

1 **Alternative splicing of COQ-2 determines the choice between ubiquinone and  
2 rhodoquinone biosynthesis in helminths**

3 **June H. Tan<sup>1</sup>, Margot Lautens<sup>1</sup>, Laura Romanelli-Cedrez<sup>2</sup>, Jianbin Wang<sup>3,4</sup>, Michael R. Schertzberg<sup>1</sup>,  
4 Samantha R. Reinl<sup>5</sup>, Richard E. Davis<sup>3</sup>, Jennifer N. Shepherd<sup>5\*</sup>, Andrew G. Fraser<sup>1\*</sup>, Gustavo Salinas<sup>2\*</sup>**

5 <sup>1</sup>The Donnelly Centre, University of Toronto, Toronto, Canada; <sup>2</sup>Laboratorio de Biología de Gusanos. Unidad  
6 Mixta, Departamento de Biociencias, Facultad de Química, Universidad de la República - Institut Pasteur de  
7 Montevideo, Montevideo, Uruguay; <sup>3</sup>Department of Biochemistry and Molecular Genetics, RNA Bioscience  
8 Initiative, University of Colorado School of Medicine, Aurora, Colorado, USA; <sup>4</sup>Department of Biochemistry  
9 and Cellular and Molecular Biology, University of Tennessee, Knoxville, USA; <sup>5</sup>Department of Chemistry and  
10 Biochemistry, Gonzaga University, Spokane, Washington, USA

11

12 **\*For correspondence:**

13 shepherd@gonzaga.edu (JNS);

14 andyfraser.utoronto@gmail.com (AGF);

15 gsalin@fq.edu.uy (GS)

16

17 **Abstract**

18 Parasitic helminths use two benzoquinones as electron carriers in the electron transport chain. In aerobic  
19 environments they use ubiquinone (UQ) but in anaerobic environments inside the host, they require rhodoquinone  
20 (RQ) and greatly increase RQ levels. The switch to RQ synthesis is driven by substrate selection by the  
21 polyprenyltransferase COQ-2 but the mechanisms underlying this substrate choice are unknown. We found that  
22 helminths make two *coq-2* isoforms, *coq-2a* and *coq-2e*, by alternative splicing. COQ-2a is homologous to COQ2  
23 from other eukaryotes but the COQ-2e-specific exon is only found in species that make RQ and its inclusion

24 changes the enzyme core. We show COQ-2e is required for RQ synthesis and for survival in cyanide in *C. elegans*.  
25 Crucially, we see a switch from COQ-2a to COQ-2e as parasites transition into anaerobic environments. We  
26 conclude that under anaerobic conditions helminths switch from UQ to RQ synthesis via alternative splicing of  
27 *coq-2*.

28

29 **Introduction**

30 Parasitic helminths are major human pathogens. Soil transmitted helminths (STHs) such as the nematodes  
31 *Ascaris*, hookworms, and whipworms infect well over a billion humans and seven out of the 18 neglected diseases  
32 categorized by WHO are caused by helminths (CDC, 2019). Despite the huge impact on global health of these  
33 infections, there are few classes of available anthelmintics and resistance is increasing in humans and is  
34 widespread in some species that infect animals — for example, *Haemonchus contortus*, a wide-spread parasite in  
35 small ruminants, has developed multidrug resistance (Jackson, Coop, 2000, Kotze, Prichard, 2016). There is thus  
36 a serious need to develop new classes of anthelmintics that target the parasites while leaving their animal hosts  
37 unaffected. One potential target for anthelmintics is their unusual anaerobic metabolism which differs from that  
38 of their hosts. When STHs are in their free-living stages of their life cycles, they use the same aerobic respiration  
39 as their hosts and use ubiquinone (referred to here as UQ and as Q in other papers) as an electron carrier in their  
40 mitochondrial electron transport chain (ETC). However, when they infect their hosts, they encounter highly  
41 anaerobic environments. This is particularly the case when they live in the host gut and *Ascaris*, for example, can  
42 live for many months in this anaerobic environment (Dold, Holland, 2011). To survive, they use an alternate form  
43 of anaerobic metabolism that relies on the electron carrier rhodoquinone (RQ). Since hosts do not make or use  
44 RQ, RQ-dependent metabolism could be a key pharmacological target since it is required by the parasite but is  
45 absent from their mammalian hosts.

