Trends in Parasitology

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REVIEWS

Review

Haem Biology in Metazoan Parasites – 'The Bright Side of Haem'

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Traditionally, host haem has been recognized as a cytotoxic molecule that parasites need to eliminate or detoxify in order to survive. However, recent evidence indicates that some lineages of parasites have lost genes that encode enzymes involved specifically in endogenous haem biosynthesis. Such lineages thus need to acquire and utilize haem originating from their host animal, making it an indispensable molecule for their survival and reproduction. In multicellular parasites, host haem needs to be systemically distributed throughout their bodies to meet the haem demands in all cell and tissue types. Host haem also gets deposited in parasite eggs, enabling embryogenesis and reproduction. Clearly, a better understanding of haem biology in multicellular parasites should elucidate organismal adaptations to obligatory blood-feeding.

Highlights

Ticks and nematodes do not code for haem biosynthetic and degrading enzymes, likely operating independent haem and iron acquisition/distribution networks.

The uptake of exogenous haem brings a selective advantage in a haem-rich environment, even in the presence of functional haem biosynthesis.

The unavailability of host haem is often manifested during embryogenesis, and the formation of progeny is conditioned by deposits of host haem.

Metazoan Parasites and Blood-Feeding

Metazoan parasites include arthropods, nematodes, and platyhelminths [1], some of which are blood-feeders. Arthropods can be parasites and/or notorious vectors of disease. According to the World Health Organization (WHO), vector-borne diseases account for >17% of all infectious diseases, causing more than 700 000 deaths annually[i]. Many blood-feeding insects and ticks ingest pathogens while taking their blood meal from an infected reservoir animal and can transmit these pathogens to a naïve host during a second blood meal. While ticks are obligatory blood-feeders, insects have two modes of blood-feeding. **Hemimetabolan haematophagous** (see Glossary) insects are obligatory blood-feeders and rely solely on the blood meal as a food source at the juvenile and adult stages (e.g., lice and kissing bugs). By contrast, the juvenile stages of **holometabolan** haematophagous insects often take meals other than blood, and only adults feed on blood (e.g., mosquitoes and fleas). Nematodes (roundworms) are a very diverse group of animals, known to inhabit all ecosystems from hot springs to animal tissues, and can be divided into five distinct clades, many of which include parasites of vertebrates [2–5]. Nematodes, such as *Haemonchus*, *Ancylostoma*, and *Necator*, are blood-feeders with direct life cycles, whereas others (e.g., filarioids and spiruroids) have to be transmitted by insect vectors (e.g., mosquitoes or black flies) to complete their cycles. Platyhelminths (flatworms), including trematodes (flukes) and cestodes (tapeworms), usually have indirect life cycles. While cestodes are not blood-feeders, some flukes are, and they often cause chronic diseases in humans and other animals. Of major health concern, for example, is human schistosomiasis, caused mainly by blood flukes, including *Schistosoma mansoni* and *Schistosoma haematobium* in Africa, the Middle East and/or South America, and *Schistosoma japonicum* in Asia [6]. Liver flukes, including species of *Clonorchis*, *Opisthorchis*, and *Fasciola*, cause complex hepatic diseases in animals, including humans, and the former two genera are carcinogenic [7,8].

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For all haematophagous parasites, the blood meal is an abundant source of nutrients. As the blood meal is a relatively consistent diet, in terms of cellular, molecular, and chemical compositions, parasites have evolved a number of specific mechanisms to secure optimal nutrient acquisition and distribution [9]. Red blood cells (erythrocytes) comprise approximately half of the blood volume and are a rich source of haemoglobin (150 mg/ml), exceeding the protein content of serum (70 mg/ml) by more than two times. The hydrolysis of haemoglobin is concomitantly linked to the release of vast amounts of haem, which is why blood-feeding parasites need to have mechanisms for the efficient disposal and detoxification of its haem excess [10,11]. In physiological amounts, haem is an essential prosthetic group of enzymes involved in vitally important biological processes, such as energy metabolism, oxygen transport and storage, cytochrome-facilitated electron transport, soluble guanylyl cyclase, and nitric oxide synthase activities [12]. In addition, haem functions as an intercellular and intracellular signalling molecule [13,14].

In this review, we focus on the beneficial aspects of haem and its biology in multicellular parasites. This article discusses the components of the haem biosynthesis pathway (Box 1) and haem biology in metazoan parasites; the evolution of somatic intertissue networks of haem distribution; and the role of haem as a nutritional source of iron in parasites. We specifically address the following three main topics:

Q1. Haem biosynthesis. (i) Do all metazoan parasites code for an **endogenous** haem biosynthesis pathway? (ii) How widespread is a lack of haem biosynthesis pathway genes in parasite lineages?

Q2. Haem acquisition and distribution. (i) Is the absence of an endogenous haem biosynthesis pathway a prerequisite for the utilization of host haem? (ii) What mechanisms have evolved to secure the acquisition of haem and its systemic distribution in parasites? (iii) What does the interference with **exogenous** haem supply cause in parasites?

Q3. Haem catabolism. (i) Are multicellular parasites canonically equipped with genetically encoded haem catabolism? (ii) Can parasites utilize iron liberated by cleavage of the haem tetrapyrrole ring?

