Horizontal gene transfer in the acquisition of novel traits by metazoans

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Horizontal gene transfer is accepted as an important evolutionary force modulating the evolution of prokaryote genomes. However, it is thought that horizontal gene transfer plays only a minor role in metazoan evolution. In this paper, I critically review the rising evidence on horizontally transferred genes and on the acquisition of novel traits in metazoans. In particular, I discuss suspected examples in sponges, cnidarians, rotifers, nematodes, molluscs and arthropods which suggest that horizontal gene transfer in metazoans is not simply a curiosity. In addition, I stress the scarcity of studies in vertebrates and other animal groups and the importance of forthcoming studies to understand the importance and extent of horizontal gene transfer in animals.

1. Introduction

In the last 20 years, horizontal gene transfer, ‘the non-genealogical transmission of genetic material from one organism to another’ according to Goldenfeld & Woese [1], has emerged as an important evolutionary force modulating the evolution of prokaryote genomes and consequently the evolution in the Bacteria and Archaea domains [2,3]. However, the importance of this process in eukaryotic evolution has been questioned (except for gene transfers from mitochondria and plastid ancestors to the eukaryotic nucleus [4,5]) considering the barriers imposed on the spread of horizontally acquired genes by the presence of the germ line.

This idea has been challenged for the past 5 years after the completion of an increasing number of eukaryotic genomes projects and today, the scientific community is beginning to acknowledge the importance of horizontal gene transfer in the evolution of unicellular eukaryotes [4,6,7]. On the other hand, more and more instances of genes showing phylogenetic incongruence and presumptive horizontally transferred are being described in more complex eukaryotes showing that horizontal gene transfer also plays a role in eukaryotic evolution leading to the acquisition of novel traits.

In this paper, I will review how horizontal gene transfer from symbiotic or free-living organisms can be also an important mechanism for (and a process of) the acquisition of novel traits by metazoans discussing the open questions and future outlooks on this topic. The discussion of the massive transfer of genes occurring as a consequence of interspecies hybridization is out of the scope of this review.

2. The number of presumptive genes horizontally transferred in metazoans is increasing

The advent of the next generation sequencers and the lower cost of sequencing projects enable the completion of genomic sequences and proteomic studies in non-model organisms, leading to insights in many important evolutionary and ecological aspects of the living world [8]. One important achievement derived from these studies is the discovery that several genes among metazoans have a phylogenetic history which is not congruent with that of the correspondent species. These genes show phylogenetic proximity between distant organisms and are suspected of horizontal gene transfer.
Two years ago, Dunning Hotopp [9] wrote the first review on horizontal gene transfer between bacteria and animals. Since then, an increasing number of genes in metazoans acquired by horizontal gene transfer, in an increasing number of species and groups and from an increasing number of potential donors have been proposed.

In this review, I will try to summarize the current and changing state of art with respect to the importance of acquired genes in the different metazoans groups and their role in the adaptation to particular lifestyles.

3. Genes horizontally acquired by sponges and cnidarians

Porifera and Cnidaria are metazoan phyla which include aquatic organisms living in close association with viruses, bacteria and unicellular eukaryotes [10–12] and whose life cycles encompass in some cases a vegetative phase mediated by budding or excision of somatic cells. These characters increase the possibility of genetic exchange and of the vertical transmission of acquired sequences by these basal metazoans.

Until now, only a single case of an ancient horizontally transferred gene has been reported in Porifera. A proteomic survey of the calcified spicules in *Astroclera willeynana* shows the acquisition by the ancestor of *A. willeynana* and *Amphipnedon guenslandica* [13] from bacteria of one gene encoding a protein involved in biomineralization processes. The gene is well expressed in spherulite forming cells, suggesting that it might be involved in the biocalcification process in coralline dermospoages.