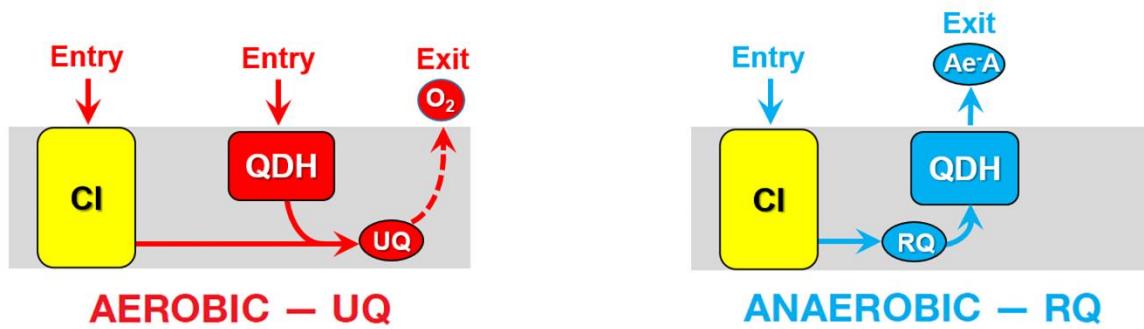
46 RQ is an electron carrier that functions in the mitochondrial ETC of STHs (Van Hellemond et al., 1995). RQ  
47 is a prenylated aminobenzoquinone that is similar to UQ (Figure 1), but the slight difference in structure gives

48 RQ a lower standard redox potential than UQ (-63 mV and 110 mV, respectively) (Unden, Bongaerts, 1997, Erabi  
49 et al., 1976). This difference in redox potential means that RQ, but not UQ, can play a unique role in anaerobic  
50 metabolism. In aerobic metabolism, UQ can accept electrons from a diverse set of molecules via quinone-coupled  
51 dehydrogenases — these include succinate dehydrogenase and electron-transferring-flavoprotein (ETF)  
52 dehydrogenase. In RQ-dependent anaerobic metabolism, RQ does the reverse — it carries electrons to these same  
53 dehydrogenase enzymes and drives them in reverse to act as reductases that transfer electrons onto a diverse set  
54 of terminal acceptors (Tielens, Van Hellemond, 1998, van Hellemond et al., 2003). UQ thus allows electrons to  
55 enter the ETC via dehydrogenases; RQ can drive the reactions that let electrons leave the ETC and this difference  
56 in direction of electron flow is driven by the difference in redox potential (Figure 1A). This ability of RQ to  
57 provide electrons to an alternative set of terminal electron acceptors allows helminths to continue to use a form  
58 of mitochondrial ETC to generate ATP without oxygen. In this RQ-dependent anaerobic metabolism, electrons  
59 enter the ETC from NADH through complex I and onto RQ, and this is coupled to proton pumping to generate  
60 the proton motive force required for ATP synthesis by the F0F1ATPase (van Hellemond et al., 2003). The  
61 electrons are carried by RQ to the quinone-coupled dehydrogenases which are driven in reverse as reductases and  
62 the electrons thus exit onto a diverse set of terminal acceptors, allowing  $\text{NAD}^+$  to be regenerated and the redox  
63 balance to be maintained. This RQ-dependent metabolism does not occur in the hosts and it is thus an excellent  
64 target for anthelmintics (Kita, Nihei & Tomitsuka, 2003).

