Aiptasia sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians

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Symbiosis, defined as the persistent association between two distinct species, is an evolutionary and ecologically critical phenomenon facilitating survival of both partners in diverse habitats. The biodiversity of coral reef ecosystems depends on a functional symbiosis with photosynthetic dinoflagellates of the highly diverse genus Symbiodinium, which reside in coral host cells and continuously support their nutrition. The mechanisms underlying symbiont selection to establish a stable endosymbiosis in non-symbiotic juvenile corals are unclear. Here we show for the first time that symbiont selection patterns for larvae of two Acropora coral species and the model anemone Aiptasia are similar under controlled conditions. We find that Aiptasia larvae distinguish between compatible and incompatible symbionts during uptake into the gastric cavity and phagocytosis. Using RNA-Seq, we identify a set of candidate genes potentially involved in symbiosis establishment. Together, our data complement existing molecular resources to mechanistically dissect symbiont phagocytosis in cnidarians under controlled conditions, thereby strengthening the role of Aiptasia larvae as a powerful model for cnidarian endosymbiosis establishment.
including gene expression, nutrient transfer, cell organization, and cell division, is largely unclear. Progress in understanding these processes mechanistically has been, and still is, slow because molecular tools for corals and their larvae are sparse. For example, the identification of key players involved in symbiosis establishment is still in its infancy; previous comparative transcriptomics in coral larvae were unable to find many candidates. One reason may be presumably low symbiont-to-host-cell ratios that masked signals from symbiont-carrying endodermal tissue. Furthermore, many corals spawn only once annually, severely limiting larval access and optimization of experiments.

The small sea anemone Aiptasia is an emerging model for coral symbiosis. Similar to corals, Aiptasia produces symbiont-free offspring that then establish symbiosis with various Symbiodinium strains but not others, suggesting that “symbiosis specificity” is a common, lineage-independent phenomenon among symbiotic cnidarians. Importantly, defined clonal lines are available for Aiptasia and for Symbiodinium, including axenic strains representing four of the nine described major Symbiodinium clades A-I. We recently established a robust protocol to induce Aiptasia spawning in the laboratory. We then described when and where during larval development symbionts are phagocytosed by endodermal cells, defining reproducible experimental conditions for analyzing symbiont acquisition, and developed a set of experimental tools. These resources, in conjunction with various transcriptomic, proteomic, and genomic resources for Aiptasia, Symbiodinium, and corals (for comparative analyses), provide the foundation to use Aiptasia larvae as a platform to study the molecular mechanisms of symbiosis establishment in cnidarians.

Here we further develop the Aiptasia larval system as a model for uncovering fundamental aspects of cnidian symbiont selection at the molecular level. We directly compared symbiont selection patterns between larvae of Aiptasia and of two major reef-building coral species: Acropora digitifera and Acropora tenuis. We found that the overall patterns of “symbiosis specificity” are maintained between lineages, suggesting common underlying mechanisms of symbiont selection in Aiptasia and corals. Using Aiptasia larvae as a model, we find that both uptake into the gastric cavity and phagocytosis into the endoderm play a role in distinguishing between compatible and incompatible symbionts, and we identified a set of genes likely involved in symbiosis establishment by RNA-Seq. We conclude that Aiptasia is a powerful system to dissect the complex molecular mechanisms underlying initial symbiont selection.