Box 1. Haem Biosynthesis

Haem, an essential cellular cofactor, is produced via a genetically encoded, endogenous pathway comprising eight enzymatic steps in most heterotrophic eukaryotes [65] (Figure I). Following transcription in the nucleus and translation in the cytosol, enzymes involved in haem biosynthesis are distributed across the cytosol, delivered to the mitochondrial matrix and/or become tethered to the mitochondrial membranes [66]. It has been known for almost four decades that some eukaryotic, unicellular parasites are haem auxotrophs that acquire haem from the diet. This dependence has been caused by partial or complete loss of genetic coding for haem biosynthesis complement. Such genetic reduction has been reported for protists, including *Trypanosoma* and *Leishmania* (kinetoplastids) [67] or *Theileria*, *Babesia*, and *Cryptosporidium* (apicomplexans) [68]. While the loss of haem biosynthesis appears to be a common trait of parasitic kinetoplastids, this is not the case for apicomplexan parasites such as *Plasmodium* and *Toxoplasma*, which do code for enzymes involved in haem biosynthesis [69]. Despite the full genetic coding for haem biosynthesis, blood stages of *Plasmodium falciparum* utilize host haem [70]. Genetic mutants of haem biosynthesis can survive the blood stage form, suggesting a selective advantage in using exogenous haem in a haem-rich environment [70]. The loss of ability to synthesize haem has been reported also for some multicellular parasites [16,24,27], which is supported by genomic and/or transcriptomic predictions [15,16,24,71,72]. The complexity of metazoans, with numerous different tissue and cell types, means that the loss of such an essential pathway could seriously impact the differentiation and/or development of these organisms. Thus, not only a functional pathway of haem acquisition but also somatic distribution of haem is crucial to meet the haem demands of all cell types.

Glossary

Auxotroph: an organism incapable of synthesizing molecules required for its own metabolism.
Endogenous: originating within an organism.
Exogenous: coming into an organism from outside.
Haematophagy: blood-feeding (obligatory or facultative).
Haemosome: a specialized, membrane-delimited, organelle of the tick digest cell that collects excess acquired haem.
Hemimetaboly: an insect metamorphotic process in which juveniles resemble adults and compete for the same type of diet; that is, all stages would be obligatory blood feeders (e.g., kissing bugs and bed bugs).
Hemiptera: a diverse group of true bugs, some of which are blood-feeders.
Holometaboly: an insect metamorphotic process in which juveniles differ from adults and, therefore, do not compete for the same type of diet; that is, only adults feed on blood (e.g., mosquitoes and sand flies).
iK5: an initiative to sequence the genomes of 5000 arthropod species.
Inert haem: an end product of haem(oglobin) metabolism; parasites make the excess of cellular haem inert by means of aggregation in haemozoins (*Plasmodium*, *Schistosoma*, *Rhodnius*) or haemosomes (ticks) in order to prevent cytotoxicity.
Labile haem: an affinity-driven entity of redox-active haem molecules which are interchangeably bound to small molecules, peptides, and proteins, and are available for haem-dependent processes in the cell.

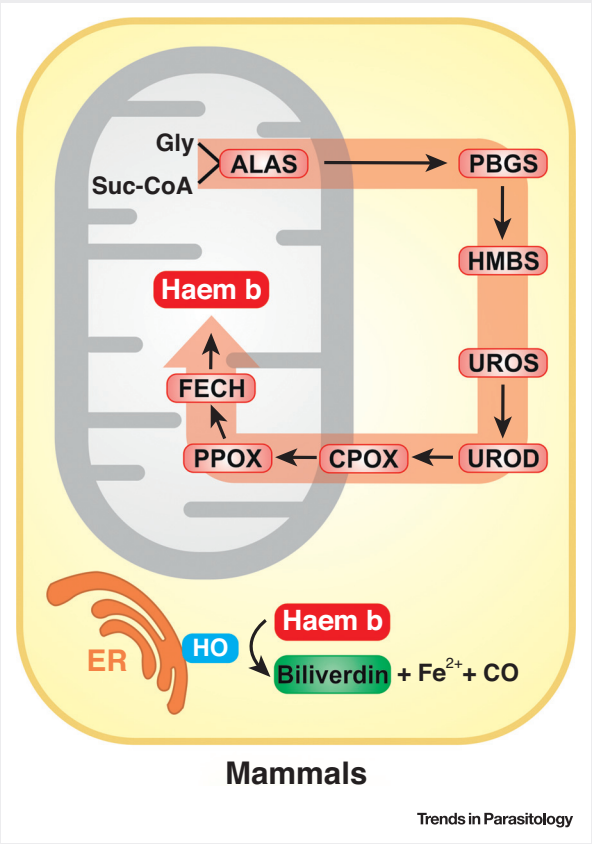

Figure I. Depiction of the Haem Biosynthesis Pathway in Mammals. This schematic illustration depicts an endogenous haem biosynthesis pathway of mammals, typical for most heterotrophic eukaryotes. Genetically encoded enzymes (black) catalyze the formation of intermediate products of haem b production (white in red box); mitochondria (grey), cytosol (yellow). ER, endoplasmic reticulum; CO, carbon monoxide; Gly, glycine; Suc-CoA, succinyl coenzyme A. ALAS, 5-aminolevulinate synthase; PBGS, porphobilinogen synthase; HMBS, hydroxymethylbilane synthase; UROS, uroporphyrinogen synthase; UROD, uroporphyrinogen decarboxylase; CPOX, coproporphyrinogen oxidase; PPOX, protoporphyrinogen oxidase; FECH, ferrochelatase; HO, haem oxygenase.