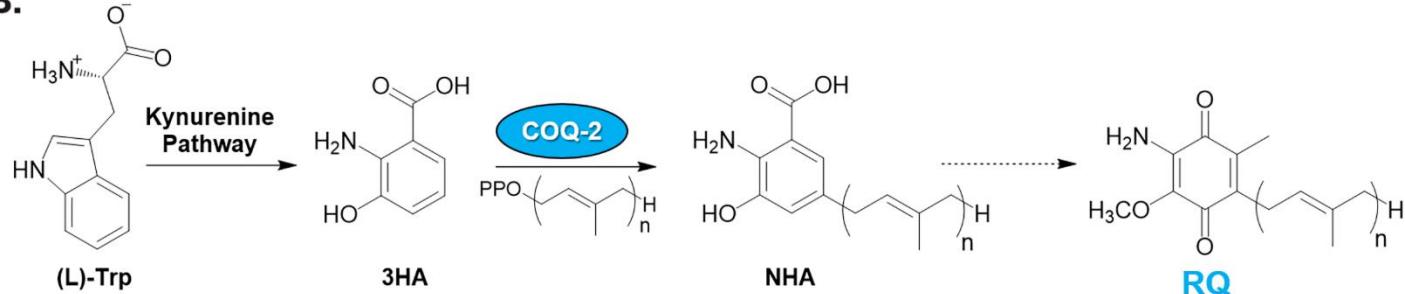
65 In the animal kingdom, RQ is present in several facultative anaerobic lineages that face environmental anoxia  
66 or hypoxia as part of their lifecycle. Among animals, RQ has only been described in nematodes, platyhelminths,  
67 mollusks and annelids (Van Hellemond et al., 1995). Key steps of RQ biosynthesis in animals were recently  
68 elucidated (Roberts Buceta et al., 2019, Del Borrello et al., 2019). In contrast to bacteria and protists, where RQ  
69 derives from UQ (Brajcich et al., 2010, Stairs et al., 2018), RQ biosynthesis in animals requires precursors derived  
70 from tryptophan via the kynurenine pathway (Figure 1B). Studies performed by two teams in *C. elegans* have  
71 recently demonstrated that animals that lack a functional kynureninase pathway (e.g. strains carrying mutations

72 in *kynu-1*, the sole kynureninase) are unable to synthesize RQ (Roberts Buceta et al., 2019, Del Borrello et al.,  
73 2019). It is presumed that 3-hydroxyanthranilic acid (3HA here, also sometimes referred to as 3HAA) from the  
74 kynurenine pathway is prenylated in a reaction catalyzed by COQ-2. The prenylated benzoquinone ring can then  
75 be modified by methylases and hydroxylases (COQ-3, COQ-5 and COQ-6) to form RQ. This proposed pathway  
76 is analogous to the biosynthesis of UQ from 4-hydroxybenzoic acid (4HB) or *para*-aminobenzoic acid (pABA)  
77 (Figure 1C). The key insight from these previous studies is that the critical choice between UQ and RQ synthesis  
78 is the choice of substrate by COQ-2 — if 4HB is prenylated by COQ-2, UQ will ultimately be made, but if 3HA  
79 is used, the final product will be RQ. In most parasitic helminths there is a major shift in quinone composition as  
80 they move from aerobic to anaerobic environments e.g. RQ is less than 10% of *H. contortus* total quinone when  
81 the parasite is in an aerobic environment but is over 80% of total quinone in the anaerobic environment of the  
82 sheep gut and similar shifts occur in other parasites (Van Hellemond et al., 1995, Sakai et al., 2012, Luemmen et  
83 al., 2014). Somehow, COQ-2 must therefore switch from using 4HB to using 3HA as a substrate but the  
84 mechanism for this substrate switch is completely unknown. Understanding this mechanism is important — if we  
85 could interfere pharmacologically with the switch to RQ synthesis, it could lead to a new class of anthelmintics.  
86 In this study, we reveal that two variants of COQ-2, derived from alternative splicing of mutually exclusive exons,  
87 are the key for the discrimination in the RQ/UQ biosynthesis. The removal of one of the mutually exclusive exons,  
88 present only in species that synthesize RQ, abolishes RQ biosynthesis in *C. elegans*. The analysis of COQ-2  
89 RNA-seq data from parasites revealed that the RQ-specific exon expression is increased in hypoxic lifestages,  
90 while the alternative exon is increased in normoxic lifestages. We thus conclude that the alternative splicing of  
91 COQ-2 is the key mechanism that regulates the switch from UQ to RQ synthesis in the parasite life-cycle. We  
92 also propose that the reason that RQ is only synthesised in helminths, annelids, and molluscs is due to the  
93 independent evolution of COQ-2 alternative splicing in these animal lineages.

