evolutionary rabbit hole. Proc Natl Acad Sci 112:10169–10176. https://doi.org/10.1073/pnas.1421388112

Bergin C, Wentrup C, Brewig N, Blazejak A, Erséus C, Giere O, Schmid M, De Wit P, Dubilier N (2018) Acquisition of a novel sulphur-oxidizing symbiont in the gutless marine worm *Inanidrilus exumae*. Appl Environ Microbiol 84. https://doi.org/10.1128/AEM.02267-17

Bettencourt R, Rodrigues M, Barros I, Cerqueira T, Freitas C, Costa V, Pinheiro M, Egas C, Santos RS (2014) Site-related differences in gene expression and bacterial densities in the mussel *Bathymodiolus azoricus* from the Menez Gwen and Lucky Strike deep-sea hydrothermal vent sites. Fish Shellfish Immunol 39:343–353. https://doi.org/10.1016/j.fsi.2014.05.024

Brandt JP, Ringstad N (2015) Toll-like receptor signaling promotes development and function of sensory neurons required for a *c. elegans* pathogen-avoidance behavior. Curr Biol 25:2228–2237. https://doi.org/10.1016/j.cub.2015.07.037

Braun A, Spona-Friedl M, Avramov M, Elsner M, Baltar F, Reinthaler T, Herndl GJ, Griebler C (2021) Reviews and syntheses: Heterotrophic fixation of inorganic carbon—significant but invisible flux in environmental carbon cycling. Biogeosciences 18:3689–3700. https://doi.org/10.5194/bg-18-3689-2021

Brennan JJ, Gilmore TD (2018) Evolutionary origins of toll-like receptor signaling. Mol Biol Evol 35:1576–1587. https://doi.org/10.1093/molbev/msy050

Bright M, Bulgheresi S (2010) A complex journey: transmission of microbial symbionts. Nat Rev Microbiol 8:218–230. https://doi.org/10.1038/nrmicro2262

Bulgheresi S (2011) Calling the roll on *Laxus oneistus* immune defense molecules. Symbiosis 55:127–135. https://doi.org/10.1007/s13199-012-0157-3

Bulgheresi S, Gruber-Vodicka HR, Heindl NR, Dirks U, Kostadinova M, Breiteneder H, Ott JA (2011) Sequence variability of the pattern recognition receptor Mermaid mediates specificity of marine nematode symbioses. ISME J 5(6):986–98. https://doi.org/10.1038/ismej.2010.198

Bulgheresi S (2016) Bacterial cell biology outside the streetlight. Environ Microbiol 14

Bulgheresi S, Schabussova I, Chen T, Mullin NP, Maizels RM, Ott JA (2006) A new c-type lectin similar to the human immunoreceptor DC-SIGN mediates symbiont acquisition by a marine nematode. Appl Environ Microbiol 72:2950–2956. https://doi.org/10.1128/AEM.72.4.2950-2956.2006

Chen F, Krasity BC, Peyer SM, Koehler S, Ruby EG, Zhang X, McFall-Ngai MJ (2017) Bactericidal permeability-increasing proteins shape host-microbe interactions. mBio 8. https://doi.org/10.1128/mBio.00040-17

Chomicki G, Kiers ET, Renner SS (2020a) The evolution of mutualistic dependence. Annu Rev Ecol Evol Syst 51:409–432. https://doi.org/10.1146/annurev-ecolsys-110218-024629

Chomicki G, Werner GDA, West SA, Kiers ET (2020b) Compartmentalization drives the evolution of symbiotic cooperation. Philos Trans R Soc B Biol Sci 375, 20190602. https://doi.org/10.1098/rstb.2019.0602

Cummings RD, McEver RP (2009) C-type Lectins. In: Varki A, Cummings RD, Esko JD, Freeze HH, Stanley P, Bertozzi CR, Hart GW, Etzler ME (eds) Essentials of glycobiology. Cold Spring Harbor Laboratory Press, Cold Spring Harbor (NY)

