mTOR Complex 1 Implicated in Aphid/Buchnera Host/Symbiont Integration

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ABSTRACT Obligate nutritional endosymbioses are arguably the most intimate of all interspecific associations. While many insect nutritional endosymbioses are well studied, a full picture of how two disparate organisms, a bacterial endosymbiont and a eukaryotic host, are integrated is still lacking. The mTOR pathway is known to integrate nutritional conditions with cell growth and survival in eukaryotes. Characterization and localization of amino acid transporters in aphids suggest the mTOR pathway as a point of integration between an aphid host and its amino acid-provisioning endosymbiont Buchnera aphidicola. The mTOR pathway is unannotated in aphids and unstudied in any nutritional endosymbiosis. We annotated mTOR pathway genes in two aphid species, Acyrthosiphon pisum and Myzus persicae, using both BLASTp searches and Hidden Markov Models. Using previously collected RNAseq data we constructed new reference transcriptomes for bacteriocyte, gut, and whole insect tissue for three lines of M. persicae. Annotation of the mTOR pathway identified homologs of all known invertebrate mTOR genes in both aphid species with some duplications. Differential expression analysis showed that genes specific to the amino acid-sensitive mTOR Complex 1 were more highly expressed in bacteriocytes than genes specific to the amino acid-insensitive mTOR Complex 2. Almost all mTOR genes involved in sensing amino acids showed higher expression in bacteriocytes than in whole insect tissue. When compared to gut, the putative glutamine/arginine sensing transporter ACYPI000333, an ortholog of SLC38A9, showed 6.5 times higher expression in bacteriocytes. Our results suggest that the mTOR pathway may be functionally important in mediating integration of Buchnera into aphid growth and reproduction.

KEYWORDS mTORC1 symbiosis Myzus persicae transcriptome

Two species living together in symbiosis can reciprocally impact each other’s evolution (Ehrlich and Raven 1964). The most intimate symbioses are those where one species resides inside the cells of the second species in obligate endosymbiosis (Wernegreen 2004). In plant-sap feeding insects such obligate endosymbioses are typically nutritional and feature hosts that require their endosymbiont for reproduction, and endosymbionts that are unable to survive outside their host. Despite the apparent harmony between members of these nutritional endosymbioses the partners must balance conflict and cooperation for the relationship to persist (Bennett and Moran 2015; Wernegreen 2004). The host must not completely reject the intracellular symbiont population but instead must provision the metabolites required for endosymbiont survival. In turn, the endosymbiont must persist within the host cell without imposing a fatal cost upon the host. Members of an endosymbiosis face unique and separate selection pressures that govern their evolution as discrete species, while existing in a robust state of integration (Bennett and Moran 2015; Keeling and McCutcheon 2017). Despite the evolutionary, ecological, agricultural and economic importance of many insect obligate nutritional endosymbioses a full picture of how these relationships are functionally regulated by their members is lacking. Therefore an understanding of how these endosymbiotic interactions evolved, are maintained, and indeed how they might be targeted for human control is also wanting.

The most-studied insect model of a host endosymbiont relationship is that of aphids and their endosymbiont, Buchnera aphidicola. Buchnera are housed within specialized aphid organs called bacteriomes, where they are contained inside bacteriocyte cells.
amino acids in aphids have recently been identified, and competitively inhibited by arginine, a host-controlled supply of the precursor glutamine via a glutamine-specific amino acid transporter which is highly expressed in bacteriocytes and competitively inhibited by arginine, a Buchnera-produced end-product amino acid (Price et al. 2014; Price et al. 2015). Second, microbial small RNAs that are conserved across Buchnera from different aphid species have been proposed to function in Buchnera gene regulation (Hansen and Degnan 2014). In particular, life-stage-dependent differential expression of amino acid biosynthesis pathways within Buchnera has been hypothesized to result from post-transcriptional small RNA regulation within Buchnera (Hansen and Degnan 2014). Third, microRNAs (miRNAs) encoded in aphid genomes have been found to show bacteriocyte-specific expression (Feng et al. 2018). Remarkably, of the 14 miRNAs highly or differentially expressed within aphid bacteriocytes, ten have previously been characterized to play roles in host/microbe interactions (Feng et al. 2018). The emerging picture of host/endo-symbiont regulation is one of multiple concurrent and overlapping mechanisms of integration. While some of these mechanisms have been characterized we do not yet have a full mechanistic understanding of host/endo-symbiont integration. In particular, the mechanisms that link Buchnera amino acid output, the currency of this nutritional endosymbiotic relationship, to symbiont growth and proliferation are yet to be identified.