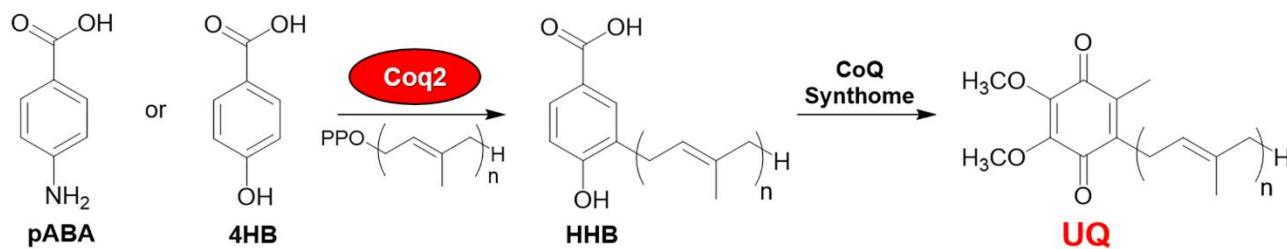
**A.**



**B.**



**C.**



94 **Figure 1.** Rhodoquinone and ubiquinone biosynthesis and function in electron transport chains. (A) In aerobic  
95 metabolism, ubiquinone (UQ) shuttles electrons in the ETC from Complex I and quinone dehydrogenases  
96 (QDHs), such as Complex II and electron-transferring flavoprotein (ETF), which are ultimately transferred to  
97 oxygen. In anaerobic metabolism, rhodoquinone (RQ) reverses electron flow in QDHs and facilitates an early  
98 exit of electrons from the ETC at anaerobic electron acceptors (Ae-A), such as fumarate. (B) The RQ  
99 biosynthetic pathway in *C. elegans* requires *L*-tryptophan, a precursor in the kynureneine pathway. *L*-Trp is  
100 transformed into 3-hydroxyanthranilic acid (3HA) in four steps. It is proposed that 3HA is a substrate for COQ-  
101 2, producing 3-hydroxy-5-nonenylanthranilic acid (NHA) where n = 9. Transformation of NHA to RQ  
102 requires several shared proteins from the UQ biosynthetic pathway. (C) Yeast can use either *p*-aminobenzoic  
103 acid (pABA) or 4-hydroxybenzoic acid (4HB) as precursors to UQ. Prenylation is facilitated by Coq2 to form 3-  
104 hexaprenyl-4-hydroxybenzoic acid (HHB), where n = 6. Further functionalization occurs through a CoQ  
105 synthome (Coq3-Coq9 and Coq11) to yield UQ.

106 **Results**

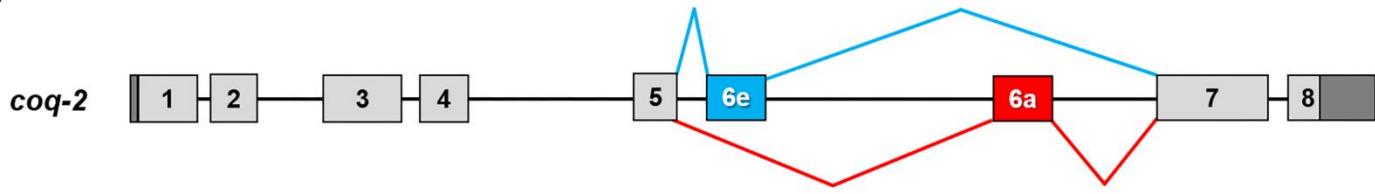
107 **The *C. elegans* COQ-2 polyprenyl transferase required for quinone biosynthesis has two major**  
108 **alternative splice forms**

109 Our groups previously showed that if COQ-2 uses 4HB as a substrate, this will lead to synthesis of UQ; however,  
110 if it uses 3HA this will ultimately yield RQ. As parasites move from aerobic environments to the anaerobic niches  
111 in their host, they change their quinone composition from high UQ to high RQ. For this to occur, COQ-2 must  
112 switch its substrate from 4HB to 3HA but the mechanism for this switch is unknown.

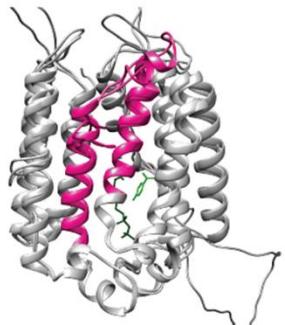
113 We identified two distinct splice forms of *C. elegans* *coq-2*: *coq-2a* and *coq-2e*. These are annotated in the  
114 genome and confirmed by RNA-seq, by nanopore sequencing, and by targeted validation studies (Ramani et al.,  
115 2011, Kuroyanagi, Takei & Suzuki, 2014, Roach et al., 2020, Li et al., 2020). These two isoforms differ by the  
116 mutually exclusive splicing of two internal exons (6a and 6e), both of 134 nucleotides (see Figure 2A). We note  
117 that mutually exclusive splicing of cassette exons is very rare in the *C. elegans* genome and fewer than 100 such  
118 splicing events have ever been identified (Ramani et al., 2011, Kuroyanagi, Takei & Suzuki, 2014). Both *coq-2a*  
119 and *coq-2e* splice forms are abundant in *C. elegans* across all stages of development, where ~30-50% of *coq-2* is  
120 the *coq-2e* isoform (Ramani et al., 2011, Gerstein et al., 2010, Grun et al., 2014).

