Developmental Origin and Evolution of Bacteriocytes in the Aphid–Buchnera Symbiosis

Christian Braendle\textsuperscript{1,2,\ast}, Toru Miura\textsuperscript{3,\ast}, Ryan Bickel\textsuperscript{1}, Alexander W. Shingleton\textsuperscript{1}, Srinivas Kambhampati\textsuperscript{4}, David L. Stern\textsuperscript{1,\ast}

1 Department of Ecology and Evolutionary Biology, Princeton University, Princeton, New Jersey, United States of America, 2 Laboratory for Development and Evolution, University Museum of Zoology, Cambridge, United Kingdom, 3 Department of Biology, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan, 4 Department of Entomology, Kansas State University, Manhattan, Kansas, United States of America

Symbiotic relationships between bacteria and insect hosts are common. Although the bacterial endosymbionts have been subjected to intense investigation, little is known of the host cells in which they reside, the bacteriocytes. We have studied the development and evolution of aphid bacteriocytes, the host cells that contain the endosymbiotic bacteria \textit{Buchnera aphidicola}. We show that bacteriocytes of \textit{Acyrthosiphon pisum} express several gene products (or their paralogues): Distal-less, Ultrabithorax/Abdominal-A, and Engrailed. Using these markers, we find that a subpopulation of the bacteriocytes is specified prior to the transmission of maternal bacteria to the embryo. In addition, we discovered that a second population of cells is recruited to the bacteriocyte fate later in development. We experimentally demonstrate that bacteriocyte induction and proliferation occur independently of \textit{B. aphidicola}. Major features of bacteriocyte development, including the two-step recruitment of bacteriocytes, have been conserved in aphids for 80–150 million years. Furthermore, we have investigated two cases of evolutionary loss of bacterial symbionts: in one case, where novel extracellular, eukaryotic symbionts replaced the bacteria, the bacteriocyte is maintained; in another case, where symbionts are absent, the bacteriocytes are initiated but not maintained. The bacteriocyte represents an evolutionarily novel cell fate, which is developmentally determined independently of the bacteria. Three of five transcription factors we examined show novel expression patterns in bacteriocytes, suggesting that bacteriocytes may have evolved to express many additional transcription factors. The evolutionary transition to a symbiosis in which bacteria and an aphid cell form a functional unit, similar to the origin of plastids, has apparently involved extensive molecular adaptations on the part of the host cell.

Introduction

Endosymbiosis is common in insects, with more than 10\% of insect species relying upon intracellular bacteria for their development and survival (Baumann et al. 2000). Full genome sequencing of the endosymbiotic bacteria, \textit{Buchnera aphidicola}, of several species of aphids has revealed extensive gene loss (Shigenobu et al. 2000; Tamas et al. 2002; van Ham et al. 2003), but has failed to reveal the genetic basis for the interaction between the bacteria and host cells. The key adaptations that allow incorporation of the bacteria into host cells may therefore be encoded by the host genome.

The symbiotic bacteria of aphids, \textit{B. aphidicola}, live within large polyploid cells, called bacteriocytes, which are grouped into organ-like structures, called bacteriomes, located adjacent to the ovarioles. During most of the aphid lifecycle, embryos develop parthenogenetically from unfertilized diploid oocytes, and multiple embryos develop serially within a single ovariole (Dixon 1985) (Figure 1A). Maternal bacteria are transferred directly to the developing blastoderm-stage embryos through an opening in the posterior of the embryo (Buchner 1965; Miura et al. 2003) (Figure 1B). Several researchers have described this transovarial transfer of bacteria (e.g., Uichanco 1924; Klevenhusen 1927; Toth 1933, 1938; Lampel 1958; Buchner 1965), but the details of bacteriocyte development have remained unclear.