On the other hand, several cases have been postulated in Cnidarians. The acquisition by the starlet sea anemone, *Nematostella vectensis*, of bacterial genes involved in the shikimic acid [14] and glyoxilate pathways [15] has been proposed from bioinformatics studies performed on the *Nematostella* genome. In addition, an interesting gene encoding a protein with mismatch repair activity has been postulated to be targeted to octocorals mitochondrial DNA [16], however, the gene donor or the relevance of gene function remains unknown.

But it is the survey of the *Hydra magnipapillata* genome that sheds light on the magnitude of horizontal gene transfer in Cnidarians [17]. Seventy one genes show closer relationships with bacterial genes, and 51 of them lack homologues in metazoans other than *Nematostella*. Moreover, 90 transposable elements that are of potential horizontal gene transfer origin have been identified. Among the putative horizontally transferred genes, a number of them encode sugar-modifying enzymes. Several bacterial phyla could be the potential donors of these genes that seem to have been recently transferred to the *Hydra* genome considering its lower intron number in comparison with *Hydra* genes.

A recent study reports the acquisition of bacterial toxin genes of the aerolysin (pore-forming toxins) family by *Nematostella, Hydra* and other eukaryotes such as plants, fungus and insects [18] probably involved in both defence and predation mechanisms.

Considering that a considerable number of gene loss events are necessary to explain the distribution of these genes across the eukaryotic phylogeny in the case where these genes were present in the common ancestor of the eukaryotes, this scenario seems less parsimonious than that of independent acquisitions from different sources. The ability to bring immediate benefits to the receptor and the fact that the proteins codified by these genes are not part of protein complexes (complexity hypothesis [19]) may help explain the proposed recurrent horizontal gene transfer of these genes.

The cases of horizontally acquired genes discussed earlier seem few considering the life history of these organisms that encompass a vegetative phase and their close association with viruses, bacteria and unicellular eukaryotes [10–12] living in symbiosis or in associated communities. Future surveys of genomes or proteomes in other groups of Porifera and Cnidaria will surely reveal the importance and extent of horizontal gene transfer among these basal metazoans.

4. Horizontal gene transfer in rotifers

The acquisition of a great number of genes by horizontal transfer in rotifers of the class Bdelloidea is one of the most interesting cases of gene transfer in metazoans. These organisms are microscopic invertebrates inhabiting freshwater habitats, showing an extreme tolerance to desiccation at the adult stage and whose reproductive mode is exclusively parthenogenetic [20].

A survey of a small part of the *Adineta vaga* genome [21] by screening of fosmid libraries revealed a high number of genes with sequences more similar to bacteria, fungi and plant homologues than to metazoan ones. These genes, located in the chromosomal subtelomeric regions encompass genes which encode for different specialized functions.

Two recent studies reinforce the massive gene transfer to bdelloid rotifer genomes. The first one [22] analysing the transcriptome of *Adineta ricciae* confirms the presence of a great number of foreign genes in the genome of these organisms that are truly transcribed. This study shows that around 10% of genes in the transcriptome of this species are of foreign origin and that 533 organisms could have contributed to the bdelloid genome as sources of horizontal gene transfer. The second study [23], dealing with the analysis of the *Adineta vaga* genome reveals the presence of 8% of foreign genes in this species, showing evidence of both ancient and recent transfers. Interestingly, glycosil hydrolases genes account for an important fraction of these presumptive transferred genes.

The majority of acquired genes seem to encode enzymes and many of them are involved in biochemical functions which are absent in other metazoans, such as those related to toxic compound degradation, resource acquisition or antioxidant biosynthesis.

Although the selective advantage for bdelloid rotifers, which is provided by the acquisition of particular genes, is unknown at present, it has been suggested that the massive horizontal gene transfer from bacteria, fungi and plants inhabiting the same environment provides genetic variability which compensates for the unisexual reproductive mode of these animals and enables tolerance against desiccation. In this sense, some of the genes acquired by *Adineta ricciae* seem to be upregulated by water loss, suggesting that they might be involved in the desiccation stress response [24].