The highly conserved mechanistic target of rapamycin (mTOR) signaling pathway (Figure 1) links a cell’s intracellular amino acid availability, extracellular growth factors, and available energy levels to cellular responses that include protein synthesis, lipid synthesis, cell proliferation, cytoskeleton organization, autophagy, and mitochondrial regulation and proliferation (Crespo and Hall 2002; Laplante and Sabatini 2012; Morita et al. 2015; Sarbassov et al. 2004; Shimobayashi and Hall 2016; Goberdhan et al. 2016). This signaling pathway centers around two complexes: mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2). The complexes form around the mTOR protein, but differ in protein composition such that each complex has distinct functions. mTORC1 is activated in response to sufficient levels of amino acids and responds by signaling for protein, nucleotide, and lipid synthesis, and blocking autophagy; mTORC2 allows cellular growth when nutritional conditions will support growth (Goberdhan et al. 2016). The genes and complexes upstream of mTORC1 and mTORC1 itself are referred to in this paper as the amino acid sensing pathway. In contrast, mTORC2 integrates growth factors and other extracellular signals with cell survival and cytoskeleton organization (Ebnerr et al. 2017; Wullschleger et al. 2006). The mTOR pathway represents a conspicuous candidate for integrating amino acid-provisioning, endosymbiont growth and population size to their metabolic output within host tissues. Currently, mTOR is unannotated and unstudied in any nutritional endosymbiosis.

In this study we present an annotation of the central components of the mTOR pathway and its associated amino acid-sensing cellular machinery in the aphids Myzus persicae and Acyrthosiphon pisum. In addition, we re-analyze previously collected RNAseq data generated from M. persicae bacteriome, gut, and whole insect tissue reporting differential and ranked expression of mTOR pathway components from three genetically distinct M. persicae lines.

**MATERIALS AND METHODS**

Annotation of the mTOR pathway in Myzus persicae and Acyrthosiphon pisum was done using both BLASTp searches in which annotated mTOR proteins from Drosophila melanogaster were used as queries (accessed from FlyBase), and Hidden Markov Models of mTOR-associated genes (accessed from PantherDB (Mi et al. 2016)) against the M. persicae G006 (NCBI taxid: 13164) and A. pisum LSR1 (NCBI taxid: 7029) reference genomes. Gene models and gene duplications identified using our annotation pipeline were validated using PhylomeDB to confirm orthology and paralogy relationships (Huerta-Cepas et al. 2014).

We used the RNAseq that was generated by Duncan et al. 2016 to construct reference transcriptomes from three different M. persicae genotypes based on M. persicae G006 v1.0 official gene set from aphidbase (bippaa.genouest.org/is/aphidbase). We used RNAseq generated in Feng et al. 2018 and Liagüéry et al. 2018 to produce reference transcriptomes for A. pisum based on the 2.1b official gene set on aphidbase. We did not use the previous de novo transcriptomes because they were unsuitable for differentiating between known duplicated genes. For M. persicae bacteriocyte and gut tissue transcriptomes, 300 aphids were dissected and pooled and 10 aphids were used for whole insect transcriptomes as described in Duncan et al. 2016. Quality control was carried out in FastQC (Anders 2010) along with the TrimGalore v0.4.3 (Kruger 2012) package. Reads were aligned using the Hisat2 v2.0.0 (Kim et al. 2015) package. Differential expression analysis was carried out using the nbinomtest() function in DESeq2 in SeqMonk (Andrews 2007; Love et al. 2014). For additional more detailed methods please see supplemental material.

**Statistical Analysis**

A principle component analysis was performed with ggplot2 in R (R Development Core Team 2010; Wickham 2009) on the M. persicae transcriptomes because they were unsuitable for differentiating between known duplicated genes. For M. persicae bacteriocyte and gut tissue transcriptomes, 300 aphids were dissected and pooled and 10 aphids were used for whole insect transcriptomes as described in Duncan et al. 2016. Quality control was carried out in FastQC (Anders 2010) along with the TrimGalore v0.4.3 (Kruger 2012) package. Reads were aligned using the Hisat2 v2.0.0 (Kim et al. 2015) package. Differential expression analysis was carried out using the nbinomtest() function in DESeq2 in SeqMonk (Andrews 2007; Love et al. 2014). For additional more detailed methods please see supplemental material.