Davidson CR, Best NM, Francis JW, Cooper EL, Wood TC (2008) Toll-like receptor genes (TLRs) from *Capitella capitata* and *Helobdella robusta* (Annelida). Dev Comp Immunol 32:608–612. https://doi.org/10.1016/j.dci.2007.11.004

de Mendoza A, Sebé-Pedrós A, Ruiz-Trillo I (2014) The evolution of the GPCR signaling system in eukaryotes: modularity, conservation, and the transition to metazoan multicellularity. Genome Biol Evol 6:606–619. https://doi.org/10.1093/gbe/evu038

de Velde WV, Zehirov G, Szatmari A, Debreczeny M, Ishihara H, Kevei Z, Farkas A, Mikulass K, Nagy A, Tiricz H, Satiat-Jeunemaître B, Alunni B, Bourge M, Kucho K, Abe M, Kereszt A, Maroti G, Uchiumi T, Kondorosi E, Mergaert P (2010) Plant peptides govern terminal differentiation of bacteria in symbiosis 327:6

Dierking K, Pita L (2020) Receptors mediating host-microbiota communication in the metaorganism: the invertebrate perspective. Front Immunol 11:1251. https://doi.org/10.3389/fimmu.2020.01251

Dirks U, Gruber-Vodicka HR, Leisch N, Sterrer W, Ott JA (2011) A new species of symbiotic flatworms, *Paracatenula galateia* sp. nov. (Platyhelminthes: Catenulida: Retronectidae) from Belize (Central America). Mar Biol Res 201:769–777. https://doi.org/10.1080/17451000.2011.574880

Dirks U, Gruber-Vodicka HR, Leisch N, Bulgheresi S, Egger B, Ladurner P, Ott JA (2012) Bacterial symbiosis maintenance in the asexually reproducing and regenerating flatworm *paracatenula galateia.* PLoS ONE 7:e34709. https://doi.org/10.1371/journal.pone.0034709

Dubilier N, Mülders C, Ferdelman T, de Beer D, Pernthaler A, Klein M, Wagner M, Erséus C, Thiermann F, Krieger J, Giere O, Amann R (2001) Endosymbiotic sulphate-reducing and sulphide-oxidizing bacteria in an oligochaete worm. Nature 411:298–302. https://doi.org/10.1038/35077067

Dubilier N, Bergin C, Lott C (2008) Symbiotic diversity in marine animals: the art of harnessing chemosynthesis. Nat Rev Microbiol 6:725–740. https://doi.org/10.1038/nrmicro1992

Dziarski R, Gupta D (2018) How innate immunity proteins kill bacteria and why they are not prone to resistance. Curr Genet 64:125–129. https://doi.org/10.1007/s00294-017-0737-0

Fenchel T, Finlay BJ (1989) *Kentrophoros*: a mouthless ciliate with a symbiotic kitchen garden. Ophelia 30:75–93

Fisher RM, Henry LM, Cornwallis CK, Kiers ET, West SA (2017) The evolution of host-symbiont dependence. Nat Commun 8:15973. https://doi.org/10.1038/ncomms15973

Foissner W (1995) *Kentrophoros* (Ciliophora, Karyorelictea) has oral vestiges: a reinvestigation of *K. fistulosus* (Fauré-Fremiet, 1950) using protargol impregnation. Arch Für Protistenkd 146:165–179. https://doi.org/10.1016/S0003-9365(11)80107-7

Giere O, Krieger J (2001) A triple bacterial endosymbiosis in a gutless oligochaete (Annelida): ultrastructural and immunocytochemical evidence. Invertebr Biol 120:41–49

Giere O, Langheld C (1987) Structural organisation, transfer and biological fate of endosymbiotic bacteria in gutless oligochaetes. Mar Biol 93:641–650. https://doi.org/10.1007/BF00392801

Giere O, Conway N, Gastrock G, Schmidt C (1991) Regulation of gutless annelid ecology by endosymbiotic bacteria. Mar Ecol Prog Ser 68:287–299. https://doi.org/10.3354/meps068287

Giere O, Windoffer R, Southward EC (1995) The bacterial endosymbiosis of the gutless nematode, *Astomonema southwardorum* : ultrastructural aspects. J Mar Biol Assoc UK 75:153–164. https://doi.org/10.1017/S0025315400015265