Components Integral to the Haem Biosynthesis Pathway in Multicellular Parasites (Q1)

Arthropods

The non-blood-feeding mites, *Tetranychus urticae*, *Metaseiulus occidentalis*, and *Varroa destructor*, encode a full enzymatic complement to synthesize haem (Figure 1) [15–17]. Through analyses of genomic data sets representing many arthropods (**iK5**), it has become evident that parasitism is associated with genome remodeling and a reduction of some metabolic pathways [18,19]. The genome of *Ixodes scapularis*, the black-legged tick which transmits Lyme disease and anaplasmosis in the USA, has a reduced set of genes coding for haem-synthesizing enzymes [16,19]. Only genes encoding coproporphyrinogen-III oxidase (*cpox*), protoporphyrinogen oxidase (*ppox*), and ferrochelatase (*fech*) of the canonical haem biosynthesis pathway have been retained in the tick genome (Figure 1; see also Tables S2 and S3 in the supplemental information online). In addition, uroporphyrinogen decarboxylase (*urod*) is also present in the *I. scapularis* genome database (ISCW020804; Tables S2 and S3). Even though this gene does not contain introns, it is adjacent to other likely bacterial genes, some of

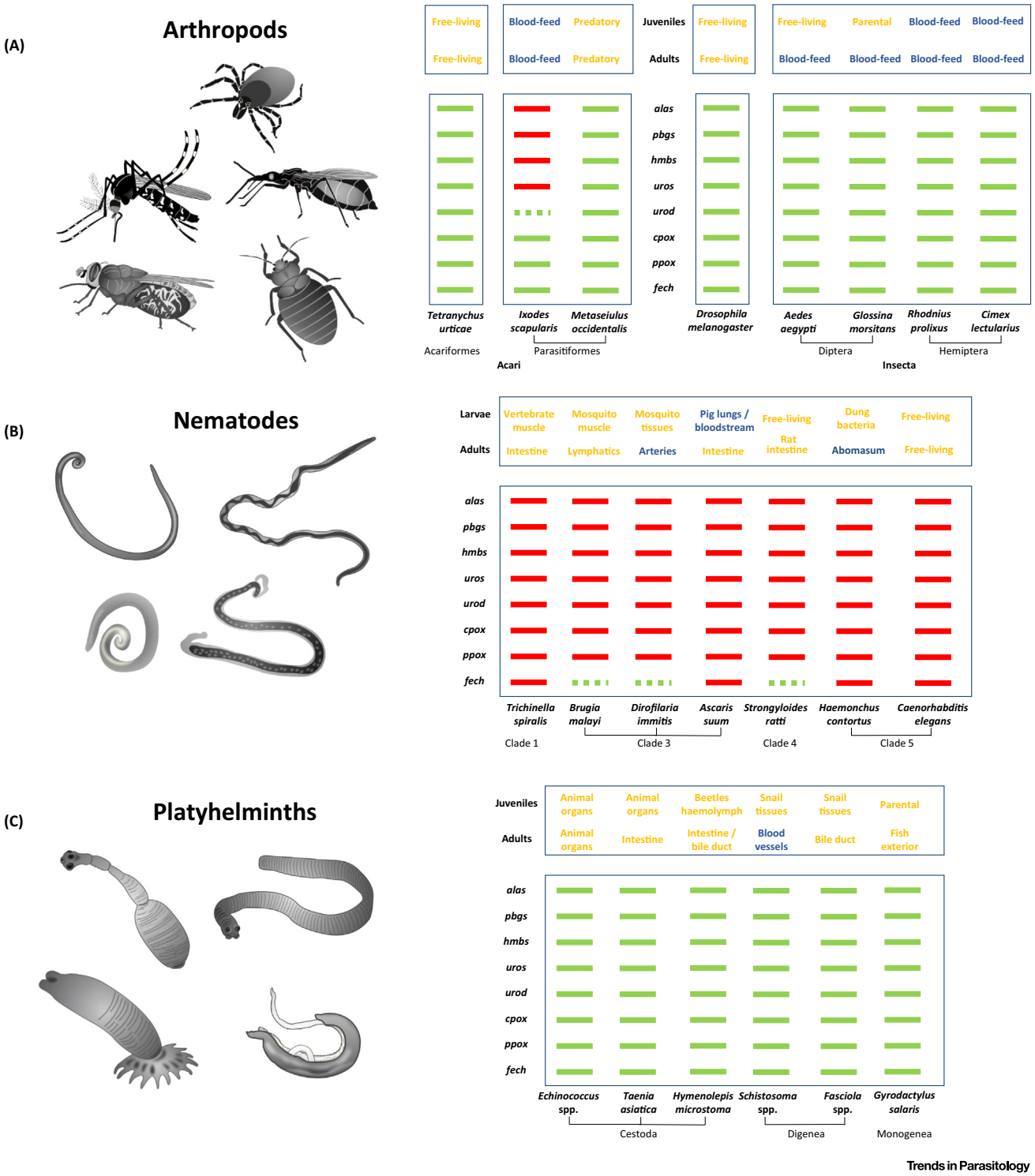

Figure 1. Salient Information on Parasite Nutrient Source and Genes Inferred to Be Involved in Haem Biosynthesis. This figure refers to selected model organisms and parasitic arthropods (A), nematodes (B), and platyhelminths (C). The green lines indicate the presence of a gene, the red lines indicate the absence of a gene in the genome of a species; dashed green lines indicate the presence of a gene of bacterial origin; blue text indicates blood-feeding. For the meanings of gene abbreviations see the legend of Figure I in Box 1. A summary of materials and methods used is given in the supplemental information online together with protein/gene identifications (IDs; cf. Tables S1–S3, and S5).