**Data availability**

M. persicae RNAseq data can be accessed on the NCBI Sequence Read Archive under BioProject PRJNA296778. A. pisum RNAseq data (see discussion) can be accessed on the NCBI Sequence Read Archive under BioProject PRJNA315109 and PRJNA385573. Aphid lines are available upon request. Supplemental files available at FigShare. Figure S1 contains PCA analysis of transcriptomes. Figure S2 contains the complete comparison of mTOR genes between gut and bacteriocyte transcriptomes. Figure S3 contains the syntenic-based alignment of duplicated genes between A. pisum and M. persicae. Table S1 contains the sequence identity of mTOR genes in A. pisum and M. persicae. Sequence data are available at aphidbase (https://bippaa.genouest.org/is/aphidbase/). Table S2 gives the sequences and HMMs used for gene annotation.
These can be accessed at flybase (http://flybase.org/), and on pantherDB (http://pantherdb.org/) and eggnog mapper (Huerta-Cepas et al. 2016) (http://eggnogdb.embl.de).

File S1 contains additional more detailed methods used in this study. Supplemental material available at Figshare: https://doi.org/10.25387/g3.6855089.

**RESULTS**

Aphids show novel duplications in the highly conserved mTOR pathway

*A. pism* and *M. persicae* both retain the mTOR genes that are widely conserved across invertebrates (Figure 2 A & B, Table S1). Genes missing from the aphid genomes (DEPTOR, PROTOR, Te2, IKKx, TBC1D7, FLCN, FNIP1, and RNF152) are mostly found only in vertebrates. Both aphid species show gene duplications. We identified four RHEB orthologs, and two orthologs of both Rag A/B, and Skp2 in the A. pism genome that are not shared with either the grape phylloxera, *Daktulosphaira vitifoliae* or the fruitfly, *Drosophila melanogaster*. We identified two RHEB orthologs, and two Nup44A orthologs in the M. persicae genome that are not shared with either *D. vitifoliae* or *D. melanogaster*. Based on their synteny, two of the RHEB duplications are inferred to have been present in the common ancestor of *A. pism* and *M. persicae* (Figure S3A).

The mTOR pathway is expressed in *M. persicae* bacteriome tissue

We identified all *M. persicae* mTOR-associated genes in the bacteriocyte transcriptomes of three genetically distinct *M. persicae* lineages (Figure 3). Most, 28 of 34, mTOR-associated genes ranked in the top half of expressed genes, with one of two RHEB orthologs and vATPase ranking in the top 10% of expressed genes. The mTORC1-specific genes: Raptor and PRAS40, are more highly expressed in bacteriocyte tissue than the mTORC2-specific genes, Rictor and mSin1 (Figure 3A).

mTOR genes show bacteriocyte-specific expression patterns

In *M. persicae* mTORC1 the genes, Raptor and PRAS40, are more highly expressed in bacteriome tissue than in whole insect tissue, while the mTORC2-specific genes, Rictor and mSin1, are less highly expressed in bacteriome tissue than in whole insect tissue (Figure 4A). The majority of genes (12 of 20) in the mTOR amino acid sensing pathway, including all members of the Regulator complex (LAMTOR1-5), Sestrin (SESN), putative arginine transporter SLC38A9, and vATPase (Figure 4B) show significantly higher expression in bacteriome tissue than in whole insect tissue. Notably, the putative arginine transporter SLC38A9 is also more highly expressed in bacteriome tissue than in gut tissue (Figure 5).
DISCUSSION

Aphids have a complete invertebrate mTOR pathway
Annotation of the mTOR pathway in aphids shows that, with a few exceptions, they possess the mTOR related genes that would be expected in invertebrates, and that the aphid complement of mTOR related genes is similar to that of two other arthropods, the grape phylloxera, *D. vitifoliae* (a close relative of aphids, but lacking an endosymbiont) and the fruit fly, *D. melanogaster* (Figure 2). Given that the mTOR pathway is heavily conserved in all eukaryotes (Hall 2008), it is unsurprising that aphids share many gene orthologs with the non-arthropods *Caenorhabditis elegans* and *Mus musculus*. Most genes absent from the aphid mTOR pathway are commonly absent from other annotated invertebrates (Figure 2), although TBC1D7, FLCN, and FNIP may represent more recent gene losses within Sternorrhyncha or Hemiptera (the insect suborder and order to which aphids belong) as these genes are present in the fruit fly, *D. melanogaster*. The loss of these three genes is not expected to interrupt the functions of the genes present, although the losses of FLCN and FNIP represent loss of an amino acid-sensitive mTORC1 associated complex (Tsun et al. 2013).