On the other hand, it has been proposed that desiccation and rehydration periods can induce a transient competence state for DNA uptake in the gut cells, permitting the acquisition of foreign DNA in the form of food particles or free DNA absorbed in mineral surfaces [20,23]. The immediate...
vicinity between the gut and the ovary in these animals could enable the parallel uptake of foreign DNA by oocytes as well as the integration of genes and other genetic elements in the germ line.

Comparative studies using facultative asexual rotifers of the class Monogononta (results from the transcriptomic study discussed above [22] show that acquired genes in Monogononta are less numerous than in Bdelloidea and additional sequence information from new genomic projects will contribute in the near future to understanding the role of horizontally acquired genes in the evolution of these animals and of the mechanisms leading to an unprecedented amount of horizontal gene transfer in this metazoan group.

5. Acquisition of adaptive genes by nematodes

The phylum Nematoda encompasses several groups of roundworms that exhibit different lifestyles from free-living species to necromenic association with other invertebrates (feeding on the bacteria that thrive on dead animals), or plant or animal parasitism. Most of these are important plant pests or involved in human diseases.

Over the last few years, several studies have shown good examples of horizontal gene transfer in this metazoan group (see reviews in [25,26]).

Analyses of the complete genome of the necromenic nematode Pristionchus pacificus and transcriptomic studies reveal that several functional cellulase genes have been transferred to the ancestor of the Pristionchus genus from bacterial donors [27–29]. These cellulase genes are unrelated to the cellulase genes found in plant-parasitic nematodes (see below). Moreover, several gene duplication and gene loss events occurred after the acquisition of cellulase genes by the ancestor of Pristionchus.

In addition, a survey specifically designed to detect horizontal gene transfer has revealed a high number of genes and other genetic elements (most of them retrotransposons) in the genome of this nematode whose codon usage, phylogenetic relationships and genomic localization suggest an insect origin [30], thereby highlighting the importance of horizontal gene transfer from different sources in necromenic nematodes.

These organisms live in close association with insects and feed on bacteria and fungi that decompose the insect body after the insect’s death. In this sense, cellulase genes could be involved in the cellulose degradation of the biofilm that is formed by bacteria on which these nematodes feed and consequently be an important adaptive acquisition for the lifestyle of these metazoans. However, the significance of putatively genes acquired from insects is unclear and more studies are needed to unravel the role of these genes in the biology of necromenic nematodes.

Animal-parasitic nematodes infecting vertebrates cause both human and domestic animal diseases like filariasis among other important threats affecting human health. Several transfer events from the endosymbiont bacteria Wolbachia to the genome of animal-parasitic nematodes have been described [31,32], but the significance of the transferred genes is not clear. On the other hand, gene transfers from endosymbionts to Onchocercids nematode genomes can be facilitated by the close association between bacteria and nematode germ line cells at the female stage. A recent study [32] shows the presence of transcriptionally active Wolbachia-related genes in Wolbachia-free Onchocercid genomes suggesting an ancient transfer occurring before the loss of the symbiont in these nematodes.

Moreover, the presence of a functional and essential ferrochelatase gene acquired from non-Wolbachia bacteria has been recently reported in the human parasitic nematode Brugia malayi [33]. However, the acquisition mechanism of this gene from non-symbiogenic bacteria is not obvious. Moreover, the function of this acquired gene is not clear considering that the nematode endosymbiont Wolbachia has a complete biosynthesis haem group pathway.

Further studies are needed to understand the significance and role of acquired genes by animal–parasitic nematodes.

The ability to parasitize plants has emerged independently at least three times during nematode evolution [26], and it may therefore be considered to be a bona fide evolutionary novelty [34]. Several studies reveal that horizontal gene transfer may be an important contributor to the acquisition by nematodes of a plant–parasitic lifestyle.

A number of cellulase genes probably acquired from different sources have been found in genomes of plant-parasitic nematodes [35–37]. Interestingly, these cellulase genes have experienced multiple post-transfer duplication events [37] and are different from those present in necromenic nematodes (see above), thereby suggesting that they might be involved in degradation of the plant cell wall.