Results
Symbiosis patterns are similar in Aiptasia and Acropora. To date, a direct comparison of symbiosis establishment patterns between Aiptasia and corals is missing. To assess similarities and differences between Aiptasia and two major reef-building corals of the genus Acropora, A. tenuis and A. digitifera, we compared symbiont selection at early life stages using defined Symbiodinium strains. We incubated Aiptasia and coral larvae with four clonal, axenic Symbiodinium strains – SSA01, SSA02 (both clade A), SS01, clade B), SSE01 (clade E) – and the non-clonal, non-axenic strain CCMP2556 (clade D) for 10 days. We found that the strains SSA02, SS01, and CCMP2556 efficiently infected hosts, whereas strain SSE01 was found in lower proportions of the larval populations (Fig. 1a–c). Strain SSA01 (S. tenuis) was able to efficiently establish symbiosis with Aiptasia larvae but was rarely found in Acropora larvae (Fig. 1b–d). In line with the observation that the Symbiodinium strains SSA02, SS01, and CCMP2556 efficiently infect coral larvae, microscopic analysis shows that many Symbiodinium cells were taken up per larva, especially when compared to strains SSE01 and SSA01 (Fig. 1f; Supplementary Fig. S1).

Symbiosis establishment patterns are maintained between larva and polyp stages in Aiptasia, to test whether such similarities hold true for Acropora larvae and juvenile polyps, we exposed juvenile Acropora polyps to the five different Symbiodinium strains for four days, after which algae were removed from the environment; progression of infection was monitored for six additional days (Fig. 2; Supplementary Fig. S2). We found that, as in conspecific larvae, polyps were efficiently infected by the Symbiodinium strains SS01, SSA02, and CCMP2556 within the monitored time period (Fig. 2a). Symbiosis establishment occurred rapidly, with most polyps hosting algae after only 5 days and comparably robust populations after 10 days (Fig. 2a). Residential algal populations increased after the removal of environmental algae, indicating in hospite symbiont proliferation (Fig. 2a). Symbiodinium strains SSA01 and SSE01 failed to effectively infect polyps of either coral species, as these algae were nearly undetectable in polyps (Fig. 2; Supplementary Fig. S2).

Symbiosis patterns established during early life stages may change over longer time periods. Therefore, we tested whether the two symbiont types that infect Acropora most efficiently, SS01 and CCMP2556, were maintained over longer time periods in polyps under laboratory conditions. We exposed A. tenuis and A. digitifera larvae to either Symbiodinium strain for nine days, induced metamorphosis, and monitored the in hospite algal populations in the polyps with light microscopy. At 2 days post-metamorphosis (dpm). At 2 dpm, the majority of polyps hosted symbionts: for A. tenuis, 35 out of 35 polyps had SS01 and 34 of 34 had CCMP2556; for A. digitifera, 12 of 16 had SS01 and 26 of 27 had CCMP2556. At 49 dpm, 100% of the polyps were infected. At 49 dpm, 100% of polyps remained infected, although polyp budding and mortality led to different polyp numbers: for A. tenuis, 43 polyps with SS01 and 41 with CCMP2556; for A. digitifera, 25 polyps with SS01 and 18 with CCMP2556. Although all polyps were infected in the duration of the experiment, the populations of both CCMP2556 and SS01 appeared to decrease during the monitored time period, SS01 apparently more drastically than CCMP2556 (Fig. 3).

Early acquisition steps are more efficient for compatible symbionts than incompatible symbionts. Our comparative analysis showed striking similarities in symbiont selection between A. tenuis, A. digitifera, and Aiptasia, with high compatibility of SS01 and low compatibility of SSE01. To better understand the common principles underlying the broad preference for SS01 over SSE01, we used Aiptasia larvae as a model and compared two distinct steps during the early phase of symbiosis establishment: uptake of symbionts into the
gastric cavity and phagocytosis of symbionts into the endodermal tissue. We infected Aiptasia larvae 6–7 days post-fertilization (dpf) for four days with either SSB01, SSE01, or inert fluorescent polystyrene beads (7 μm diameter) and again found that SSB01 was taken up more efficiently by Aiptasia larvae (an average 65% of larvae contained one or more symbionts) than SSE01 and inert beads (19% and 10%, respectively) (Fig. 4a). When distinguishing the localization of algae inside larvae (Fig. 4b), we found that the majority of SSB01 algae appeared to be integrated in the endoderm (63%; 1072/1693), compared to only 33% (65/198) of SSE01 algae (Fig. 4c). Interestingly, of the beads that were taken up by larvae, a higher proportion (49%; 48/98) than SSE01 were found in the endoderm (Fig. 4c). Because SSE01 has been thought to be free-living, its endodermal localization was surprising. We therefore imaged larvae at the tissue and cellular level using confocal microscopy to demonstrate that, indeed, SSB01 and SSE01 are found intracellularly in the endodermal cells (Fig. 4d,e).