Gruber-Vodicka HR, Dirks U, Leisch N, Baranyi C, Stoecker K, Bulgheresi S, Heindl NR, Horn M, Lott C, Loy A, Wagner M, Ott J (2011) *Paracatenula*, an ancient symbiosis between thiotrophic

Alphaproteobacteria and catenulid flatworms. Proc Natl Acad Sci 108:12078–12083. https://doi.org/10.1073/pnas.1105347108

Hinzke T, Kleiner M, Meister M, Schlüter R, Hentschker C, Pané-Farré J, Hildebrandt P, Felbeck H, Sievert SM, Bonn F, Völker U, Becher D, Schweder T, Markert S (2021) Bacterial symbiont subpopulations have different roles in a deep-sea symbiosis. eLife 10, e58371. https://doi.org/10.7554/eLife.58371

Holzer P, Farzi A (2014) Neuropeptides and the microbiota-gut-brain axis. In: Lyte M, Cryan JF (eds) Microbial endocrinology: the microbiota-gut-brain axis in health and disease. Springer New York, New York, NY, pp 195–219. https://doi.org/10.1007/978-1-4939-0897-4_9

Ingole B, Singh R, Sautya S, Dovgal I, Chatterjee T (2010) Report of epibiont *Thecacineta calix* (Ciliophora: Suctorea) on deep-sea Desmodora (Nematoda) from the Andaman Sea. Indian Ocean Mar Biodivers Rec 3:e46. https://doi.org/10.1017/S1755267209990777

Jäckle O, Seah BKB, Tietjen M, Leisch N, Liebeke M, Kleiner M, Berg JS, Gruber-Vodicka HR (2019) Chemosynthetic symbiont with a drastically reduced genome serves as primary energy storage in the marine flatworm *Paracatenula*. Proc Natl Acad Sci 116:8505–8514. https://doi.org/10.1073/pnas.1818995116

Kawai T, Akira S (2010) The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 11:373–384. https://doi.org/10.1038/ni.1863

Kleiner M, Wentrup C, Lott C, Teeling H, Wetzel S, Young J, Chang Y-J, Shah M, VerBerkmoes NC, Zarzycki J, Fuchs G, Markert S, Hempel K, Voigt B, Becher D, Liebeke M, Lalk M, Albrecht D, Hecker M, Schweder T, Dubilier N (2012) Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. Proc Natl Acad Sci 109:E1173–E1182. https://doi.org/10.1073/pnas.1121198109

Kleiner M, Wentrup C, Holler T, Lavik G, Harder J, Lott C, Littmann S, Kuypers MMM, Dubilier N (2015) Use of carbon monoxide and hydrogen by a bacteria–animal symbiosis from seagrass sediments. Environ Microbiol 17:5023–5035. https://doi.org/10.1111/1462-2920.12912

Kleiner M, Dong X, Hinzke T, Wippler J, Thorson E, Mayer B, Strous M (2018) Metaproteomics method to determine carbon sources and assimilation pathways of species in microbial communities. Proc Natl Acad Sci 115:E5576–E5584. https://doi.org/10.1073/pnas.1722325115

Krieger J (2000) Funktion und Übertragung endosymbiontischer Bakterien bei darmlosen marinen Oligochaeten. Diss Univ Hamburg, p 80, Shaker Verlag Aachen, ISBN 3-8265-8671-9

Leduc D (2013) One new genus and two new deep-sea nematode species (Desmodoridae, Stilbonematinae) from phosphorite nodule deposits on Chatham Rise, Southwest Pacific Ocean. Mar Biodivers 43:421–428. https://doi.org/10.1007/s12526-013-0171-6

Leisch N, Dirks U, Gruber-Vodicka HR, Schmid M, Sterrer W, Ott JA (2011) Microanatomy of the trophosome region of *Paracatenula* cf. *polyhymnia* (Catenulida, Platyhelminthes) and its intracellular symbionts. Zoomorphology 130:261–271. https://doi.org/10.1007/s00435-011-0135-y