We have identified bacteriocyte-specific markers that allow us to track the proliferation of bacteriocytes throughout the development of the pea aphid \textit{Acyrthosiphon pisum} (Harris) (Hemiptera: Aphididae). Using these markers, we aimed to determine the developmental origin of bacteriocytes and to what extent bacteria are required for the formation of the bacteriocytes. We also tested whether the observed patterns of bacteriocyte development are evolutionarily conserved among distantly related aphid species. We show that three transcription factors are expressed in a specific temporal order during early bacteriocyte development of the pea aphid. The final population of bacteriocytes originates from two distinct populations of nuclei recruited at different times...
of development. Furthermore, we experimentally demonstrate that the specification and proliferation of bacteriocytes occur independently of \textit{B. aphidicola}. In distant relatives of the pea aphid, we found that the two-step determination of bacteriocytes is conserved. We also investigated two cases involving the loss of \textit{B. aphidicola}. In the first case, in which the bacterial symbionts have been replaced with extracellular, eukaryotic symbionts, bacteriocyte development appears to proceed normally. In a second case, in which males do not inherit \textit{B. aphidicola}, the bacteriocytes have been lost.

**Results**

Three Transcription Factors Are Expressed in a Specific Temporal Order during Early Bacteriocyte Development

We tested five cross-reacting antibodies (see Materials and Methods) for their expression patterns in aphids. In every case, we observed antibody staining in the expected population of cells in the developing embryo (also see Miura et al. 2003). In addition, we found that three of the antibodies stained nuclei that form bacteriocytes of \textit{A. pisum}. We infer
that these antibodies are recognizing the homologues, or possibly paralogues, of their respective target proteins. The three proteins are expressed in a specific temporal order. We first observe expression of the Distal-less (Dll) protein (FlyBase ID: FBgn0000157) (Panganiban et al. 1994) in syncytial nuclei at the posterior of the blastoderm embryo just prior to the invasion of bacteria into the embryo (Figure 1C). As the bacteria enter the embryo, these nuclei associate with the bacteria and start to express a second protein, Ultrabithorax (Ubx) (FBgn0003944) or Abdominal-A (Abd-A) (FBgn0000014) or both, detected by the FP6.87 antibody (Kelsh et al. 1994) (Figure 1D–1F). The bacteria can be easily observed as spheres 2–4 µm in diameter (Buchner 1965) that stain with phalloidin (Figure 1D–1F). As the transfer of bacteria to the embryo is being completed, expression of the Engrailed (En) protein (FBgn0000577) (Patel et al. 1989) is detected (Figure 1G).

Two Populations of Cells Are Recruited to the Bacteriocyte Fate at Different Times in Development

The early embryo contains approximately eight bacteriocyte nuclei that express Dll (Figure 1C), whereas the adult aphid contains 60–90 uninucleate polyploid bacteriocytes (Baumann et al. 2000) that also express Dll (data not shown). We found that the increase in bacteriocyte number occurs through two mechanisms. First, we infer that the original bacteriocyte nuclei divide, apparently in a syncytium and perhaps synchronously, through two rounds of division because we observe that the number of Dll-expressing nuclei increases from approximately eight to 16 by stage 12 and then to approximately 32 by stage 13 (data not shown). By stage 14, these original bacteriocytes have formed cell membranes and become polyploid (Figure 2A). At stage 13, a second population of approximately 40–60 cells located near the
posterior end of the dorsal germband begins to express Dll (Figure 2B). The nuclei of these cells are visibly smaller than those of the original bacteriocytes (Figure 2A–2E). Based on observations of multiple fixed specimens, we infer that these cells then migrate across the germband (Figure 2E) and intercalate between the original bacteriocytes (Figure 2C and 2D). The bacteria are presumably then subdivided among all of the Dll-expressing nuclei and the final bacteriocytes are formed.

Bacteriocytes Are Specified and Maintained When the Bacteria Have Been Experimentally Removed

The observations described in the first section suggest that the initial specification of the bacteriocyte may occur independently of *B. aphidicola*. We tested this idea by eliminating *B. aphidicola* from pea aphids by feeding aphids on an artificial diet containing antibiotics. We found that the embryos within these aposymbiotic aphids specify the bacteriocyte cell fate, as revealed by Dll expression, and maintain the bacteriocyte cell fate in the absence of bacteria (Figure 3). In addition, we have observed that the number of bacteriocytes in aposymbiotic embryos increases precisely as described for symbiotic embryos, including the second wave of bacteriocytes (Figure 3F; data not shown).