**Figure 1. Symbiosis patterns are similar between Aiptasia and Acropora.** (a–c) Percentage of infected larvae of Aiptasia (a) and the corals Acropora digitifera (b) and Acropora tenuis (c) after exposure to the indicated Symbiodinium strains at 10,000 algal cells/ml for 10 days. Strains used in this study are SSB01 (clade B), SSA01 (clade A), CCMP2556 (clade D), SSA02 (clade A), and SSE01 (clade E). Infected larvae contain one or more algal cells. Error bars are SEM of 3 replicate experiments. (d) Representative images of Symbiodinium infections in A. digitifera larvae. Left panels are brightfield images, right panels are red autofluorescence of algal photosynthetic pigments. Note the diffuse weak autofluorescence of larvae that is distinct from the bright puncta of the algae.
In other systems, phagocytosis efficiency is dependent on size and shape of the phagocytosed particle, with larger particles being phagocytosed less efficiently than smaller ones\(^{36}\). As noted previously, SSE01 cells...
are relatively large, especially compared to SSB01 cells (Fig. 4b). To ask whether the cell size of SSE01 may correlate with phagocytosis efficiency, we compared cell sizes of these algae found in the gastric cavity and in the endoderm. SSE01 cells in the endoderm were significantly smaller than algae in the gastric cavity (8.5 μm vs. 11 μm), whereas such a difference could not be detected for SSB01 cells (Fig. 4f). Together, our results indicate that the compatible symbiont strain SSB01 is more efficiently taken up into the gastric cavity and phagocytosed into the endoderm, with the latter process potentially influenced by algal cell size.

Identification of candidate genes involved in symbiosis by RNA-Seq. Identification and functional characterization of key players involved in symbiosis establishment and selection during larval stages is crucial...
to understand these processes at the molecular level. We therefore used RNA-Seq to compare gene expression between symbiotic and non-symbiotic *Aiptasia* larvae. *Aiptasia* larvae (5 dpf) were either infected with SSB01 or kept non-symbiotic as a control for five days. We identified 19,771 genes (of a total of 26,039 genomic gene models) expressed in at least one of the four replicates (two symbiotic and two non-symbiotic samples) with an FPKM value over 0. Of these genes, the difference in expression levels were significant for 351 genes between the two states (False Discovery Rate ≤ 0.1). The majority of differentially expressed genes (n = 219) are down-regulated in the symbiotic state, resulting in an average log2 fold change of −2.3. However, the log2 fold changes ranged from −10.9 to 10.8. (Supplementary Table S1).

To assess and illustrate clustering and variation between replicates, gene expression data was plotted as a multidimensional scaling plot (MDS plot) (Supplementary Fig. S3).

**Figure 5. Differential expression of genes putatively involved in symbiosis establishment in *Aiptasia* larvae.**

Heatmap of 41 significantly differentially expressed genes. Gene expression was calculated over two replicates of aposymbiotic (A1, A2) and symbiotic (S1, S2) samples each. Up-regulation of gene expression is shown in yellow, down-regulation in blue. Genes were categorized by KEGG pathway annotations (purple bars) potentially involved in symbiosis establishment: Phagocytosis (ko04145: Phagosome, ko04666: Fc-gamma mediated phagocytosis), Cytoskeleton (ko04812: Eukaryotic cytoskeleton proteins, ko04810: Regulation of actin cytoskeleton), Endocytosis (ko04144), Lysosome (ko04068), P13K-Akt signaling (ko04151), FoxO signaling (ko04068) and Compound transport. KEGG annotation was automated based on homology.