121 To examine how the alternative splicing of COQ-2 might affect its function, we threaded the predicted COQ-  
122 2a and COQ-2e protein sequences onto the solved crystal structure of the archaeal *Aeropyrum pernix* COQ-2  
123 orthologue (PDB:4OD5). We found that the splicing change causes a switch in two alpha-helices at the core of  
124 the COQ-2 structure (Figure 2B). This is a region of the protein that is thought to form a hydrophobic tunnel  
125 along which the aromatic substrates must pass to the active site for the key polyprenylation reaction (Desbats et  
126 al., 2016) suggesting that the change in splicing could affect COQ-2 substrate selection and thus could explain a  
127 shift from UQ to RQ synthesis. We therefore examined whether similar COQ-2 alternative splicing is seen in  
128 parasitic helminths and how the different splice forms compare to COQ-2 sequences in parasite hosts which do  
129 not make RQ.

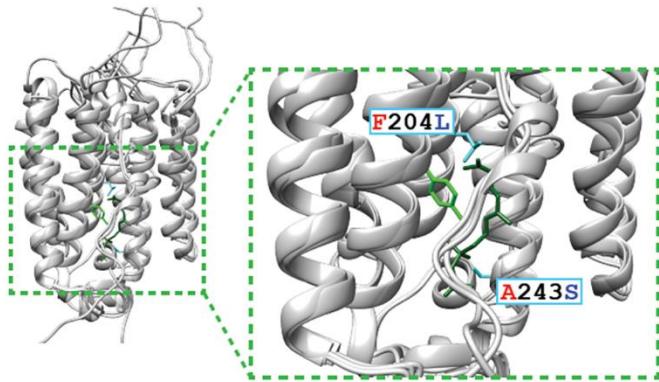
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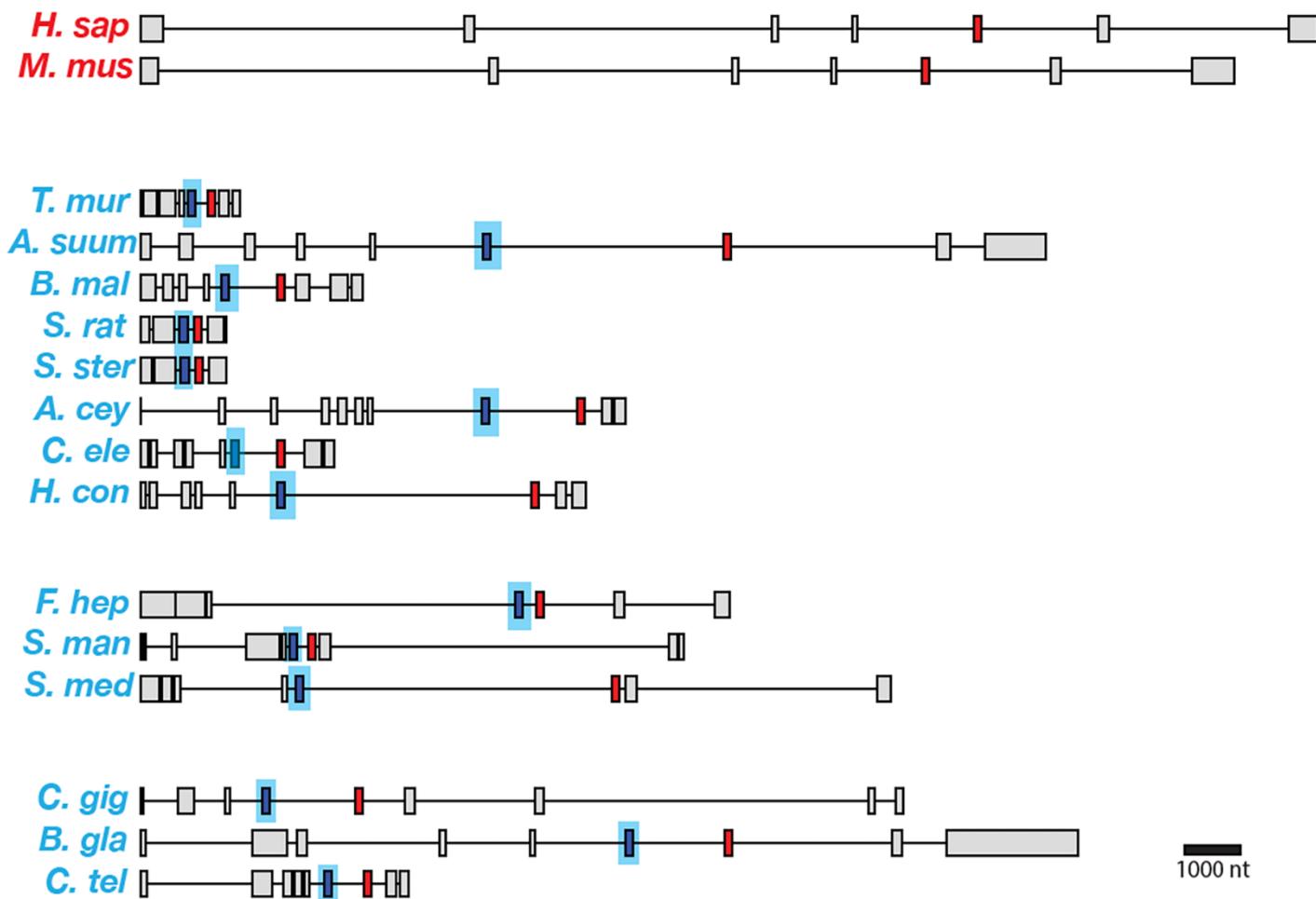