Other plant cell wall-degrading enzymes presumably acquired by horizontal gene transfer, such as xylanases, pectate lyases and polygalacturonases have been found in these nematodes [37]. A number of experimental data (reviewed in Danchin & Rosso [25] have shown that these genes are transcribed, their products are functional and excreted to plant tissues and the gene inactivation by knock-down leads to a reduction in the success of infection.

On the other hand, horizontally transferred genes from bacteria encoding proteins participating in the biosynthesis of several types of vitamin B have been described [38,39]. These are probably involved in the processing of plant resources.

Moreover, genes involved in plant cell defence suppression and detoxification, such as chorismate mutases or cyanate lyases, or in establishment of the nematode feeding site, have also probably been acquired by horizontal gene transfer (reviewed in Haegeman et al. [26]).

A recent study comparing proteomes of Meloidogyne hapla and Meloidogyne incognita with 14 metazoan genomes [40] has shown that at least 3.34% of the genes encoding proteins in genomes of these root-knot nematodes are of non-metazoan origin and encode mainly proteins related to the plant parasitism lifestyle.

Overall, these studies suggest that horizontal gene transfer has played an important role in the acquisition of plant-parasitic lifestyle in nematodes. However, the important question here is how did nematodes acquire these genes from bacteria, taking into consideration that many of the extant plant-parasitic nematodes lack symbionts and that the possible source of transfer are bacteria that are not phylogenetically related to Wolbachia or other past endosymbionts? One possibility proposed by Danchin [25,41] is that an ancestor of these nematodes could have been bacteriovorous, feeding on plant pathogenic bacteria. In this scenario, genetic material from digested bacteria could have been acquired by nematodes using plasmid or viruses as vectors. However, the question remains of how did these genes become part of the nematode germ line?
6. Mollusc genes from algal origin: are they really horizontally transferred genes?

A controversial case of horizontal gene transfer is the presence of genes from algal origin in kleptoplastic molluscs. Several species of sachoglossan opisthobranch gastropods live in an interesting symbiosis with chloroplast acquired from their algal food. Chloroplasts captured from algae (called kleptoplasts) remain intact and are capable of photosynthesis in cells of the digestive tubule of these sacoglossan sea slugs for some period of time. In a few species, photosynthesis continues for as long as 1 year in the absence of algal food [42]. In one of these species, Elysia chlorotica, PCR experiments have suggested the presence of genes derived from the algae Vaucheria litorea in the nuclear genome of the sea slug [43]. Further transcriptome analyses however have revealed controversial results.

A partial transcriptome analysis of two other sea slug species, Elysia timida and Plakobranchus ocellatus, has revealed no evidence of transcripts corresponding to algal genes [44]. Another report has also failed to detect evidence of the transcription of presumptive transferred genes in E. chlorotica [45]. A new study however, which extends the sequencing coverage has recently revealed the presence of at least 111 transcripts in the transcriptome of E. chlorotica matching 52 of the nuclear-encoded genes of V. litorea, including one that was identified by PCR in previous studies [46]. The last note of this story may be found in a very recent paper that has failed to find evidence of algal acquired genes in genome reads of E. chlorotica fertilized eggs [47], suggesting that horizontal gene transfer is not the primary reason for the maintenance of photosynthesis in these animals.

The studies discussed above illustrate important aspects of horizontal gene transfer studies. It is not sufficient to identify presumptive horizontally transferred genes. It is necessary to test and ensure that these genes are transcribed efficiently and that they can play a role in the receptor’s biology. Moreover, it is necessary to search in the germ line cells for the presence of presumptive horizontally transferred genes.