Recent transcriptomic and proteomic approaches in different systems have identified a set of symbiosis-specific candidate genes, and we found many similar genes among our list of differentially expressed genes (DEG); for example, the lysosomal Niemann-Pick disease type C2 (NPC2) protein transmembrane receptors that may play a role in symbiont recognition, and small GTPases potentially involved in endocytotic vesicle transport during phagocytosis (Fig. 5). However, our dataset also identified many new genes encoding factors potentially involved in symbiosis establishment. Using the functional annotations of the Kyoto Encyclopedia of Genes and Genomes (KEGG), we found in total 41 DEGs related to phagocytosis, endocytosis, lysosomes, signaling pathways, cytoskeletal reorganization, and transport of compounds between partners. Among these genes, 9 genes are up- and 32 are down-regulated in the symbiotic state (Fig. 5). Thus, RNA-Seq of whole larvae is sufficiently sensitive to identify many genes putatively involved in symbiosis establishment.
Discussion

“Symbiosis specificity”, defined as non-random host/symbiont combinations, may have broad ecological implications for coral reef ecosystems; however, this remains a matter of debate, with interpretations of the symbiosis ranging from specific and evolutionarily fixed to largely random and highly adaptive (e.g. during environmental change) [44-53]. Reaching a unified understanding of fundamental principles is difficult because of the complexity of the phenomenon: it is likely influenced by coral taxa, developmental stage, varying microhabitats of corals and symbionts, and local availability of symbionts. These factors, together with limited experiments under controlled laboratory conditions to standardize experimental design and replication, slow the progress of directly testing influences of host and symbiont. The molecular mechanisms underlying “symbiosis specificity” are likewise unclear and remain impossible to dissect without molecular tools and approaches.

To dissect “symbiosis specificity” using Aiptasia larvae as a model, we provide here the first direct comparison of symbiont uptake specificity in Aiptasia and Acropora under laboratory conditions using defined Symbiodinium strains. We tested two strains (SSB01 and SSA01) originating from Aiptasia [21,23,25] (two strains (SSA02 and CCMP2556) isolated from corals [21], and the presumably free-living strain SSE01 [21,33,35]. We find that patterns of symbiont selectivity are very similar between these taxa, indicating low lineage-constrained selectivity for Symbiodinium during early development stages (larval and juvenile polyp) independent of whether the algal strain originated from Aiptasia or a coral host. Of the Symbiodinium strains tested, three (SSB01, SSA02 and CCMP2556) are positively selected and one (SSE01) is negatively selected by Aiptasia and both Acroporids, even when the latter strain is present at high concentrations. This indicates that fundamental mechanisms of positive and negative symbiont selection are generally conserved between Aiptasia and Acropora during early life stages. SSA01 is a notable exception: it is efficiently acquired by progeny of its original host (Aiptasia) but rejected by both Acropora species tested. This comparative dataset is an important step towards dissecting fundamental and conserved principles (as well as differences) underlying host/symbiont compatibility and incompatibility in cnidarians.

Symbiosis establishment is a complex process comprising multiple steps, including symbiont uptake into the gastric cavity, phagocytosis by host cells, integration into host cell function, long-term persistence, and proliferation [45]. Each step, alone or in conjunction with others, may play a role during the establishment of suitable host/symbiont combinations. Further, each step may be influenced by specific molecular mechanisms as well as physical factors [46,54, this study]. In the first step, encounter efficiency may be important for initial uptake into the gastric cavity; for example, we have previously shown that symbiont uptake efficiency is concentration-dependent in Aiptasia larvae [44]. However, symbionts are presumably sparse in the natural environment and direct chemical attraction may facilitate host/symbiont meeting [44]. Once inside the gastric cavity, algae and host cell-cell contact is important to trigger phagocytosis. To date, it is unclear whether symbiont phagocytosis is restricted to certain cells with distinct receptors or whether it is an unspecific, evolutionarily conserved feeding mechanism in nutritive cells [45,58]. Many cell types are capable of engulfing particles, but phagocytosis requires fundamental changes in cell shape and architecture and, accordingly, particle size and shape directly influence cells’ phagocytosis efficiencies [46]. Despite having entered host cells, symbionts may still fail to populate hosts; various examples indicate that coral larvae initially take up heterologous symbionts that are ultimately not maintained over longer time periods [12,23] (Fig. 3). It is unknown whether this lack of long-term compatibility is due to symbiont expulsion, digestion, or a lack of proliferation capacities.