Aphids show novel duplications within the mTOR pathway

The genomes of both, *M. persicae* and *A. pisms* showed duplications that are not present in other invertebrates including the closely related grape phylloxera, *D. vitifoliae*. *Acrithosiphon pisum* contains four RHEB orthologs, two of which are shared with *M. persicae* and assumed to be ancestral (Figure S3). In addition, *A. pisms* has two Rag A/B, and Skp2 orthologs, and *M. persicae* two Nup44A orthologs. All of these duplicated genes with the exception of the two RHEB orthologs unique to *A. pisms* are detected in wingless parthenogenetic female aphid transcriptomes (Feng et al. 2018; NCBI BioProject PRJNA315109). The two RHEB orthologs unique to *A. pisms* were not detected in the transcriptomes associated with this study but are found in an alate male aphid transcriptome (data from Jaquiery et al., 2018. BioProject PRJNA385573), suggesting male-specific function associated with these *A. pisms*-specific duplications (ACYPI005487 and ACYPI006392).

Acquisition of novel genomic material into host genomes by processes that include gene duplication and lateral gene transfer appears to be a feature of coevolution in host/endosymbiont systems (Wilson & Duncan 2015). Previous work in obligate sap-feeding insects, including aphids has shown that duplications in the amino acid-auxin-permease family (AAAP) of transporters has occurred after endosymbiont colonization while work with the amino acid-polyamine-organocation (APC) transporter family has shown that duplications have occurred both before and after endosymbiont colonization (Duncan et al., 2016). While duplications that occurred before endosymbiont colonization cannot have been driven by symbiosis, post colonization duplication events may have been driven by host/endosymbiont coevolution, and indeed many duplicated amino acid transporters from both the AAAP and APC transporter families show bacteriocyte-specific expression (Duncan et al., 2016).

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**Figure 2** Presence and absence of genes in (A) the mTOR complexes, and inputs from the AMPK/MAKP/Akt pathway and (B) the mTOR amino acid sensing pathway. Genes were identified by Hidden Markov Models, and confirmed by PhyloMeDB and KEGG. A filled square indicates the gene is present in the species’ genome. Where duplications have occurred the number of gene copies is shown in the square. An empty square indicates the gene is absent from a species’ genome. The empty spaces for vAT-Pase indicates that genes could not be confidently annotated using Hidden Markov Models. The HMMs were selected to perform annotation in aphid species, and as the vAT-Pase family contains many genes annotation using these HMMs was not reliable for *C. elegans* or *M. musculus*. 

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Amino acid-sensitive mTORC1 appears to be more important in bacteriomes than the amino acid-insensitive mTORC2

The mTOR pathway can take multiple signals as inputs, with amino acid-sensing signals being parsed through mTORC1, and growth factor signals through mTORC2 (Goberdhan et al. 2016; Laplante and Sabatini 2012; Shimobayashi and Hall 2016). The higher expression of mTORC1-specific genes compared with mTORC2-specific genes both within bacteriocytes, and in comparison to whole insect tissue suggests that the role of mTORC1 in bacteriocyte tissue is more...
Bacteriocytes are specialized cells that function to house Buchnera, a bacteria with a genome streamlined for essential amino acid production (Moran and Mira 2001; Wernegreen 2002). Both A. pisum and M. persicae strains of Buchnera have undergone severe genome reduction, losing DNA repair mechanisms, cell membrane and surface synthesis pathways, and some non-essential amino acid synthesis pathways (Jiang et al. 2013; Shigenobu et al. 2000). Based on genomic analyses the main function retained by Buchnera for host benefit is production of host-essential amino acids and vitamins. Given the wide range of inputs the mTOR pathway is able to take, it is interesting that the amino acid-sensitive mTORC1 shows high expression within bacteriocytes. The high expression of mTORC1-specific genes suggests that amino acid sensing through mTORC1 plays a role in mediating the relationship between aphids and Buchnera. mTORC1 has two points of amino acid integration: Sestrin and SLC38A9. Sestrin activates mTORC1 via the GATOR complexes (Figure 1), and detects leucine, methionine, isoleucine and valine by binding to them and thereby interfering with Sestrin’s GATOR2 inhibition mechanism (Wolfson et al. 2016). In aphids these four amino acids are four of five amino acids that are hypothesized based on genomic analysis (Shigenobu et al. 2000; Wilson et al. 2010) and some experimental validation (Russell et al. 2013) to be products of aphid/Buchnera collaborative amino acid biosynthesis. Biosynthesis of three of these four amino acids, isoleucine, leucine and valine, appears to take place almost entirely within Buchnera, with the exception of the final step in each of these pathways which is proposed to be carried out by branched-chain-amino-acid transaminase (BCAT), a eukaryotic gene encoded in the aphid genome, whose functional ortholog is notably absent in Buchnera (International Aphid Genomics Consortium 2010; Jiang et al. 2013; Price et al. 2015; Russell et al. 2013; Wilson et al. 2010). This evidence for a host-catalyzed final step of branched chain amino acid biosynthesis supports intimate coordination between the aphid and Buchnera in completion of amino acid biosynthesis pathways (Hansen and Moran 2011), but also opens the metabolic pathway to host control. Recent work identifying microRNA (miRNA) targets in aphid bacteriocytes suggests that expression of BCAT is miRNA regulated, thereby implicating host regulatory checks in the production of the branched-chain amino acids (Feng et al. 2018). With respect to symbiont control, it is of interest that the genes for leucine biosynthesis are located on a plasmid within Buchnera from M. persicae and A. pisum, a genomic location that potentially uncouples leucine production rates from Buchnera genome copy number, a feature of symbiont genome evolution that possibly reveals some level of endosymbiont control of leucine production (Baumann et al. 1999;
Jiang et al. (2013; Shigenobu et al. 2000). The fourth amino acid that feeds inputs into mTOR via Sestrin is methionine. Methionine also appears to be collaboratively synthesized by aphids and Buchnera, although in this case, the final biosynthesis step is predicted to be carried out in Buchnera, with intermediate metabolites provisioned by the aphid host (Russell et al. 2013).