7. Horizontal gene transfer in arthropods: transfer from endosymbiont bacteria and the acquisition of carotenoid biosynthesis pathways and other adaptive traits

Endosymbiosis between bacteria and arthropods is a widespread phenomenon [48]. Among insects for example, at least 20% of species host endocellular α-proteobacteria of the genus Wolbachia, which are maternally transmitted and closely associated with insect germ line cells. This makes arthropods in general and insects in particular good candidate organisms for horizontal gene transfer detection in metazoans.

Fragment of the Wolbachia genome (approx. 30% with respect to the gene repertoire) have been found in the genome of the bean beetle Callosobruchus chinensis. However, although a low transcriptional level could be detected, most of the genes in these transferred fragments have been pseudogenized [49,50].

Moreover, nearly the entire Wolbachia pipientis genome seems to have been transferred to the Drosophila ananassae genome [31] and sequences of Wolbachia can also be detected in other drosophilidae species. Similar to Callosobruchus, some of the transferred genes show a low transcriptional level.

Likewise, Wolbachia genes can be detected in genomes of parasitoid wasps of the genus Nasonia [31,51]. Overall, these studies show widespread horizontal gene transfer from endosymbiotic bacteria to insects. However, the pseudogenization of a large number of acquired genes and the low transcriptional level exhibited by horizontally transferred genes obscures the importance of these genes for the host. Some authors even question the importance of the genetic material acquired from Wolbachia, suggesting that these insertions resemble the presence of ‘numts’ (non-functional fragments of mitochondrial origin) in animal genomes [52].

Recent studies have revealed cases of horizontally transferred genes from non-endosymbiotic bacteria, whose significance seems more explicit to the life history of insects. This is the case for the identification of genes involved in the adaptation to the herbivorous lifestyle. Two studies have recently identified in two beetle species genes that are probably involved in plant cell wall degradation. One mannanase gene (HhMAN1) that is phylogenetically related to intestinal bacteria genes of the Bacillus clade has been identified in the coffee berry borer beetle, Hypothenemus hampei, an invasive coffee pest [53]. Moreover, two xylanase genes have been identified in the mustard leaf beetle, Phaedon cochleariae [54], that are probably acquired through horizontal gene transfer from γ-proteobacteria followed by a tandem duplication of the transferred gene.

In both cases, transferred genes are functional and they have been ameliorated showing hallmarks of authentic eukaryotic genes, such as polyadenylation sequences. Moreover, in both cases, genes are flanked by two eukaryotic transposons whose role in the elusive mechanism of transfer is unclear owing to the absence of similarities with prokaryotic transposons. It also remains unclear how acquired genes have been incorporated to the germ line of these insects. Despite the fact that some authors consider some plant cell wall-degrading enzymes were present in the last common ancestor of bilaterian metazoans [55] the phylogenetic distance between the two presumptive prokaryotic donors for the HhMAN1 in H. hampei and xylanase genes in P. cochleariae suggest that these cases are bona fide examples of horizontal gene transfer in insects. Further studies will probably reveal more examples of acquired genes related to plant feeding in herbivorous Coleoptera.

Another interesting example of the acquisition of a novel trait by horizontal gene transfer is the presence of carotenoid biosynthesis genes in two different insect groups [56,57] and in one arachnid species [58].

Carotenoids are organic pigments whose physiological functions encompass photosynthesis facilitation, protection against photo-oxidation, display coloration and light detection. It has been traditionally assumed that only plants, microorganisms and fungi were capable of synthesizing carotenoids, the animals obtaining these pigments from their diets.

In aphids (Insecta, Hemiptera), carotenoids are involved in colour polymorphisms that affect the interaction with both predators and parasites. Three years ago, Moran & Jarvik [56] found in the genome of the pea aphid, Acyrthosiphon pisum, several functional genes encoding for carotenoid biosynthesis proteins that were phylogenetically related to fungal genes. This study provided the first example of
carotenoid biosynthesis by metazoans, suggesting that biosynthesis genes may be acquired by the ancestor of several extant aphids from a fungus through ancient horizontal gene transfer events. Carotenoid biosynthesis genes seem to have undergone duplication, recombination and selection events following this ancient transfer, yielding a wide array of carotenoid profiles which characterize the different aphid species [59].