which do contain a predicted intron (ISCW020798, ISCW020784). Therefore, it is not clear whether it represents an acquired tract of bacterial DNA or a bacterial contamination [16]. Extensive transcriptomic studies performed by us and others on the closely related *Ixodes ricinus* (European Lyme disease vector) and other tick species (Table S4) have confirmed transcript expression of genes encoding the last three mitochondrial enzymes involved in haem biosynthesis, suggesting active functionality. Yet, RNAi-mediated silencing of *fech* did not reveal any impact on feeding physiology and reproduction of adult *I. ricinus* [20]. Analyses of the recent transcriptomic data sets for the soft tick *Ornithodoros rostratus* (Argasidae) have revealed that this species has retained porphobilinogen synthase (*pbgs*), in addition to *cpox, ppox*, and *fech* [15].

Using the genome of *Drosophila melanogaster* as a reference, we identified all gene homologues pertaining to the haem biosynthesis pathway in the blood-feeding dipterans *Aedes aegypti* and *Glossina morsitans*, and the hemipterans *Rhodnius prolixus* and *Cimex lectularius* (Table S1; Figure 1). Outside of the dipterans and hemipterans, we also studied the transcriptome of the obligatory blood-feeding flea, *Ctenocephalides felis* (BioProject: PRJNA219547). Homologues of all eight haem biosynthesis enzymes were identified in this flea, supporting the relative conservation of the haem biosynthesis pathway across insect orders, irrespective of whether they are obligatory blood feeders or not.

Nematodes

Using arthropod genes from *A. aegypti* as references, we searched for homologous encoded enzymes in the haem biosynthesis pathway of selected representatives of nematode clades that infect vertebrates, including *Trichinella spiralis* (Clade I); *Brugia malayi*, *Dirofilaria immitis*, *Ascaris suum* (Clade III); *Strongyloides ratti* (Clade IV); *Haemonchus contortus* and the free-living *Caenorhabditis elegans* (Clade V). All species studied lacked a complete gene complement for enzymes involved in haem biosynthesis (Figure 1). The nematode species are, therefore, exclusively dependent on haem availability, as distinct from endogenous haem biosynthesis. Yet, in two species, namely parasitic *Ancylostoma ceylanicum* and free-living *Diploscapter pachy*, we identified enzymes encoded by intron-containing homologues of *urod* genes of bacteria (species of *Fluviicola* and *Sphingomonas*, respectively) (Table S5). In addition, for several species, we and others [21,22] detected a distant homologue of bacterial FECH (Table S5). Phylogenetic analysis revealed that the ferrochelatase gene of *B. malayi* (*bmfech*) is most closely related to α-proteobacterial ferrochelatases, suggesting that filarial nematodes have acquired this gene by lateral gene transfer [23]. Interestingly, the *B. malayi* ferrochelatase (*Bm*FECH) has been reported to be essential for the parasite's viability, suggesting that this enzyme might serve as a potential target for filariasis control [23].

Platyhelminths

Genomes of the blood flukes (schistosomes) *S. mansoni*, *S. japonicum*, and *S. haematobium*, and of the liver fluke *Fasciola hepatica* (Table S1), have a complete gene complement representing the haem biosynthesis pathway (Figure 1), indicating that these worms likely depend on *de novo* haem biosynthesis at least at some point in their life cycle. This finding seems to contrast with the original proposal, based on enzymatic activity measurements, that *S. mansoni* has an inactive haem biosynthesis pathway [24]. Also, the genomes of tapeworms infective to humans and other mammals (e.g., *Echinococcus granulosus*, *Echinococcus multilocularis*, and *Taenia asiatica*), or specifically to rodents (*Hymenolepis microstoma*) [25], as well as those of monogeneans (*Gyrodactylus salaris*), all have a full complement of genes encoding enzymes involved in this pathway [26] (Figure 1).

In summary, current evidence indicates that ticks have gradually lost part of the genetic complement coding for haem biosynthesis, which might relate to obligatory blood-feeding by individual developmental stages. In obligatory blood-feeding and other insects, such a loss seems not to have occurred, which, surprisingly, is consistent with platyhelminths which encode a full haem biosynthetic pathway. Interestingly, free-living and parasitic nematodes studied to date have lost the genetic complement coding for this pathway.

Acquisition and Somatic Distribution of Haem (Q2)

Arthropods

The lack of endogenous haem biosynthesis was experimentally proven for the hard ticks, and it is recognized that these ticks acquire haem from host haemoglobin [16,27]. Some indirect evidence suggests that ticks acquire host haemoglobin in their midgut through receptor-mediated uptake [28,29]. Haem acquired from digested haemoglobin is transported into ovaries for haemoprotein assembly during embryogenesis, which has been shown to be essential for tick reproduction [16]. Work has shown that adult *I. ricinus* females fed on serum lay dead eggs that fail to reproduce; by contrast, embryogenesis is normal when ticks are fed on serum supplemented with bovine haemoglobin [16]. Serum feeding by soft ticks, such as *Ornithodoros moubata*, also leads to reduced reproduction (unpublished data), suggesting a dependence on host haemoglobin for effective reproduction. There are also intriguing examples of soft ticks (genera *Antricola* and *Nothoaspis*) that feed on blood only at the larval and possibly early nymphal stages, but not at the adult stage, yet they produce large numbers of progeny (Ben Mans, personal communication, March 13, 2017). How these ticks secure haem for reproduction is unclear.