Here we identified a putative Sestrin gene in the genomes of M. persicae and A. pisum (Figure 2). This putative Sestrin has not yet been characterized as leucine responsive (similar to the mammalian Sestrins 1 & 2, which are capable of binding leucine) or non-responsive (similar to the mammalian Sestrin 3, which is incapable of binding leucine) (Lee et al. 2016; Saxton et al. 2016; Wolfsin et al. 2016). The single Sestrin found in Drosophila has been shown to work weakly responsive to leucine treatments (Wolfsin et al. 2016), and it has been shown that genes downstream of the A. pisum mTORC1 are activated in response to leucine and methionine treatments (Gao et al. 2018). An amino acid responsive Sestrin (as proposed in Figure 1), a function conserved from Drosophila and present in mammals, would present a point of integration for these four collaboratively synthesized amino acids into mTOR signaling.

**Buchnera produced amino acids may be integrated into mTORC1 signaling by the SLC38A9/vATPase complex**

One of the several points of signaling into mTORC1 occurs through the lysosomal vATPase/SLC38A9 complex that detects glutamine, arginine, and asparagine (Jewell et al. 2015; Wang et al. 2015; Yao et al. 2017). We found transcripts of both vATPase and SLC38A9 present and highly expressed in aphid bacteriocytes, and highly expressed in bacteriocytes relative to other aphid tissues (Figures 1, 3B, 4B, & 5). The non-essential amino acid glutamine plays an important role in aphid/Buchnera endosymbiosis as it is hypothesized to be used as a common precursor for Buchnera-produced amino acids (Sasaki and Ishikawa 1995). Buchnera from M. persicae (Buchnera Mp), unlike Buchnera from A. pisum, have retained a gene encoding an asparaginase in their genome, suggesting that Buchnera Mp might also be able to use host provisioned asparagine as an amino acid precursor in addition to glutamine (Jiang et al. 2013; Shigenobu et al. 2000). Host glutamine provisioning has previously been proposed to be regulated via the amino acid transporter ApGLNT1, that localizes to the bacteriocyte plasma membrane (Price et al. 2014). ApGLNT1 has high affinity for glutamine transport, but transport of glutamine is competitively inhibited by arginine (Price et al. 2014). Within the aphid/Buchnera endosymbiosis, arginine is produced by Buchnera (Hansen and Moran 2011; International Aphid Genomics Consortium 2010; Shigenobu et al. 2000). Host sensing of arginine via a SLC38A9 ortholog would integrate Buchnera’s output of host-essential amino acids into mTORC1 signaling, while SLC38A9 sensing of glutamine and asparagine would represent additional host controls on the aphid provisioning of universal amino acid precursors to Buchnera. Remarkably, SLC38A9 itself may be under another level of control: differential expression of SLC38A9 orthologs is predicted to be at least one target of miRNA mediated regulation of gene expression in aphids (Feng et al. 2018). While aphid orthologs of SLC38A9 and vATPase are yet to be functionally characterized, or localized within bacteriomes, immunolocalization and functional characterization of these genes will reveal whether they colocalize and maintain their ancestral function in aphids.