Indeed, our analyses in Aiptasia larvae directly comparing the broadly compatible symbiont strain SSB01 and the incompatible strain SSE01 confirm the collective influence of multiple steps for the efficiency of symbiont acquisition. Despite our observation that SSE01 cells are phagocytosed into Aiptasia larvae endodermal cells, we repeatedly find virtually no larvae stably infected with SSE01 under the tested conditions. This failure stands in contrast to the consistent high infection rates of SSB01 symbionts in larvae. SSB01 is taken up into the gastric cavity more efficiently than SSE01 (alternatively, the SSE01 expulsion rate may be higher); phagocytosis of SSB01 is more efficient than that of SSE01; and while the number of SSB01 algae inside larvae increases over time, SSE01 fail to persist in larvae at detectable levels (this study). Phagocytosis efficiency may be directly related to symbiont size, as SSE01 is phagocytosed at the lowest rates, yet our comparison of SSB01, SSE01, and small inert beads indicates that entering or persisting in the gastric cavity is not (Fig. 4a,c). It is noteworthy that SSE01, similar to other Symbiodinium types in Clade E, is thought to be free-living [23,33,35]. Our data suggest that the inability of SSE01 to establish stable symbiosis in cnidarians is not governed by the inhibition of phagocytosis into host cells, but may rather be a consequence of the inability of the two partners to initiate a functional molecular cross-talk after intracellularization. The direct involvement of more complex molecular mechanisms during symbiont selection is supported by our observation that SSA01 acceptance by cnidian hosts is opposite for Aiptasia and Acroporids: Aiptasia larvae take up SSA01 cells with high efficiency, but Acropora tenuis and Acropora digitifera do not (Fig. 1).

In the future, it will be also interesting to extend the analysis of changes in “symbiosis specificity” over longer time periods. Other studies have shown that juvenile A. tenuis larvae exhibit different specificity throughout ontogeny, with nonhomologous symbiont types (in clade D) being taken up first and replaced only after several months by the homologous symbionts (in clade C) that dominate adult colonies [34,58]. Our observations that populations of nonhomologous symbiont strains SSB01 (clade B) and CCMP2556 (clade D) declined over time in Acropora polyps are consistent with this phenomenon (Fig. 3). However, Acropora polyps are notoriously difficult to keep in the laboratory, and the polyps we monitored under laboratory conditions suffered substantial mortality. Further, the low number of polyps in our experiment prevents us from drawing hard conclusions; additional experiments are needed under controlled and optimized growth conditions for juvenile Acropora. Alternatively, similar long-term experiments could be done with Aiptasia and defined Symbiodinium strains under controlled conditions, which would greatly increase reproducibility and comparability and further reveal the complex dynamics of symbiosis specificity over time. Likewise, Aiptasia may help reveal the influence of
environmental factors (e.g. elevated temperature) on symbiosis specificity, further highlighting the need for a laboratory-based model system to dissect “symbiosis specificity” in a systematic, controlled way.

To dissect distinct molecular mechanisms involved in “symbiosis specificity” and symbiosis establishment in general, the identification of key players is crucial. Here we used RNA-Seq as a proof-of-concept to identify symbiosis-specific genes in *Aiptasia* larvae, and we found over 300 genes that are differentially expressed in symbiotic versus non-symbiotic larvae. Notably, a suite of those genes are involved in pathways and biological processes that were previously identified to play a role in the cnidarian-dinoflagellate endosymbiosis, validating this approach48,9,29,32,39–43. RNA-Seq is an easy, rapid, and cost-effective technique, and in the future it can be combined with cell biological analyses to reveal the functions of identified candidates in the symbiosis.