Upon acquisition, cellular haem can become redox-inactive, **inert haem**, or become available for haem-dependent processes as so-called **labile haem** [14]. Some haem facilitates haem-based metabolism in various cellular compartments or affects transcription in the cell nucleus (Box 2), and some of it is destined for somatic distribution. In the tick midgut, however, most acquired haem is detoxified in **haemosomes** of digest cells [30]. ATP-binding cassette

Box 2. Haem as a Blood-Feeding Messenger

Apart from a canonical perception that haem is an enzymatic cofactor, haem is also now recognized as a signalling molecule, regulating a plethora of other molecules involved in various cellular processes [73,74]. Nuclear haem sensors, that is, haem-binding transcription factors, represent different protein families [75]. In parasites, haem can trigger processes associated with a parasitic lifestyle, including blood digestion, stress response, defence mechanisms, and immune evasion.

Recently, it was shown for ticks that blood feeding triggers an increase in the transcription of genes encoding detoxification enzymes and haem-scavengers in the midgut, while the transcription of genes linked to glucose import and antioxidant enzymes decreases [76]. For example, the transcription of a tick-specific delta-class glutathione-*S*-transferase (*Ir*GST1) increases, in a dose-dependent manner, with amounts of dietary haemin [77]. This transferase binds haem *in vitro* and is hypothesized to prevent excessive bioavailability of haem by a 'mopping-up' process within cells of the tick midgut [77]. Other haem-inducible transcripts comprise genes encoding sulfotransferase (Ir-110976), cytochrome P450 (IrSigP-112182), peritrophic membrane chitin-binding protein (Ir-113572), and secreted mucin (Ir-119139) [76]. Whether or not the transcription of these midgut-associated genes is under the control of a currently unknown haem-sensing transcription factor remains to be established.

In the mosquito *Aedes aegypti*, haem-induced transcription represents detoxification enzymes (e.g., glutathione-*S*-transferase) and proteins involved in immune pathways [13]. Haem-sensing in the midgut of *A. aegypti* also has a permissive effect on the proliferation of the microbiota in the mosquito [78]. The sensing of the host blood through haem-signalling is mediated by the haem-dependent nuclear receptor E75. RNAi-mediated silencing of E75 in mosquitoes has been shown to result in decreased transcription of a vitellogenin gene [79]. These data imply that haem functions as an important signalling molecule, serving as a sensor for the availability of a blood meal to enable egg development [79].

Box 3. Participation of Symbionts in Haem Provision

Blood-feeding parasites acquire a nutritionally unbalanced diet and, therefore, often need to rely on symbionts to supplement nutrients that are lacking [80]. During complex life cycles, parasites find themselves deprived of nutrients and thus may need to extract metabolic products from their symbiont(s). For example, the endosymbiont *Wolbachia* might provide haem to *Brugia malayi*, when exogenous levels of haem are low [45] – as could occur following the migration of microfilariae from the mosquito gut and subsequent formation of infective larvae in mosquito thoracic muscles and salivary glands. This proposal arises from the authors' previous work on the inhibition of haem biosynthesis [81]. Using submillimolar and low millimolar concentrations, respectively, of *N*-methyl-mesoporphyrin and succinyl acetone (inhibitors of the haem biosynthesis enzymes ferrochelatase and porphobilinogen synthase, respectively), the motility of both male and female adults was dramatically reduced in *in vitro* culture. However, the addition of haemin to worms in culture did not rescue motility, suggesting that the effect of the inhibitor might be insufficient and/or nonspecific [81]. The specificity of succinyl acetone (3 mM) has been questioned elsewhere [82]. Beside succinyl acetone, a high throughput chemical screen led to the identification of another potent inhibitor of *Wolbachia* porphobilinogen synthase (formerly δ-aminolevulinic acid dehydratase); hence, its name *w*ALADin1 [82]. This compound elicits a filaricidal effect in the *Wolbachia*-harboring nematode *Litomosoides sigmodontis*, but not in the *Wolbachia*-free nematode *Acanthocheilonema viteae*, suggesting that the antifilarial activity of wALADin1 is linked to *Wolbachia* [82]. Whether inhibition of *Wolbachia* haem biosynthesis causes the death of the symbiont, which affects the worm in a similar manner to filaricidal treatment with doxocycline antibiotics [83], or whether inhibition of *Wolbachia* haem biosynthesis has a direct effect on the worm because of a reduced availability of haem to the worm is still unclear.

transporter B10 has been shown to facilitate the export of haem from digestive vesicles, which is then transferred to haemosomes by an as yet unidentified cytosolic protein [31]. Intertissue transport of haem in ticks is made via large (~300 kDa) lipid-transfer proteins [32]. The genome of *I. scapularis* codes for five carrier proteins (CPs) and two vitellogenins (VGs) [19], which are orthologues of those found also in *I. ricinus* [16]. Even though these proteins are similar in structure, they differ substantially in their expression profiles [32]. Results obtained thus far suggest that CP homologues serve as a constitutive haem transporter to peripheral tissues, whereas VGs take over the critical function of transporting maternal haem to ovaries, to secure embryogenesis and reproduction [16]. The binding of haem by VGs seems to be specific to ticks, as it does not occur in mites or insects studied to date [32]. Which protein domain of tick CPs and/or VGs is responsible for haem binding is still unknown.