The Two-Step Determination of Bacteriocytes Is Evolutionarily Conserved

The two-step determination of bacteriocytes described in the previous sections appears to be a conserved feature of the aphids. Using the anti-Dll antibody, we examined development of the bacteriocytes in two species of aphids that diverged from *A. pisum* (subfamily Aphidinae) approximately 80–150 million years ago (von Dohlen and Moran 2000): *Pemphigus spyrothecae* (Eriosomatinae) and *Tuberaphis styraci* (Hormaphidinae) (discussed below). In both cases, Dll is expressed in a small number of bacteriocyte nuclei of the blastoderm-stage embryo and additional Dll-expressing cells are recruited later. In *P. spyrothecae*, one or two nuclei are originally determined as bacteriocytes, as suggested by Lampel (1958) (Figure 4A). These nuclei become highly polyploid prior to bacterial invasion and do not divide (Figure 4B and 4C). A second population of bacteriocytes is determined at approximately stage 14 (Figure 4D). These surround the original bacteriocyte (Figure 4E) and appear to divide the bacteria into independent bacteriocytes.

Bacteriocytes Develop in Aphids in Which the Bacteria Have Been Replaced with Extracellular Eukaryotic Symbionts

*B. aphidicola* has been lost in the lineage leading to *T. styraci* and has been replaced by a yeast-like symbiont (Buchner 1965; Fukatsu and Ishikawa 1992a; Fukatsu et al. 1994). These symbionts live in the hemolymph and occasionally invade cells of the fat body (Buchner 1965). Previous studies have therefore claimed that these species lack bacteriocytes (Buchner 1965; Fukatsu and Ishikawa 1992a). We found that these aphids contain one or two nuclei in the posterior of the blastoderm embryo that express Dll (Figure 5A). These nuclei divide once or twice and then become polyploid. At approximately stage 14, we observed a second population of Dll-expressing cells that migrate to the original Dll-expressing cells (Figure 5B). Therefore, *T. styraci* appears to retain the

**Figure 4.** Expression of Dll in Bacteriocytes and the Pattern of Bacteriocyte Development Are Conserved in Parthenogenetic Females of *P. spyrothecae*

Confocal micrographs of *P. spyrothecae* parthenogenetic embryos stained with anti-Dll antibody (red).

(A) Dll is first detected in stage 6 embryos in one or two nuclei posterior to the cellular blastoderm (arrow).

(B) By stage 8, the bacteria have been transferred to and entirely fill the embryo (red arrowhead). The Dll-expressing nuclei (arrow) have become highly polyploid.

(C and D) At stage 12, only the original bacteriocyte nuclei are observed expressing Dll (white arrow), but by stage 14 (D) additional nuclei (blue arrow) closely apposed to the dorsal germband express Dll.

(E) By stage 15, these new nuclei surround the original bacteriocyte, and at later stages the bacteria are divided into individual cells.

DOI: 10.1371/journal.pbio.0000021.g004
The aphid bacteriocyte expresses three transcription factors:Dll, En, and Ubx or Abd-A. These transcription factors play important roles during later stages of development in insects. For example, Dll is required for limb development, En is required for segmentation, and Ubx and Abd-A are the products of Hox genes, required for patterning thoracic and abdominal body regions (Kuner et al. 1985; Hidalgo 1996; Weatherbee et al. 1999; Panganiban and Rubenstein 2002). We know of no other cases in other insects in which any of these three transcription factors are expressed at such early stages of development as we have observed in the bacteriocytes (approximately cellular blastoderm). We cannot exclude the possibility that bacteriocytes evolved from a cell type that expressed this combination of transcription factors, but there are no obvious candidate cell types, such as fat cells or viteloophages, in other insects that fulfill this criterion. We do not yet know whether these genes are involved in the determination of bacteriocytes. However, bacteriocytes may require a novel combination of transcription factors to regulate the symbiont population and to mediate transovarial transmission.