130 **Figure 2.** *C. elegans* *coq-2* gene model. (A) The *coq-2* gene contains two mutually exclusive exons, 6e (blue box)  
131 and 6a (red box), that are alternatively spliced (blue and red lines, respectively) generating two COQ-2  
132 isoforms. Light grey boxes represent coding sequences of exons 1-5 and 7-8, black lines represent introns, and  
133 dark grey boxes denote 5' and 3' untranslated regions of exons 1 and 8. (B) Alternative splicing of COQ-2  
134 changes the enzyme core. The sequences of *C. elegans* *coq-2a* and *coq-2e* were threaded onto the crystal  
135 structure of the apo-form of the *A. pernix* COQ-2 homologue (PDB: 4OD5) in Chimera using Modeller. The  
136 region switched by mutually exclusive alternative splicing is shown magenta. (C) The alternative exons found  
137 in all RQ-synthesising species have two residues that are invariant (L204 and S243 show in cyan; *C. elegans*  
138 numbering) that are near the binding site of the two substrates. Substrates are the polyprenyl tail (dark green;  
139 geranyl S-thiolodiphosphate in the crystal structure), and the aromatic ring (light green; *p*-hydroxybenzoic acid  
140 (4HB) in the crystal structure). Note that COQ-2 is rotated from panel B to panel C for clarity.

141 **Parasitic helminths have a distinct splice form of *coq-2* that is not present in any of the parasitic hosts**

142 *C. elegans* has two major isoforms of *coq-2* which are the result of mutually exclusive alternative splicing of  
143 two internal exons and affects the core of the enzyme. If this alternative splicing affects the choice of COQ-2  
144 substrate and thus the switch from UQ to RQ synthesis, we reasoned that parasitic helminths that make RQ should  
145 have a similar gene structure and that the hosts that do not make RQ should not. We used both gene predictions  
146 and available RNA-seq data to examine the *coq-2* gene structure and splicing in parasitic helminths and their  
147 hosts (human, sheep, cow, and rat respectively). As shown in Figure 3, all the parasites examined have a similar  
148 *coq-2* gene structure with the same two mutually exclusive internal exons — note that while many of the parasite  
149 gene structures were not correctly annotated, all the relevant exon junctions in Figure 3 were manually annotated  
150 and confirmed with RNA-seq data (see Materials and Methods). Remarkably, we find a similar gene structure  
151 with the same mutually exclusive exons in all parasites known to make RQ. Furthermore, annelids and molluscs  
152 are the only other phyla known to make RQ and their *coq-2* orthologues also show mutually exclusive alternative  
153 splicing of homologous exons. This gene structure is only seen in species that make RQ — we find no evidence  
154 in any available data for similar alternative splicing in any mammalian hosts (human and mouse are shown as  
155 representatives in Figure 3) or in other lineages that lack RQ, such as yeasts. This suggests that this *coq-2*  
156 alternative splicing could indeed be linked to the ability to synthesize RQ.