Although all insect genomes examined thus far code for haem biosynthesis pathway components, reproduction in some blood-feeding hemipteran insects, such as *R. prolixus*, seems to be highly dependent on the haem acquired from host blood. Experiments have shown that feeding this insect with haemoglobin-depleted plasma results in reduced fecundity in females, and that this effect can be reversed by adding haemin to dietary plasma [33]. Haem transport to the ovaries in *R. prolixus* is facilitated by a low-molecular-weight (15 kDa) haemolymphatic haem-binding protein, referred to as RHBP [33,34]. RNAi-mediated silencing of the *rhbp* gene has been shown to lead to oviposition of white, unviable eggs which are devoid of haem deposits; embryogenesis in these eggs does not take place, possibly due to the stark contrast of low levels of available haem with a high demand for haem for energetically-intensive embryogenesis [35]. That *R. prolixus* embryos depend primarily on maternal haem to complete embryogenesis suggests that host haem acquisition and somatic distribution do not have to be linked exclusively to an absence of endogenous haem biosynthesis.

In contrast to **hemipteran** blood-feeders, mosquitoes are capable of providing sufficient haem to their progenies via endogenous synthesis. Studies have shown that *Aedes albopictus* and *A. aegypti* fed on buffered bovine serum albumin (BSA) lay clutches of eggs that are similar in size to those fed on blood alone [36,37]. However, the viability of the eggs laid by *A. aegypti* fed exclusively on BSA was significantly less than when fed on serum or BSA supplemented with ferric chloride [36]. To fuel endogenous haem biosynthesis, mosquitoes obtain most iron from

the cleavage of host haemoglobin haem [38]. Such haem-derived iron (notintact haem) is thus an essential nutrient factor for mosquito reproduction [36,39].

Nematodes

It has been long assumed that nematode development is conditioned by the presence of haem-containing dietary components [40,41]. Two modes of haem acquisition have been considered in nematodes as a substitute of the lost endogenous haem biosynthesis: (i) exogenous/environmental acquisition of haem from the worm's diet, and (ii) haem supply via intracellular symbionts within worm tissues. The acquisition of dietary haem is exemplified by the free-living nematode *C. elegans*, where seminal work described molecules that secure haem acquisition, somatic distribution, and subcellular trafficking. Genome-wide microarray analysis of *C. elegans* cultured in a medium with low, intermediate (optimal), or high concentrations of haem led to the identification of differentially transcribed genes, some of which were novel and annotated as haem-responsive genes – *hrg*s [42]. Seven HRG paralogues of different function were described in *C. elegans* [42–44], but one orthologue of HRG-1 has been detected in some parasitic nematodes, such as *Bm*-HRG-1 in *B. malayi* – a causative agent of lymphatic filariasis