Leisch N, Verheul J, Heindl NR, Gruber-Vodicka HR, Pende N, den Blaauwen T, Bulgheresi S (2012) Growth in width and FtsZ ring longitudinal positioning in a gammaproteobacterial symbiont. Curr Biol 22:R831–R832. https://doi.org/10.1016/j.cub.2012.08.033

Leisch N, Pende N, Weber PM, Gruber-Vodicka HR, Verheul J, Vischer NOE, Abby SS, Geier B, den Blaauwen T, Bulgheresi S (2017) Asynchronous division by non-ring FtsZ in the gammaproteobacterial symbiont of *Robbea hypermnestra*. Nat Microbiol 2:16182. https://doi.org/10.1038/nmicrobiol.2016.182

Login FH, Balmand S, Vallier A, Vincent-Monégat C, Vigneron A, Weiss-Gayet M, Rochat D, Heddi A (2011) Antimicrobial peptides keep insect endosymbionts under control 334:4

Lu X, Wang M, Qi J, Wang H, Li X, Gupta D, Dziarski R (2006) Peptidoglycan recognition proteins are a new class of human bactericidal proteins. J Biol Chem 281:5895–5907. https://doi.org/10.1074/jbc.M511631200

McFall-Ngai M et al (2013) Animals in a bacterial world, a new imperative for the life sciences. PNAS 110:3229–3236. https://doi.org/10.1073/pnas.1218525110

McQuillen R, Xiao J (2020) Insights into the structure, function, and dynamics of the bacterial cytokinetic FtsZ-ring. Annu Rev Biophys 49:309–341. https://doi.org/10.1146/annurev-biophys-121219-081703

Mendell JE, Clements KD, Choat JH, Angert ER (2008) Extreme polyploidy in a large bacterium. Proc Natl Acad Sci 105:6730–6734. https://doi.org/10.1073/pnas.0707522105

Moran MA, Miller WL (2007) Resourceful heterotrophs make the most of light in the coastal ocean. Nat Rev Microbiol 5:792–800. https://doi.org/10.1038/nrmicro1746

Musat N, Giere O, Gieseke A, Thiermann F, Amann R, Dubilier N (2007) Molecular and morphological characterization of the association between bacterial endosymbionts and the marine nematode *Astomonema* sp. from the Bahamas. Environ Microbiol 9:1345–1353. https://doi.org/10.1111/j.1462-2920.2006.01232.x

Ott J, Rieger G, Rieger R, Enderes F (1982) New mouth less interstitial worms from the sulfide system: symbiosis with prokaryotes. Mar Ecol 3:313–333. https://doi.org/10.1111/j.1439-0485.1982.tb00282.x

Ott JA, Novak R, Schiemer F, Hentschel U, Nebelsick M, Polz M (1991) Tackling the sulfide gradient: a novel strategy involving marine nematodes and chemoautotrophic ectosymbionts. Mar Ecol 12:261–279. https://doi.org/10.1111/j.1439-0485.1991.tb00258.x

Ott J, Bright M, Bulgheresi S (2004) Symbioses between Marine Nematodes and Sulphur-oxidizing Chemoautotrophic Bacteria. Symbiosis 36:103–126

Ott JA, Leisch N, Gruber-Vodicka HR (2014) *Eubostrichus fertilis* sp. n., a new marine nematode (Desmodoridae: Stilbonematinae) with an extraordinary reproductive potential from Belize, Central America. Nematology 16:777–787. https://doi.org/10.1163/15685411-00002807

Paredes GF, Viehboeck T, Lee R, Palatinszky M, Mausz MA, Reipert S, Schintlmeister A, Maier A, Volland J-M, Hirschfeld C, Wagner M, Berry D, Markert S, Bulgheresi S, König L (2021) Anaerobic sulphur oxidation underlies adaptation of a chemosynthetic symbiont to oxic-anoxic interfaces. mSystems 6. https://doi.org/10.1128/mSystems.01186-20

Paredes GF, Viehboeck T, Markert S, Mausz MA, Sato Y, Liebeke M, König L, Bulgheresi S (2022) Differential regulation of degradation and immune pathways underlies adaptation of the ectosymbiotic nematode Laxus oneistus to oxic-anoxic interfaces. Sci Rep 13:12(1):9725. https://doi.org/10.1038/s41598-022-13235-9