We have demonstrated that two cell populations express Dll in spatially and temporally distinct patterns before incorporating bacteria. Our observation of the initial putative bacteriocytes in the blastoderm embryo is consistent with observations of earlier researchers, who suggested—based on morphological observations—that the nuclei located at the posterior of the embryo constitute the future bacteriocyte nuclei (Lampel 1958; Buchner 1965). In addition, we have found that the second population of presumptive bacteriocytes appears to migrate across the germ band to the original bacteriocytes, where they take up bacteria. This is an unusual process that has not to our knowledge been described previously. In contrast, earlier studies indicated that bacteriocyte proliferation occurs solely by cell division or by budding of small nuclei from an existing polyploid bacteriocyte nucleus (e.g., Lampel 1958). We have not yet performed experiments that would allow us to positively identify the embryonic origin of this second population of cells. Based on their position—posterior to the germ cells and dorsal—these cells may be the descendants of the nuclei of the central syncytium (syncytial nuclei in the center of the blastoderm embryo) (see Miura et al. 2003).

Our results suggest that B. aphidicola is required for neither bacteriocyte induction nor for the origin and migration of the second population of bacteriocytes. While bacteria do not seem to be required for the developmental maintenance of this cell type, the bacteria may provide signals to the cells that are involved in mediating the symbiosis at the physiological level. Nonetheless, the absence of an effect of the bacteria on
bacteriocyte development contrasts with other symbioses where the bacteria induce specific developmental changes in host tissues (McFall-Ngai and Ruby 1991). We investigated two cases in which B. aphidicola have been lost during the evolution of aphids. Given our observations that bacteria are not required for the developmental maintenance of bacteriocytes, it is possible that the bacteriocyte cell type might be lost if it had no other function. This does not appear to be the case. In the lineage including T. styriaci, B. aphidicola was lost and a eukaryotic "yeast-like" symbiont has been gained (Buchner 1965; Fukatsu and Ishikawa 1992a; Fukatsu et al. 1994). Buchner (1965) suggested that the bacteriocytes of Cerataphis freycinetiae, another species in the same lineage, are originally specified, become polyploid and then degenerate. We found Dll-expressing putative bacteriocyte nuclei to be specified and maintained over extensive periods of embryonic development in T. styriaci. Buchner documented considerable variation in the details of symbiotic transmission and bacteriocyte development, and it is possible that bacteriocyte development proceeds along different paths in these two species.

We also examined the development of bacteriocytes in males of P. spyrothecae. The males do not have bacteria and we have observed, consistent with observations of earlier researchers (Lampel 1958; Buchner 1965), that bacteriocytes are not maintained in this morph. We found that bacteriocytes initially express Dll, but this expression is not maintained, which is consistent with Lampel's and Buchner's observations that the original bacteriocytes appear to be present but are not maintained. In addition, we found that the second wave of bacteriocytes is also initiated, as shown by weak, weak Dll expression. It is not clear whether these cells are subsequently respecified or are eliminated.

B. aphidicola are derived from free-living bacteria (Baumann et al. 2000), and both the bacteriocyte and the symbiont must have evolved mechanisms for integrating the bacteria into the workings of the cell. The aphid—Buchnera symbiosis represents a particularly intimate form of symbiosis. In some symbioses, the bacteria reside both intra- and intercellularly and actively invade the host cell (Dale et al. 2001). In contrast, B. aphidicola always exist either within host cells, within a membrane-bound maternal package, or with host nuclei in a syncytium. This advanced stage of symbiosis is similar to the presumptive early stages of plastid evolution.

Materials and Methods

Aphid rearing and collecting. Colonies of A. pisum were reared on broad bean (Vicia faba) or alfalfa (Medicago sativa) (Miura et al. 2003). P. spyrothecae were collected from galls on Populus nigra var. italica in Cambridge and London, United Kingdom. T. styriaci were collected from galls on Styrax obassia in Gunma Prefecture, Japan. Asexual aphid embryos of various developmental stages were dissected and fixed as described previously (Miura et al. 2003).