We propose that *Aiptasia* larvae, together with the suite of compatible and incompatible symbiont strains, constitute a powerful platform to elucidate fundamental mechanisms of the distinct steps involved in symbiosis establishment in cnidarians. We envision exploiting the *Aiptasia* larvae model by taking advantage of the easily controllable laboratory system and available molecular and cell biological tools, including RNA-Seq, to mechanistically dissect how stable host/symbiont combinations are established. Moreover, we can begin to dissect the various contributions of environmental conditions, host and symbiont genotype, and ontogeny to the phenomenon of “symbiosis specificity”. Ultimately, such laboratory experiments may also help to better understand and predict the potential of certain symbiotic associations for the resilience and adaptive capacities of coral reefs to environmental change.

**Methods**

**Collection and maintenance of *Acropora planulata* larvae.** Colonies of *Acropora digitifera* and *Acropora tenuis* were collected with permission by the Okinawa prefecture (#27-1) at Sesoko Island (26°37′41″N, 127°51′38″E, Okinawa, Japan). Corals were kept in tanks with running natural seawater and under partially shaded natural light at Sesoko Marine Station (University of Ryukyus, Okinawa, Japan). After spawning on May 31 2015, bundles of symbiont-free gametes from multiple colonies of each coral species were mixed and the resulting planula larvae were maintained in plastic bowls at approximately 1000 larvae/L in 10 μm-filtered natural seawater (FNSW). FNSW was exchanged daily.

**Aiptasia culture conditions and spawning induction.** Spawning of *Aiptasia* clonal lines CC7 and F003 (for symbiosis establishment studies) and clonal lines CC7 and H2 (for transcriptomic comparisons) was induced as previously described22. *Aiptasia* larvae were kept in filter-sterilized artificial seawater (FASW) in glass beakers at 26°C on a 12 h light:12 h dark (12L:12D) cycle.

**Symbiodinium cultures and conditions.** For infection of cnidarian hosts, we used the following clonal and axenic *Symbiodinium* strains: SSB01 (clade B), SSA01 (clade A), SSA02 (clade A), and SSE01 (clade E)21,23 as well as the non-clonal, non-axenic *Symbiodinium* culture CCMP2556 (clade D) from the scleractinian coral *Orbicella faveolata* (formerly *Montastrea faveolata*)22 purchased from the National Center for Marine Algae and Microbiota (NCMA, Bigelow Laboratory for Ocean Sciences, Maine, USA). All cultures were grown in cell culture flasks in IMK medium27 at 26°C on a 12L:12D cycle under 20–25 μmol m⁻² s⁻¹ of photosynthetically active radiation (PAR), as measured with an Apogee PAR quantum meter (MQ-200; Apogee, Logan, USA).

**Symbiosis establishment experiments in *Acropora* and *Aiptasia*.** *Aiptasia* larvae. For infection experiments, *Aiptasia* larvae 2 days post-fertilization (dpf) were distributed into 6-well plates at 300 larvae in 5 ml of FASW per well. *Symbiodinium* cells were added to each well at a final concentration of 10,000 algal cells/ml; FASW was used as a negative control. Three biological replicates (e.g. spawning crosses) were used per algae/host combination. Plates were kept in incubators at 26°C under white fluorescent bulbs at 20–25 μmol m⁻² s⁻¹ on a 12L:12D cycle with regular exchange of FASW; *Symbiodinium* cells were re-added after each wash. After ten days of exposure to *Symbiodinium*, larvae were fixed for 30 min in 4% formaldehyde in seawater, washed 3 times in PBS, and mounted in 87% glycerol in PBS for analysis. Over 45 *Aiptasia* larvae were counted per algae/host replicate.