Pende N, Leisch N, Gruber-Vodicka HR, Heindl NR, Ott J, den Blaauwen T, Bulgheresi S (2014) Size-independent symmetric division in extraordinarily long cells. Nat Commun 5:4803. https://doi.org/10.1038/ncomms5803

Pende N, Wang J, Weber PM, Verheul J, Kuru E, Rittmann SK-MR, Leisch N, VanNieuwenhze MS, Brun YV, den Blaauwen T, Bulgheresi S (2018) Host-polarized cell growth in animal symbionts. Curr Biol 28:1039-1051.e5. https://doi.org/10.1016/j.cub.2018.02.028

Petersen JM, Zielinski FU, Pape T, Seifert R, Moraru C, Amann R, Hourdez S, Girguis PR, Wankel SD, Barbe V, Pelletier E, Fink D, Borowski C, Bach W, Dubilier N (2011) Hydrogen is an energy source for hydrothermal vent symbioses. Nature 476:176–180. https://doi.org/10.1038/nature10325

Petersen JM, Kemper A, Gruber-Vodicka H, Cardini U, van der Geest M, Kleiner M, Bulgheresi S, Mußmann M, Herbold C, Seah BKB, Antony CP, Liu D, Belitz A, Weber M (2017) Chemosynthetic symbionts of marine invertebrate animals are capable of nitrogen fixation. Nat Microbiol 2:16195. https://doi.org/10.1038/nmicrobiol.2016.195

Polz MF, Felbeck H, Novak R, Nebelsick M, Ott JA (1992) Chemoautotrophic, sulphur-oxidizing symbiotic bacteria on marine nematodes: morphological and biochemical characterization. Microb Ecol 24:313–329. https://doi.org/10.1007/BF00167789

Pradel E, Zhang Y, Pujol N, Matsuyama T, Bargmann CI, Ewbank JJ (2007) Detection and avoidance of a natural product from the pathogenic bacterium *Serratia marcescens* by *Caenorhabditis elegans*. Proc Natl Acad Sci 104:2295–2300. https://doi.org/10.1073/pnas.0610281104

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan M-W, Ray KP, Solari R, Johnson CD, Ewbank JJ (2001) A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. Curr Biol 11:809–821. https://doi.org/10.1016/S0960-9822(01)00241-X

Royet J, Gupta D, Dziarski R (2011) Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. Nat Rev Immunol 11:837–851. https://doi.org/10.1038/nri3089

Rubin-Blum M, Antony CP, Borowski C, Sayavedra L, Pape T, Sahling H, Bohrmann G, Kleiner M, Redmond MC, Valentine DL, Dubilier N (2017) Short-chain alkanes fuel mussel and sponge *Cycloclasticus* symbionts from deep-sea gas and oil seeps. Nat Microbiol 2:17093. https://doi.org/10.1038/nmicrobiol.2017.93

Russell SL (2019) Transmission mode is associated with environment type and taxa across bacteria-eukaryote symbioses: a systematic review and meta-analysis. FEMS Microbiol Lett 366. https://doi.org/10.1093/femsle/fnz013

Scharhauser F, Zimmermann J, Ott JA, Leisch N, Gruber-Vodicka HR (2020) Morphology of obligate ectosymbionts reveals *Paralaxus* gen. nov.: a new circumtropical genus of marine stilbonematine nematodes. Zool Scr 49:379–394. https://doi.org/10.1111/zsc.12399

Schleifer KH, Kandler O (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. Bacteriol Rev 36:407–477. https://doi.org/10.1128/mmbr.37.2.258-258.1973

Schuelke T, Pereira TJ, Hardy SM, Bik HM (2018) Nematode-associated microbial taxa do not correlate with host phylogeny, geographic region or feeding morphology in marine sediment habitats. Mol Ecol 27:1930–1951. https://doi.org/10.1111/mec.14539