Antibody staining. A limited number of antibodies recognize the homologues of their target proteins across insects. We tested five of these antibodies in aphids and found that three stained the bacteriocyte nuclei: rabbit anti-Dll (Panganiban et al. 1994), mouse anti-En (Dale et al. 1999), and mouse anti-Ubx (Abd-A (FP.6.87) (Kelsh et al. 1994), kindly provided by G. Panganiban, N. Patel, and R. White, respectively. Two antibodies, rabbit anti-Vasa (FBgn0005206 (a gift of C.-C. Chang [Chang et al. 2002]) and mouse anti-Even-skipped (Eve) (FBgn0005270) (Patel et al. 1994) did not stain bacteriocytes, but, as expected, anti-Vasa stained the germ cells (Chang et al. 2002) and anti-Eve stained cells in the nervous system (Patel et al. 1989). Secondary antibodies conjugated with fluorescent moieties (Jackson ImmunoResearch, West Grove, Pennsylvania, United States) were tested for cross-reactivity against aphid cells by staining embryos with secondary antibodies alone. No cross-reactivity was detected. We further tested whether an additional mouse antibody (mouse anti-digoxigenin; Jackson ImmunoResearch) cross-reacted with bacteriocyte nuclei, and it did not stain any parts of the aphid embryo. In addition, the anti-Dll, anti-En, and anti-Ubx/Abd-A all stained the expected cells (Patel et al. 1989; Kelsh et al. 1994; Panganiban et al. 1994; Miura et al. 2003) in other regions of the embryos, indicating that the antibodies were working as expected. Cell outlines were visualized by staining for F-actin with fluorescein-conjugated phalloidin. Embryos were stained using standard protocols (Miura et al. 2003) and visualized on Leica SP and Zeiss confocal microscopes.

Antibiotic treatment. In the pea aphid, Buchnera can be eliminated by treating animals with antibiotics (Wilkinson 1998). First-or second-instar aphids were fed on an artificial diet containing 50 μg/ml of the antibiotic rifampicin for 72 h (e.g., Caillaud and Rahbé 1994). Aphids were then transferred to leaves of Medicago arborea in Petri-dish cultures (Miura et al. 2003). Control aphids were treated identically, except that the antibiotic was omitted from the artificial diet. Embryos that were less than 4 d old (Miura et al. 2003) were dissected from aposymbiotic aphids within 2–4 d after the end of the antibiotic treatment and stained with anti-Dll and FP0.87 antibodies and fluorescein-conjugated phalloidin. The absence of bacteria in aposymbiotic aphids was confirmed by observation with a confocal microscope (see Figure 3).

Supporting Information

Accession Numbers

The FlyBase accession numbers discussed in this paper are FBgn0000014, FBgn0000157, FBgn0000577, FBgn0000606, FBgn0000984, and FBgn0000970.

Acknowledgments

We thank J. Truman for preparing the specimens in Figure 2; M. Caillaud for help generating the aposymbiotic aphids; Y. Rahbé for providing the artificial aphid diet; H. Shibao, B. Olynyk, and V. Olynk for help collecting aphids; and N. Moran, H. Frydman, R. Blackman, C. Dale, and anonymous referees for comments on the paper. We acknowledge financial support for CB (Boehringer Ingelheim Fonds, the Roche Research Foundation, and the Janggen-Poehn-Stiftung), TM (Grants-in-Aid from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and from the Research for the Future Program of the Japan Society for the Promotion of Science), SK (United States Department of Agriculture), and DLS (Biotechnology and Biological Sciences Research Council and National Institutes of Health).

Conflicts of Interest. The authors have declared that no conflicts of interest exist.

Author Contributions. CB, TM, and DLS conceived and designed the experiments. CB, TM, RB, AW, SK, and DLS performed the experiments. CB, TM, and DLS analyzed the data. CB, TM, and DLS wrote the paper.

References


Development of Aphid Bacteriocytes

PLOS Biology | http://biology.plosjournals.org Volume 1 | Issue 1 | Page 075


Functional Analysis of RSS Spacers

Based on sheer numbers, microbes should rule the world. Most don’t cause disease, but those that do have the advantage of multiplying and mutating at a much faster rate than any multicellular organism can. So how does a slowly reproducing, trillion-celled organism like a human protect itself? By having the right weapon for the job—and that requires an incredibly diverse arsenal. A new study by a team of researchers from Yale University School of Medicine, Duke University Medical Center, and Mount Sinai School of Medicine demonstrates how the creation of that arsenal depends on a complex series of interactions between key genetic elements and proteins during the formation of the white blood cells called lymphocytes.