**The mTOR pathway presents an additional novel mechanism of host/endosymbiont integration in the face of genetic constraint**

Endosymbiosis entails a cost to the host of maintaining a symbiont within its cells. This cost will always exist for a host that houses an endosymbiont and therefore yields a situation that generates conflict between host and endosymbiont. In nutritional endosymbiosis, the endosymbiont appears to offset the cost it imposes on the host by providing a nutritional benefit to its host, a benefit that is accrued by the host at a cost to the endosymbiont. Thus, there exists a difference between what is good for the symbiotic relationship, and what is good for either member of a symbiotic relationship in isolation. Endosymbiosis can effectively be viewed as an antagonistic relationship where both members impose a cost upon each other, but in the right context they can provide a benefit to their partner that outweighs the cost (Keeling and McCutcheon 2017). Organisms in persistent endosymbiosis must evolve systems of integration and mediation to maintain mutually beneficial conditions (Leigh 2010). As described in the previous two sections, recent work in aphids reveals that multiple layers of regulation are implicated in the maintenance of beneficial conditions. These layers include within pathway metabolic collaboration (Russell et al. 2013), a negative feedback loop that regulates Buchnera amino acid precursor supply (Price et al. 2014), differential expression of Buchnera small RNAs (Hansen and Degnan 2014), aphid miRNA regulation of gene expression (Feng et al. 2018), and now mTOR.

In summary, we propose that the mTOR pathway, and specifically the amino acid sensing mTORC1, presents a compelling and logical potential candidate for an additional, novel system integrating host and symbiont within the aphid/Buchnera system. We further speculate that
if mTOR integrates the aphid/Buchnera nutritional symbiosis, it will also function in host/symbiont integration in other systems. Both cooperative or conserved genetic machinery and within pathway host/symbiont metabolic collaboration have emerged as clear and convergent signatures of host endosymbiont coevolution (Wilson and Duncan 2015). We predict that the highly conserved and nutrient sensitive mTOR pathway has been coopted in many collaborative nutritional endosymbioses as one among many integration systems, but one with important implications for regulation of endosymbiont populations within host cells. The essential next steps will require testing the model of integration we propose here through functional characterization of mTOR genes and pathway function in aphids.