The experiments in Fig. 4 were carried out with the following changes: larvae were 6–7 dpf at start of infection; *Symbiodinium* cells of strains SSB01 and SSE01 and inert polystyrene fluorescent beads (#C36950, Thermo Fisher Scientific) were added at a final concentration of 100,000 particles/ml; larvae were fixed for analysis after 4–5 days of exposure to algal cells. Over 85 *Aiptasia* larvae were counted per algae/host replicate.

**Acropora larvae.** At 4 dpf, *Acropora* larvae of either coral species were distributed into 24-well cell culture plates, with 30 larvae in 2 ml FNSW per well. *Symbiodinium* cells were added to each well at a final concentration of 10,000 algal cells/ml; FNSW was used as a negative control. Three technical replicates (i.e. wells) were used per algae/host combination. Plates were kept at ~25°C under ambient room light (~9–12 μmol m⁻² s⁻¹) on an approximate 12L:12D cycle. Larvae were washed with FNSW every one or two days as appropriate; *Symbiodinium* cells were re-added after each wash. After ten days of exposure to *Symbiodinium*, larvae were mounted in FNSW on glass slides with glass coverslips for analysis. An average of 22 larvae were counted per technical triplicate; for details, see Supplementary Table S2.

**Acropora polyps: short-term exposure.** At 6 dpf, larvae of either species were induced to metamorphose and settle in 6-well plates through overnight incubation in 5 ml FNSW supplemented with 1 μM Hym-248 neuropeptide48. The following day, 30–60 polyps had metamorphosed and settled per plate (~5–10 polyps in per well). *Symbiodinium* cells were added to each well at a final concentration of 10,000 algal cells/ml; FNSW was used as a negative control. Plates were kept at ~25°C under ambient light (~9–12 μmol m⁻² s⁻¹) on an...
Table S3.

Acropora polyps: long-term exposure. At 5 dpf, larvae of either species were distributed into plastic bowls at 250 larvae per 200 mL FNSW. Symbiodinium cells of strains SSB01 or CCMP2556 was added to each bowl at a final concentration of 10,000 algae cells/mL. FNSW was used as a negative control. Bowls were kept on the bench at ~25 °C under ambient light (~9–12 μmol m⁻² s⁻¹) on an approximate 12L:12D cycle. At 12 dpf larvae were package at a density of ~1000 larvae/L in 50 mL conical tubes and overnight shipped to the Hatta lab, Tokyo. At 14 dpf, environmental algae were removed by exchanging FNSW without re-addition of Symbiodinium. Polyps were then washed with daily exchange of seawater, which was a 50%/50% mixture of FNSW sterilized at 105 °C for 3 min and artificial seawater (Coral Pro, RedSea). Polyps were qualitatively assessed as hosting symbionts by light microscopy and photography at 2, 3, and 49 days post-metamorphosis (dpf). At 23 dpf, polyp numbers were those from the initial metamorphosis; at 49 dpf, budding and mortality led to new population sizes: for A. tenuis, 43 polyps with SSB01 and 41 with CCMP2556; for A. digitifera, 25 polyps with SSB01 and 18 with CCMP2556.

Microscopy. Acropora larvae and polyps were analyzed using a Leica S8APO stereoscope equipped with a Leica MCI100 HD color camera. Endogenous autofluorescence of Symbiodinium photosynthetic pigments was visualized using the Leica S8APO stereoscope in combination with a Stereomicroscope Fluorescence Adapter kit (SFA-LFS-Green, NightSea, USA). Microscopic analysis of Aiptasia larvae was carried out with a Nikon Eclipse Ti inverted microscope using Differential Interference Contrast (DIC) and a Nikon Plan Fluor 20x dry lens. Microscopic images of Aiptasia larvae were captured with a Nikon Eclipse 80i microscope using DIC and a Digital Sight DS-U1 color camera (Nikon Instruments).