In the last years, carotenoid biosynthesis genes of fungal origin were also identified in dimorphic flies of the family Cecidomyiidae [57] and in the two-spotted spider mite Tetramyces urticae [58]. Comparisons of the intron/exon structure of these genes in the three different arthropod groups and the patchy distribution of these genes across the arthropod phylogeny suggest [57] that the genes in the three cases come from relative fungal donor species and that they have been independently acquired by the different arthropod groups. An alternative scenario, involving one single acquisition event at the basal radiation of the arthropods followed by subsequent gene losses in different lineages, seems to be less parsimonious. Future studies will contribute to resolve the history of the acquisition of carotenoid biosynthesis genes in arthropods. The advantage of these acquisitions for arthropods is unclear taking into consideration that in some arthropods like whiteflies, carotenoids may be supplied by endosymbiotic bacteria [60], but it has been suggested that these genes could have participated in the diversification of the ecological patterns.

Similar to other cases of horizontal gene transfer discussed in this review, further studies are needed to understand both the significance of the acquisition of carotenoid biosynthesis by arthropods and the gene transfer mechanism that takes place from fungal donors to arthropods.

Nevertheless, these studies show an interesting example of acquisition of genes with known physiological functions by horizontal gene transfer and open the door to future new surprises and discoveries.

The examples discussed above (and two recent reports showing multiple ancient horizontal gene transfers from bacteria and fungi to the genomes of different lepidopteran species [61,62] and the genome of the crustacean Litopenaeus vannamei [63]) show that horizontal gene transfer in arthropods is no longer a curiosity and that more accurate screening of arthropod genomes and transcriptomes will surely unveil more evidence supporting the horizontal acquisition of genes in these metazoans.

8. Horizontal gene transfer in chordates

An interesting case of a new trait acquired by horizontal gene transfer has been found in urochordates. Until now, however, there has been a lack of clear evidence supporting the horizontal gene transfer of encoding sequences in vertebrates.

The acquisition by the urochordate Ciona intestinalis of a cellulose synthase gene of cyanobacterial origin has been proposed [64]. This acquisition gives these animals the ability (unique among animals) of cellulose biosynthesis. This can therefore be a good example of new trait acquisition by horizontal gene transfer.

In addition, it has been claimed that modern tunicates such as C. intestinalis [65] may be of a hybrid or chimeric origin. However, discussion on hybridization as a massive horizontal gene transfer event is out of the scope of this review.

In the original sequencing and analysis of the human genome, a considerable number of protein genes of possible bacterial origin were identified [66], but further studies have refuted the bacterial origin of these proteins [67,68]. There are however a few studies which suggest that vertebrates have acquired genes by horizontal gene transfer. The evidence for this is nonetheless controversial. For example, a study [15] shows a complex scenario for the evolution of glyoxilate cycle enzymes, which includes the acquisition of genes by horizontal gene transfer in non-placental vertebrates. Another study suggests that horizontal gene transfer between fish species plays a significant role in the acquisition of a particular type of antifreeze proteins by phylogenetically distant species [69]. However, the presumptive origin of the gene encoding this protein is not clear and gene loss cannot be fully discarded. Apart from the above-mentioned studies, the evidence for horizontally transferred genes in vertebrates is scarce.

However, other genetic elements, like transposable elements, could have been transferred more easily during vertebrate evolution. Increasing evidence for horizontal gene transfer of retroviruses, DNA transposons and even non-long terminal repeat retrotransposons across divergent species and geographical areas mediated by different vectors [70–73] suggest that horizontal gene transfer has modulated the evolution of animal genomes (reviewed in Ivancevic et al. [73]), introducing structural changes in the genomes of their host that may contribute to the modification of regulatory elements and lead to the acquisition of novel traits.