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LITERATURE CITED
Andrews, S., 2007 SeqMonk. A tool to visualise and analyse high throughput mapped sequence data, https://www.bioinformatics.babraham.ac.uk/projects/seqmonk/.
Andrews, S., 2010 FastQC: a quality control tool for high throughput sequence data., http://www.bioinformatics.babraham.ac.uk/projects/fastqc/.
Baumann, L., P. Baumann, N. A. Moran, J. Sandström, and M. L. Thao, 1999 Genetic Characterization of Plasmids Containing Genes Encoding Enzymes of Leucine Biosynthesis in Endosymbionts (Buchnera) of Aphids. J. Mol. Evol. 48: 77–85. https://doi.org/10.1007/PL00006447
Baumann, P., L. Baumann, C. Y. Lai, D. Rouhbakhsh, N. A. Moran et al., 1995 Genetics, physiology, and evolutionary relationships of the genus Buchnera: intracellular symbionts of aphids. Annu. Rev. Microbiol. 49: 55–94. https://doi.org/10.1146/annurev.mi.49.100195.000415
Bennett, G. M., and N. A. Moran, 2015 Heritable symbiosis: The advantages and perils of an evolutionary rabbit hole. Proc. Natl. Acad. Sci. USA 112: 10169–10176. https://doi.org/10.1073/pnas.1421388112
Crespo, J. L., and M. N. Hall, 2002 Elucidating TOR Signaling and Rapamycin Action: Lessons from Saccharomyces cerevisiae. Microbiol. Mol. Biol. Rev. 66: 579–591. https://doi.org/10.1128/MMBR.66.4.579-591.2002
Duncan, R.P., H. Feng, D.M. Nguyen, and A.C.C. Wilson, 2016 Gene Family Expansions in Aphids Maintained by Endosymbiotic and Non-symbiotic Traits. Genome Biol Evol. 8: 753–764.
Ebner, M., B. Sinkovics, M. Szczygiel, D. W. Ribeiro, and I. Yudushkin, 2017 Localization of mTORC2 activity inside cells. J. Cell Biol. 216: 343–353. https://doi.org/10.1083/jcb.201610060
Ehrlich, P. R., and P. H. Raven, 1964 Butterflies and Plants: A Study in Coevolution. Evolution 18: 586–608. https://doi.org/10.1111/j.1558-5646.1964.tb01674.x
Feng, H., L. Wang, S. Wuchty, and A. C. C. Wilson, 2018 microRNA regulation in an ancient obligate endosymbiosis. Mol. Ecol. 27: 1777–1793. https://doi.org/10.1111/mec.14464
Gao, J., H. Guo, Y. Sun, and F. Ge, 2018 Differential accumulation of leucine and methionine in red and green pea aphids leads to different fecundity in response to nitrogen fertilization. Pest Manag. Sci. 74: 1779–1789. https://doi.org/10.1002/ps.4875
Goderthian, D. C., C. Wilson, and A. L. Harris, 2016 Amino Acid Sensing by mTORC1: Intracellular Transporters Mark the Spot. Cell Metab. 23: 580–589. https://doi.org/10.1016/j.cmet.2016.03.013
Gramates, L. S., S. J. Marygold, G. D. Santos, J. M. Urbano, G. Antonazzo et al., 2017 FlyBase at 25: looking to the future. Nucleic Acids Res. 45: D663–D671. https://doi.org/10.1093/nar/gkw1016
Hall, M. N., 2008 mTOR—what does it do? Transplant. Proc. 40(10, Suppl S5–S8. https://doi.org/10.1016/j.transproceed.2008.10.009
Hansen, A. K., and P. D. Degnan, 2014 Widespread expression of conserved small RNAs in small symbiotic genomes. ISME J. 8: 2490–2502. https://doi.org/10.1038/ismej.2014.121
Hansen, A. K., and N. A. Moran, 2011 Aphid genome expression reveals host-symbiont cooperation in the production of amino acids. Proc. Natl. Acad. Sci. USA 108: 2849–2854. https://doi.org/10.1073/pnas.1013465108
Huerta-Cepas, J., S. Capella-Gutierrez, L. P. Przybycien, M. Haroutian, and N. Baldal, 2014 PhylomeDB v4: zooming into the plurality of evolutionary histories of a genome. Nucleic Acids Res. 42: D987–D992. https://doi.org/10.1093/nar/gkt1177
Huerta-Cepas, J., D. Szklarczyk, K. Forslund, H. Cook, D. Heller et al., 2016 eggNOG 4.5: a hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res. 44: D286–D293. https://doi.org/10.1093/nar/gkx1248
International Aphid Genomics Consortium, 2010 Genome sequence of the pea aphid Acrystosiphon pismum. PLoS Biol. 8: e1000313. https://doi.org/10.1371/journal.pbio.1000313
Jaquèry, J., J. Peccoud, T. Ouisse, F. Legeai, N. Prunier-Leterme et al., 2018 Disentangling the Causes for Faster-X Evolution in Aphids. Genome Biol. Evol. 10: 507–520. https://doi.org/10.1093/gbe/evy015
Jewell, J. L., Y. C. Kim, R. C. Russell, F. R. Yu, H. W. Park et al., 2015 Metabolism. Differential regulation of mTORC1 by leucine and glutamine. Science 347: 194–198. https://doi.org/10.1126/science.1259472
Jiang, Z., D. H. Jones, S. Khuri, N. F. Tsinoremas, T. Wyss et al., 2013 Comparative analysis of genome sequences from four strains of the Buchnera aphidicola Mp endosymbiont of the green peach aphid, Myzus persicae. BMC Genomics 14: 917. https://doi.org/10.1186/1471-2164-14-917
Keeling, P. J., and J. P. McCutcheon, 2017 Endosymbiosis: The feeling is not mutual. J. Theor. Biol. 434: 74–79. https://doi.org/10.1016/j.jtbi.2017.06.008
Kim, D., B. Langmead, and S. L. Salzberg, 2015 HISAT: a fast spliced aligner with low memory requirements. Nat. Methods 12: 357–360. https://doi.org/10.1038/nmeth.3317
Kruger, F., 2012 Trim Galore! https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/.
Laplante, M., and D. M. Sabatini, 2012 mTOR signaling in growth control and disease. Cell 149: 274–293. https://doi.org/10.1016/j.cell.2012.03.017
Lee, J. H., U. S. Cho, and M. Karin, 2016 Sestrin regulation of TORC1: Is Sestrin a leucine sensor? Sci. Signal. 9: re5. https://doi.org/10.1126/sci-signal.aaf2885
Leigh, E. G. Jr., 2010 The evolution of mutualism. J. Evol. Biol. 23: 2507–2528. https://doi.org/10.1111/j.1420-9101.2010.02114.x
Love, M. L. W. Huber, and S. Anders, 2014 Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15: 550. https://doi.org/10.1186/s13059-014-0550-8
Mi, H., X. Huang, A. Muruganujan, H. Tang, C. Mills et al., 2016 PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res. 45: D183–D189. https://doi.org/10.1093/nar/gkw1138
Moran, N.A., and A. Mira, 2001 The process of genome shrinkage in the obligate symbiont Buchnera aphidicola. Genome Biol 2 (12): RESEARCH0054.
Morita, M., S. P. Gravel, L. Hulea, O. Larsson, M. Pollak et al., 2015 mTOR coordinates protein synthesis, mitochondrial activity and proliferation. Cell Cycle 14: 473–480. https://doi.org/10.4161/15384101.2014.991572

Price, D. R., H. Feng, J. D. Baker, S. Bavan, C. W. Luetje et al., 2014 Aphid amino acid transporter regulates glutamine supply to intracellular bacterial symbionts. Proc. Natl. Acad. Sci. USA 111: 320–325. https://doi.org/10.1073/pnas.1306068111

Price, D. R., A. C. Wilson, and C. W. Luetje, 2015 Proton-dependent glutamine uptake by aphid bacteriocyte amino acid transporter ApGLNT1. Biochim. Biophys. Acta 1848: 2085–2091.