Two heavy hitters of the immune system—B and T cells—each produce unique protein receptors that specifically recognize and mediate the killing of the variety of potential foreign invaders, or antigens, such as bacteria, viruses, and parasites. (B cells make immunoglobulin, or antibodies, and T cells make T-cell receptors.) But these lymphocytes are unlike other cells: instead of making proteins from genes they inherited, they custom-make their genes by recombining fragments of their genes into new configurations. This genetic reshuffling process, called V(D)J recombination, yields the diversity of molecules necessary to combat the billions of different antigens they might encounter. The V, D, and J refer to different clusters of DNA sequences that follow specific rules of recombination.

While the products of recombination vary, the method does not. The fragments are spliced and then reassembled in a highly regulated process directed and controlled by a stretch of DNA (called a recombination signal sequence, or RSS) next to the gene fragment. The recombination process, the researchers show, relies on complex interactions among different parts of the signal sequences and the proteins that regulate them at key steps along the recombination pathway.

Each RSS is made up of three components: the nonamer, which controls the ability of proteins to bind to the gene fragments and initiate recombination; the heptamer, which directs the splicing of the gene fragment; and the spacer, which regulates how the gene fragments are recombined. Mutations in the DNA sequence of each of the three RSS components show that all play a critical role in the ability of the gene fragments to recombine appropriately.

While it has been established that spacers, as their name suggests, ensure that the space between the nonamer and heptamer is correct, the researchers show that spacers also regulate recombination activity by providing protein-binding sites along the DNA sequences that affect recombination. While the nonamer is the most important determinant of recombination, changes in the spacer, these researchers demonstrate, produced dramatic changes in the ability of the gene fragments to recombine.

Past studies have shown that recombination depends on the presence and sequence of specific nucleotides, but the quality of that recombination, the researchers say, can’t be understood simply by analyzing those nucleotides in isolation. Generally speaking, highly conserved sequences have functional importance. But it would be a mistake, they suggest, to think that just because a nucleotide sequence isn’t highly conserved, it’s not biologically important. Using a computer model to predict how different protein-gene interactions affect recombination, the researchers demonstrate that a fuller understanding of the process depends on observing how all these elements—including those that aren’t highly conserved—interact throughout the recombination process.


Developmental Origins and Evolution of Buchnera Host Cells

When it comes to exploiting a niche, endosymbionts take the prize. In endosymbiosis, one organism—the endosymbiont—invasive of the cells of another, in some cases taking up residence in a way that actually benefits the host. Bacteria are particularly adept at making themselves indispensable by insinuating themselves into some fundamental aspect of an organism’s biology. The endosymbiotic hypothesis proposes that this is how certain eukaryotic organelles evolved from endosymbiotic bacteria. Insights into the mechanisms governing endosymbiosis will help biologists understand how this mutually beneficial relationship evolved and provide clues to one of the fundamental questions in biology: How did the eukaryotic cell evolve?

Over 10% of insect species rely on endosymbionts for their development and survival. In this issue, David Stern and colleagues look at one of the most studied pairs, the pea aphid and Buchnera aphidicola, and discover clues to the molecular foundation of their shared fate. (Buchnera, which can no longer survive outside its host cell, is thought to produce essential amino acids that the aphid cannot get on its own.)

While it is known that Buchnera are transferred from clusters of bacteriocytes in the mother to the adjacent early-stage embryo, it has been unclear how the bacteriocytes develop. Previous studies of the bacteria’s genome have failed to explain the genetic basis of Buchnera’s ability to invade aphid cells. Consequently, Stern and colleagues have focused on the bacteriocytes, the specialized insect cells that house Buchnera, shedding light on the development of these cells as well as on the evolutionary adaptations in the aphid that made the bacteriocytes hospitable to Buchnera.

The researchers show that bacteriocytes differentiate and proliferate independently of Buchnera’s presence in the cell, and they identify three aphid transcription factors (proteins that regulate gene expression) that are expressed in three distinct stages during early-bacteriocyte development in the aphid embryo. The first protein is
expressed just before *Buchnera* enters the embryo; a second, as the bacteria invades; and a third, after the transfer is nearly complete. A second wave of the same transcription factors occurs at a later stage in aphid embryo development and increases the population of bacteriocytes.