Analysis of differential gene expression. RNA isolation and sequencing. For the analysis of differentially expressed genes, the Aiptasia larval RNA-Seq datasets described in Baumgarten et al. were obtained from the NCBI SRA database, with two replicates for aposymbiotic (accession no.: SRX757531) and symbiotic (accession no.: SRX757532) larvae. Briefly, larvae 3–4 dpf were infected with Symbiodinium strain SSB01 at a concentration of 2.5–5 × 10⁴ algae/mL for 5–6 days (FASW as negative control). Two separate crosses were used for duplicate pairs, with cross 1 containing 6,500 larvae per treatment and cross 2 containing 8,400 larvae per treatment. For all samples, total RNA was extracted using TRIzol (#15596, Thermo Fisher Scientific) and the mRNA was purified using Dynabeads oligo(dT)25 (#61002, Thermo Fisher Scientific). The sequencing libraries were prepared with the NEBNext Ultra Directional RNA Library Prep Kit (#E7420, NEB) with 180 bp insert size and sequenced on an Illumina HiSeq2000 at 2×101 bp read length. As a mapping reference for the calculation of transcript abundances, we used the Aiptasia genomic gene set (NCBI Genome ID: 40858, Accession: JXWW01000000).

Sequencing adapters and low quality reads were trimmed and filtered from the sequence reads using Trimomatic. The reads were mapped to the reference genomic gene set using bowtie2 with settings "--a -X 5000-no-unal--rdg 6,5--rfq 6,5--score-min L,6,.4--no-discardant--no-mixed--phred33". Read count abundances were calculated using eXpress with settings "--rf-stranded" and a read count table was generated using a custom perl script.

Statistical analysis. Significantly differentially expressed genes (DEGs) were subsequently calculated in R using the package edger. Specifically, to test for differential expression of genes between symbiotic and aposymbiotic Aiptasia larvae, first the variation of gene expression between replicates was determined. Two types of variation contribute to the total variation in RNA-Seq experiments and are expressed as follows: 1) technical coefficient of variation, which describes the measurement error derived from the uncertainty with which the abundance of every gene is estimated from the sequencing platform. This variation decreases with increasing total counts for each gene in an RNA sample and can be distinguished from 2) the biological coefficient of variation (BCV). The latter denotes the variation of the true, unknown expression of each gene among biological replicates, which remains even at indefinite sequencing depth. This represents the most important source of variation in RNA-Seq experiments and is calculated from the biological replication prior to the test of differential expression between...
Gene annotation. KEGG pathway identifiers of the differentially expressed genes were obtained from the gene annotations. To sort DEGs into higher level biological processes, we used the automated KEGG pathway annotations for Aiptasia. DEGs were sorted by their KEGG annotations into the processes 'Phagocytosis' (ko04145), 'Phagosome, ko04666; Fc-gamma mediated phagocytosis), 'Cytokkeleton' (ko04812: Eukaryotic cytok skeleton proteins, ko04810: Regulation of actin cytok skeleton), 'Endocytosis' (ko04144), 'Lylosome' (ko04142), 'PI3K-akt signaling' (ko04151), 'FoxO' (ko04068) as well as 'Compound transport'. The log2 fold changes of DEGs belonging to either of these groups were calculated with the predFC function in edgeR, normalized to Z-scores following the formula $z = (x-u)/s$ ($z = Z$ score; $x = fold change; u = mean fold change across all replicates; s = standard deviation across all replicates) and visualized as a heatmap in MeV.

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Author Contributions
I.W., P.A.V., E.A.H. and A.G. designed the experiments; I.W., P.A.V. and M.H. performed the experiments. S.B. and C.R.V. analyzed gene expression data. C.R.V., M.H. and A.G. provided materials, reagents and tools. I.W., S.B., P.A.V., E.A.H. and A.G. wrote the manuscript. All authors reviewed the manuscript.

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