R Development Core Team, 2010 R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna.

Rebsamen, M., L. Pochini, T. Stasyk, M. E. de Araujo, M. Galluccio et al., 2015 SLC38A9 is a component of the lysosomal amino acid sensing machinery that controls mTORC1. Nature 519: 477–481. https://doi.org/10.1038/nature14107

Russell, C. W., S. Bouvaine, P. D. Newell, and A. E. Douglas, 2013 Shared metabolic pathways in a coevolved insect-bacterial symbiosis. Appl. Environ. Microbiol. 79: 6117–6123. https://doi.org/10.1128/AEM.01543-13

Sarker, D. D., S. M. Ali, D. H. Kim, D. A. Guertin, R. R. Latek et al., 2004 Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr. Biol. 14: 1296–1302. https://doi.org/10.1016/j.cub.2004.06.054

Sasaki, T., and H. Ishikawa, 1995 Production of essential amino acids from glutamate by mycetocyte symbionts of the pea aphid, Acyrthosiphon pisum. J. Insect Physiol. 41: 41–46. https://doi.org/10.1016/0022-1910(94)00080-Z

Saxton, R. A., K. E. Knockenhauer, R. L. Wolfson, L. Chantranupong, M. E. Pacold et al., 2016 Structural basis for leucine sensing by the Sestrin2-mTORC1 pathway. Science 351: 53–58. https://doi.org/10.1126/science.aad2087

Shigenobu, S., H. Watanabe, M. Hattori, Y. Sakaki, and H. Ishikawa, 2000 Genome sequence of the endocellular bacterial symbiont of aphids Buchnera sp. APS. Nature 407: 81–86. https://doi.org/10.1038/35024074

Shimobayashi, M., and M. N. Hall, 2016 Multiple amino acid sensing inputs to mTORC1. Cell Res. 26: 7–20. https://doi.org/10.1038/cr.2015.146

Tsun, Z. Y., L. Bar-Peled, L. Chantranupong, R. Zoncu, T. Wang et al., 2013 The folliculin tumor suppressor is a GAP for the RagC/D GTPases that signal amino acid levels to mTORC1. Mol. Cell 52: 495–505. https://doi.org/10.1016/j.molcel.2013.09.016

Wang, S., Z. Y. Tsun, R. L. Wolfson, K. Shen, G. A. Wyant et al., 2015 Lysosomal amino acid transporter SLC38A9 signals arginine sufficiency to mTORC1. Science 347: 188–194. https://doi.org/10.1126/science.1257132

Wernegreen, J. J., 2002 Genome evolution in bacterial endosymbionts of insects. Nat. Rev. Genet. 3: 850–861. https://doi.org/10.1038/35024074

Wernegreen, J. J., 2004 Endosymbiosis: lessons in conflict resolution. PLoS Biol. 2: E68. https://doi.org/10.1371/journal.pbio.0020068

Wickham, H., 2009 ggplot2: Elegant Graphics for Data Analysis. New York: Springer-Verlag.

Wilson, A. C. C., P. D. Ashton, F. Calevro, H. Charles, S. Colella et al., 2010 Genomic insight into the amino acid relations of the pea aphid, Acyrthosiphon pisum, with its symbiotic bacterium Buchnera aphidicola. Insect Mol. Biol. 19: 249–258. https://doi.org/10.1111/j.1365-2583.2009.00942.x

Wilson, A. C. C., and R. P. Duncan, 2015 Signatures of host/symbiont genome coevolution in insect nutritional endosymbioses. Proc. Natl. Acad. Sci. USA 112: 10255–10261. https://doi.org/10.1073/pnas.1423305112

Wolfson, R. L., L. Chantranupong, R. A. Saxton, K. Shen, S. M. Scaria et al., 2016 Sestrin2 is a leucine sensor for the mTORC1 pathway. Science 351: 43–48. https://doi.org/10.1126/science.aab2674

Wullschleger, S., R. Loewith, and M. N. Hall, 2006 TOR signaling in growth and metabolism. Cell 124: 471–484. https://doi.org/10.1016/j.cell.2006.01.016

Yao, Y., E. Jones, and K. Inoki, 2017 Lysosomal Regulation of mTORC1 by Amino Acids in Mammalian Cells. Biomolecules 7: 51 https://doi.org/10.3390/biom7030051

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