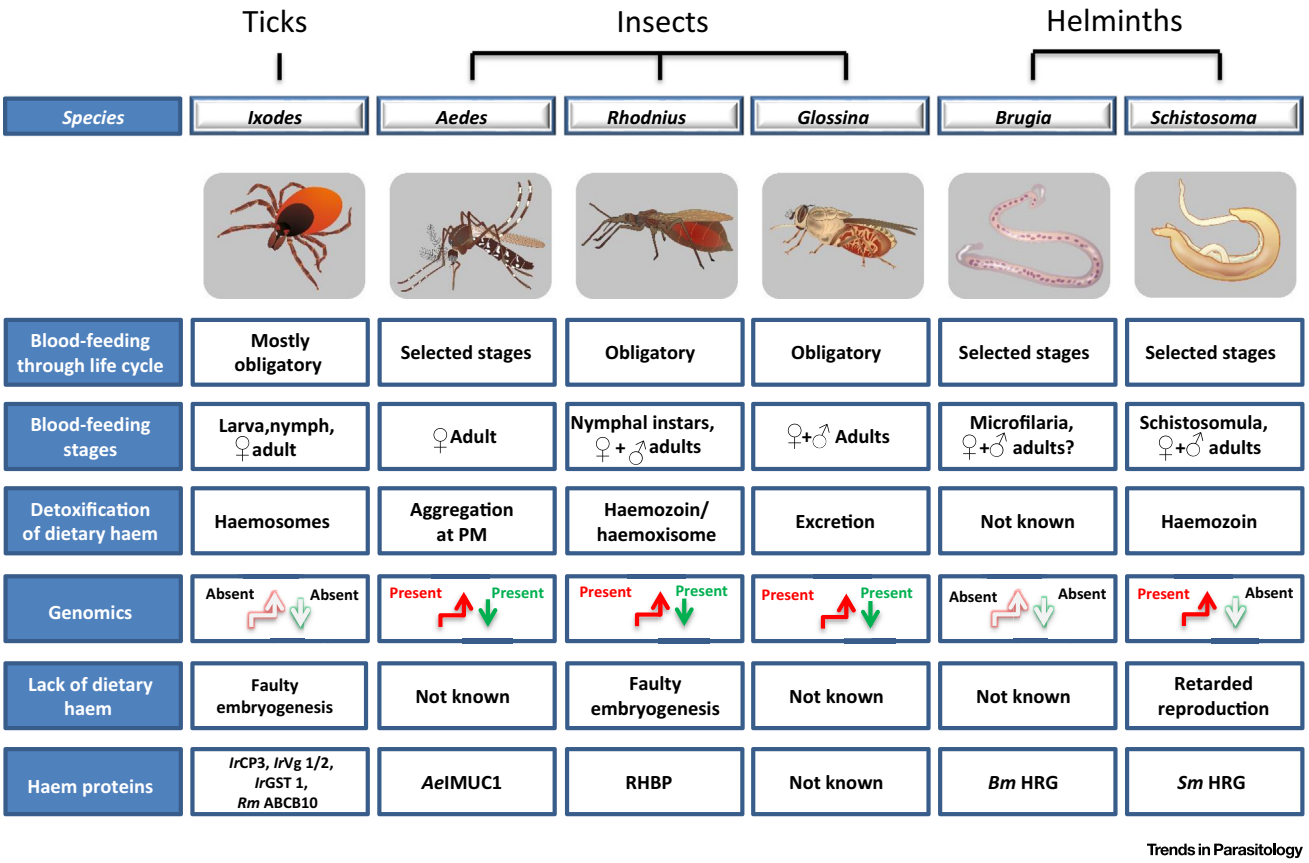

Figure 2. Summary of Blood-Feeding Demands, Haem Biosynthesis, and Degradation across Metazoan Parasites and Their Respective Haem-Interacting Proteins. A full red arrow indicates the presence of genes coding for haem biosynthesis, whereas a red contour indicates its absence. A full green arrow indicates the presence of a gene coding for haem oxygenase-based haem degradation, and a green contour indicates its absence. *Ir*CP3, *Ixodes ricinus* carrier protein 3; *Ir*Vg1/2, *I. ricinus* vitellogenin 1/2; *Ir*GST1, *I. ricinus* glutathione-*S*-transferase; *Rm*ABCB10, *Rhipicephalus microplus* ATP-binding cassette B10; *Ae*IMUC1, *Aedes aegypti* intestinal mucin 1; RHBP, *Rhodnius* haem-binding protein; *Bm*/*Sm* HRG, *Brugia malayi*/*Schistosoma mansoni* haem-responsive gene.

[45]. These authors speculated that the host blood is not the exclusive source of haem for this filarial worm, as some haem might also come from their endosymbionts (Box 3). Multidrug resistance protein MRP-5/ABCC5, involved in the transport of maternal porphyrins, haem and/or vitamin B12 [46,47], also has orthologues in parasitic nematodes.

Platyhelminths

Despite early work [24] reporting that *S. mansoni* does not synthesize haem, we could identify genes linked to haem biosynthesis components in available genomic and transcriptomic data of this blood fluke [48,49]. Even though blood flukes likely encode haem biosynthetic enzymes, *S. mansoni* schistosomule stages would rely on the acquisition of host haem [50]. Another study showed an uptake of exogenous haem by culturing *S. mansoni* in the presence of fluorescent haem analogues, which led to accumulation of the fluorescent signal in ovaries [51]. The authors further demonstrated that haem uptake into the ovaries is mediated by *Sm*HRG1, a *S. mansoni* homologue of haem-responsive genes of *C. elegans* [51]. Other haem-binding proteins have been described in *F. hepatica* and other trematodes [52]. These proteins of low molecular mass (~7.8 kDa; called MF6 monoclonal antibody recognized protein/helminth defence molecules = MF6p/HDMs) oligomerize and form high-molecular-weight complexes with haemin. The authors suggested that, besides mediating the host immune response against the trematode, MF6p/HDMs also play a role in haem homeostasis, being involved in haem scavenging, storage, and transport [52].

In summary, somatic distribution networks for haem exist in **auxotrophs** (e.g., ticks, nematodes) as well as in some parasites that do encode haem biosynthesis enzymes (e.g., kissing bugs and flukes). It is possible that the inevitable availability of exogenous haem might increase the fitness of mutations, and that incremental changes in the modus operandi of such haem recycling pathways might be selectively favored during the evolutionary adaptation to haematophagy. Such a novel paradigm – that haem is not proprietary to a cell, but can be mobilized from one cell or tissue to another – has been recognized by some authors [53].

Haem Catabolism to Secure Bioavailable Iron in Parasites (Q3)

Haem oxygenase (HO) is a single-gene-encoded enzyme that cleaves the haem ring into ferrous cation, biliverdin-IX-α, and carbon monoxide (CO). The latter products of HO cleavage, biliverdin and CO, have important pleiotropic functions, including cellular signalling and antioxidant protection [54]. The liberation of bioavailable iron through an HO-mediated cleavage is considered to be physiologically highly relevant in eukaryotic organisms, although an enzyme-independent haem cleavage *in vitro* has been reported [55]. Apart from utilizing intact host haem, parasites may thus also rely on HO-mediated products. The utilization of haem iron by parasites is well recognized.