Considering that a great part of animal genomes are made up of mobile genetic elements [66], the understanding of the role of the horizontal transfer in the widespread distribution of these elements can contribute to the better understanding of the evolution of animal genomes. In any case, knowledge on vertebrate horizontal gene transfer is far from that known for other metazoans, and further studies will clarify its extent and importance.

A new and different way forward has opened with the recently unveiled evidence on horizontal gene transfer from bacteria to human transformed somatic cells. A study led by Dunning Hotopp’s group [74] has shown that cells from acute myeloid leukaemia and stomach adenocarcinoma seem to be enriched in sequences from bacteria present in the human microbiome supporting the importance of horizontal gene transfer in cancer development. The relevance of this new research programme is clear, but the discussion of its significance is out of the scope of this review.

9. Concluding remarks

Studies discussed in this review highlight the increasing evidence for genes that show incongruent phylogenetic relationships and which are suspected of horizontal gene transfer in metazoans (table 1). In some cases (nematodes and arthropods), endosymbiont bacteria are established near to germ line cells suggesting possible mechanisms of gene transfer. In other cases, bacteria or fungi living in close association with metazoans are good source candidates of the transferred genes, and in others the possible mechanisms and vectors for gene transfer and the source of acquired genes are simply unknown. The close association between parasites and their host offers considerable opportunities for horizontal gene transfer to occur from parasites to...
the hosts and vice versa, and cases of transfers from metazoans to unicellular eukaryotes and other metazoans and from unicellular eukaryotes to metazoans has been reviewed recently by Wijayawardena et al. [75]. Acquisition of insect genes by P. pacificus [30] discussed above in this review can be a good example of these transfers. Only future studies will reveal the importance and extent of horizontal gene transfer between parasites and their hosts.

In addition, studies discussed above stress how it is not sufficient to just identify presumptive horizontal gene transfer events. It is also necessary to ensure that these presumptive genes are efficiently transcribed and that they play a role in the host.

Several cases discussed in this review (for example, genes involved in plant parasitism in nematodes or carotenoid biosynthesis genes in arthropods) fit these criteria. Besides these cases (or the cellulose synthase in urochordates), the role of presumptive transferred genes in the host is highly speculative. A best knowledge of biology of putative donors and receptors could aid in the identification of new cases of horizontal gene transfer to metazoans and contribute an explanation of the role of the transferred genes in the hosts.

The recent study by Bhattacharya et al. [47] that failed to find presumptive horizontal transferred genes in fertilized eggs of E. chlorotica highlights another important point: the need to confirm suspected cases in germ cells or fertilized eggs. Future studies using this approach will confirm the presence or the absence of suspected horizontally transferred genes in host eggs for many proposed horizontal gene transfer cases.

A recently proposed model [76] dealing with horizontal gene transfer in eukaryotes may prove useful to validate suspected cases of horizontal gene transfer and to understand how horizontal gene transfers occur in metazoans. This model associates the occurrence of horizontal gene transfer in eukaryotes with that of weak or unprotected points in the organism life cycle and suggests that horizontal gene transfer can occur in multicellular eukaryotes with exposed early developmental stages.

Future studies will surely reveal new cases of suspected horizontal gene transfer events in new metazoan groups, allowing us to understand the importance and extent of horizontal gene transfer in eukaryotes in general and in metazoans in particular and confirming the foresight of two microbiologists from 13 years ago [77].

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Table 1. Proposed horizontally transferred elements to metazoans (for references, see the main text).

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<th>donors</th>
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<td>bacteria</td>
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<tr>
<td>Cnidaria</td>
<td>genes involved in several metabolic pathways</td>
<td>bacteria</td>
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<td>sugar-modifying enzymes</td>
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<td>pore-forming toxins</td>
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<td>genes involved in several metabolic pathways</td>
<td>bacteria, fungi, plants</td>
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<td>nematodes</td>
<td>Wolbachia genetic elements</td>
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<td>genes involved in haem group biosynthesis</td>
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