This two-step specification of bacteriocytes, which occurs in related *Buchnera*-carrying aphid species, appears to be an evolutionarily conserved feature of aphids. It even occurs in an aphid species that once had a *Buchnera* endosymbiont and now has a yeast-like symbiont that lives outside the bacteriocytes. But this process is not observed in males of another aphid species that do not carry *Buchnera*. While traces of the first transcription factor activated in bacteriocytes are evident, the characteristic gene-expression pattern is not, and the aphids have no mature bacteriocytes.

While it seems that the aphid has evolved new domains of expression in the bacteriocyte for these transcription factors—none of these transcription factors is expressed at a similar stage in other insects—the researchers cannot yet say whether these genes direct the specification of bacteriocytes. Still, these transcription factors are likely to play important roles in the bacteriocyte, suggesting that the union of aphids and *Buchnera* involved significant adaptations by the host.


**Supersensitive Worms Reveal New Gene Functions**

The past ten years saw great progress in the field of molecular genetics, as new tools gave scientists the ability to investigate entire genomes instead of just one or two genes at a time. In this paper, Ronald Plasterk and colleagues developed a systemic approach using *Caenorhabditis elegans*, a tiny nematode and the first animal to have its genome sequenced, to gather functional information on nearly 400 genes.

Many of the systemic approaches to discovering gene function involve either measuring or deleting messenger RNA (mRNA), the molecule that helps translate genes into proteins. The method used here, called RNA interference, or RNAi, follows the deletion approach by taking advantage of a cellular process bearing the same name. In nature, RNAi is thought to be an important part of the innate defense machinery in plants and animals, protecting them from invaders like viruses by interrupting the manufacture of viral proteins. To do this, short double-stranded RNA molecules with complementary sequences to the target gene inhibit the gene's function by disabling mRNA, which effectively shuts down the gene. By mimicking this natural process to turn off selected genes, scientists can find clues to how those genes might normally function by watching what happens when they are taken out of the picture.

With the fully sequenced worm genome, it is possible to create interfering RNAs for all of its 20,000 or so genes. And because worms eat bacteria—which can themselves be used to deliver interfering RNAs—worms are the perfect RNAi model organism. The researchers fed the worms RNAi-producing bacteria, then observed the effects on the worm or its offspring to infer the function of the targeted gene. As previously reported, repeating this experiment for every gene in the worm genome, yields about 10% of the worms displaying abnormalities ranging from embryonic death to uncoordinated movement, suggesting defects in genes controlling development or muscle control, respectively.

Having previously identified an RNAi-hypersensitive mutant worm strain, Plasterk and his colleagues repeated the experiment in the mutants and report proposed functions for 393 previously unknown genes. The types of abnormalities observed in the short-lived mutations induced by RNAi, they say, resemble the more stable mutations seen in the collection of worm mutations cataloged by worm researchers over the years. Though the DNA alterations for many of these mutations are not yet known, researchers know roughly where they occur in the genome. And the researchers show here that they can use their RNAi experimental results along with what is known about the mutants to identify several of the sequence alterations. They also performed what is believed to be the first analysis in which independently generated large-scale RNAi results were systematically compared to see how variable such RNAi results are, and the results have implications for similar approaches not just in worms but in plants and other animals.


**Monitoring Malaria: Genomic Activity of the Parasite in Human Blood Cells**

Every year, malaria kills as many as 2.5 million people. Of these deaths, 90% occur in sub-Saharan Africa, and most are children. While four species of the single-celled organism *Plasmodium* cause malaria, *Plasmodium falciparum* is the deadliest. Harbored in mosquito saliva, the parasite infects its human host as the mosquito feeds on the victim's blood.

Efforts to control the disease have taken on an increased sense of urgency, as more *P. falciparum* strains show resistance to antimalarial drugs. To develop new drugs and vaccines that disable the parasite, researchers need a better understanding of the regulatory mechanisms that drive the malarial life cycle. Joseph DeRisi and colleagues now report significant progress toward this goal by providing the first comprehensive molecular analysis of a key phase of the parasite’s life cycle.

While *P. falciparum* is a single-celled...