Arthropods

Orthologues of the *ho* gene are present in the genomes of free-living and parasitic species of Insecta (Figure 2) and Crustacea (such as *Daphnia pulex*). On the other hand, ticks as well as free-living and parasitic mites do not have a *ho* gene [16], indicating the presence of a *ho* gene in the ancestor of arthropods, and a gradual loss of this gene from the Acari (i.e., ticks and mites). The lack of HO activity was experimentally corroborated in *I. ricinus*, in which no traces of biliverdin (haem degradation product) were detected following blood feeding. In addition, serum- and blood-feeding provided the ticks with the same amount of bioavailable iron [16]. The addition of bovine holo-transferrin to a serum meal substantially increased levels of bioavailable iron to *I. ricinus*, suggesting that host serum transferrin, and not haemoglobin, is the source of iron for ticks [16]. The transport and systemic distribution of acquired iron in

tissues of the tick is mediated by a secretory type of ferritin [56], whereas the storage and regulation of the bioavailability of iron is secured by the heavy-chain-type intracellular ferritin, which is closely related to that of mammals [57]. Unlike other species of arthropods (insects) or mammals, wherein haem and iron homeostasis is tightly interconnected, ticks maintain systemic haem and iron homeostases independently by two distinct protein networks.

Unlike ticks and mites, insects do code for HOs (Figure 2). Indeed, most of the iron in a mosquito's body, and deposited in eggs, is derived from haem from host haemoglobin [38]. In the mosquito *A. aegypti*, and the kissing bug *R. prolixus*, distinct haem degradation products, namely biglutaminyl-biliverdin [58] and dicysteinyl-biliverdin IX gamma [59], have also been identified, indicating that particular features of the HO-dependent haem degradation have arisen in haematophagous insects, but their physiological roles are presently unclear. Inhibition of HO in *A. aegypti* [60] and RNAi-silencing of the respective gene in *R. prolixus* [61] both resulted in reduced fecundity. Taken together, these data indicate that insects do have haem catabolism, and perturbation of its activity adversely impacts reproduction in these organisms.

Nematodes

It is likely that nematodes would acquire iron from a dietary nonhaem source [62]. Recently, an orthologue of divalent metal transporter 1 (DMT-1) was characterized for the filarial nematode *B. malayi*; the presence of this transporter in the worm intestine suggests its involvement in the uptake of iron from the diet [63]. It is apparent that all parasitic and free-living nematodes studied to date do not code for complete haem biosynthesis and degradation pathways (Figure 2).

Platyhelminths

Current evidence indicates that both free-living and parasitic flatworms do not code for HO (Figure 2). Flatworms are a part of the Lophotrochozoa (sister to the Ecdysozoa). The genomes of three lophotrochozoans have been sequenced and assembled, revealing that the mollusc *Lottia gigantea*, and the annelids *Helobdella robusta* and *Capitella teleta*, code for a predicted HO [64]. This information confirms the presence of this gene in this taxonomic group and suggests its loss from the platyhelminth lineage. To this end, knowledge of iron biology of platyhelminths is very limited, and it is assumed that these worms depend on nonhaem sources of iron.

In summary, while insects have retained a *ho* gene, noninsect blood-feeders acquire iron from a nonhaem source and have thus independent networks for haem and iron uptake and somatic homeostasis. It seems that, in nematodes, as well as in ticks, the loss of a *ho* gene predates the loss of the biosynthetic pathway. It is tempting to suggest that there is a link between these two events. As indicated, haem auxotrophy associates with increased absorption and transport of external haem, which is likely to increase the labile haem pool. Hence, HO-mediated haem degradation would lead to detrimental overload of free iron that cannot be utilized for haem biosynthesis [11].

Concluding Remarks

An appraisal of current knowledge in the areas of (i) the genomic reduction in haem metabolic pathways in metazoan parasites; (ii) the evolution of haem somatic distribution networks; and (iii) haem as an iron source, has raised some outstanding questions regarding haematophagy formation in multicellular parasites (see Outstanding Questions). The present genome-based prediction models reconstruct metabolic fluxes of haem – an essential cellular cofactor. These

Outstanding Questions

What is the evolutionary pressure for retaining the vestigial genes encoding the last three haem biosynthetic enzymes (CPOX, PPOX, and FECH) in ticks, and what are their natural substrates?

Unicellular parasites (such as *Trypanosoma* spp.) acquire host haemoglobin through specific receptors. Do any such receptors exist in multicellular parasites, or is haemoglobin uptake mediated by a distinct mechanism(s)?

Interference with haem distribution may elicit antiparasitic power in multicellular parasites. Haem-transporting haem-responsive genes (HRGs) and ABC transporters appear to be present in most blood-feeding multicellular parasites. Would these represent possible antiparasitic targets?

What mechanisms do platyhelminths and nematodes employ to secure iron uptake when haem is an unlikely source?

Do endosymbiotic bacteria play a role in the provision of haem to metazoan haem auxotrophs?

data indicate that, while some parasites are unable to form a haem molecule *de novo* (e.g., ticks and nematodes), other parasites clearly code for, perform, and rely on endogenous haem biosynthesis (e.g., dipteran insects). Others that code for enzymatic components of haem biosynthesis (e.g., platyhelminths and hemipteran insects) can effectively utilize host haem; their capacities to acquire host haem and distribute and/or traffic it at the tissue and subcellular levels thus represent key survival traits.

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Resources

[i]www.who.int/en/news-room/fact-sheets/detail/vector-borne-diseases

Supplemental Information

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