Seah BKB, Schwaha T, Volland J-M, Huettel B, Dubilier N, Gruber-Vodicka HR (2017) Specificity in diversity: single origin of a widespread ciliate-bacteria symbiosis. Proc R Soc B Biol Sci 284:20170764. https://doi.org/10.1098/rspb.2017.0764

Seah BKB, Antony CP, Huettel B, Zarzycki J, Schada von Borzyskowski L, Erb TJ, Kouris A, Kleiner M, Liebeke M, Dubilier N, Gruber-Vodicka HR (2019) Sulphur-oxidizing symbionts without canonical genes for autotrophic CO_2 fixation. mBio 10. https://doi.org/10.1128/mBio.01112-19

Seah BKB, Volland J-M, Leisch N, Schwaha T, Dubilier N, Gruber-Vodicka HR (2020) *Kentrophoros magnus* sp. nov. (Ciliophora, Karyorelictea), a new flagship species of marine interstitial ciliates. bioRxiv 2020.03.19.998534. https://doi.org/10.1101/2020.03.19.998534

Shi H, Bratton BP, Gitai Z, Huang KC (2018) How to build a bacterial cell: MreB as the Foreman of *E. coli* construction. Cell 172:1294–1305. https://doi.org/10.1016/j.cell.2018.02.050

Sogin E, Michellod D, Gruber-Vodicka H, Bourceau P, Geier B, Meier DV, Seidel M, Ahmerkamp S, Schorn S, D'Angelo G, Procaccini G, Dubilier N, Liebeke M (2019) Sugars dominate the seagrass rhizosphere. Biorxiv. https://doi.org/10.1101/797522

Sogin EM, Leisch N, Dubilier N (2020) Chemosynthetic symbioses. Curr Biol 30:R1137–R1142. https://doi.org/10.1016/j.cub.2020.07.050

Stanley SM (2014) Evolutionary radiation of shallow-water Lucinidae (Bivalvia with endosymbionts) as a result of the rise of seagrasses and mangroves. Geology 42:803–806. https://doi.org/10.1130/G35942.1

Swaminathan CP, Brown PH, Roychowdhury A, Wang Q, Guan R, Silverman N, Goldman WE, Boons G-J, Mariuzza RA (2006) Dual strategies for peptidoglycan discrimination by peptidoglycan recognition proteins (PGRPs). Proc Natl Acad Sci 103:684–689. https://doi.org/10.1073/pnas.0507656103

Tchesunov AV, Ingels J, Popova EV (2012) Marine free-living nematodes associated with symbiotic bacteria in deep-sea canyons of north-east Atlantic Ocean. J Mar Biol Assoc UK 92:1257–1271. https://doi.org/10.1017/S0025315411002116

Wang J, Alvarez L, Bulgheresi S, Cava F, den Blaauwen T (2021) PBP4 Is likely involved in cell division of the longitudinally dividing bacterium *Candidatus* Thiosymbion oneisti. Antibiotics 10:274. https://doi.org/10.3390/antibiotics10030274

Wippler J, Kleiner M, Lott C, Gruhl A, Abraham PE, Giannone RJ, Young JC, Hettich RL, Dubilier N (2016) Transcriptomic and proteomic insights into innate immunity and adaptations to a symbiotic lifestyle in the gutless marine worm *Olavius algarvensis*. BMC Genomics 17:942. https://doi.org/10.1186/s12864-016-3293-y

Young KD (2010) Bacterial shape: two-dimensional questions and possibilities. Annu Rev Microbiol 64:223–240. https://doi.org/10.1146/annurev.micro.112408.134102

Yu L, Wang L, Chen S (2010) Endogenous toll-like receptor ligands and their biological significance. J Cell Mol Med 14:2592–2603. https://doi.org/10.1111/j.1582-4934.2010.01127.x

Zimmermann J, Wentrup C, Sadowski M, Blazejak A, Gruber-Vodicka HR, Kleiner M, Ott JA, Cronholm B, De Wit P, Erséus C, Dubilier N (2016) Closely coupled evolutionary history of ecto- and endosymbionts from two distantly related animal phyla. Mol Ecol 25:3203–3223. https://doi.org/10.1111/mec.13554