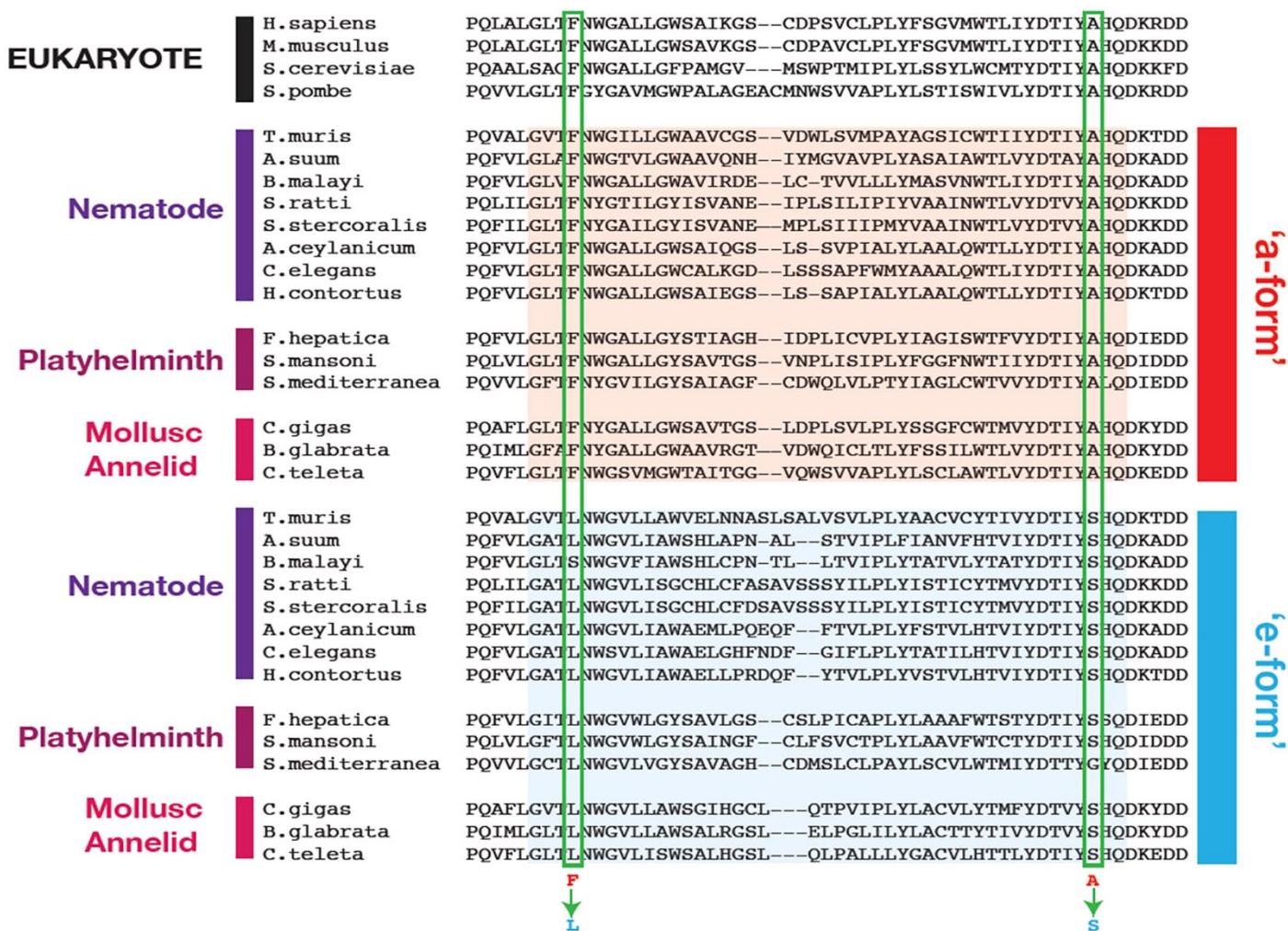
157



158 **Figure 3.** Gene models for *coq-2* orthologues in various species. Parasitic helminths, as well as annelids and  
159 molluscs, have 2 internal exons that are spliced in a mutually exclusive manner. In contrast, humans and other  
160 hosts only express one exon that is homologous to exon 6a of *C. elegans* *coq-2*. The a-form exon (red) shares  
161 greater similarity to the exon present in species that do not make RQ, while the e-form (blue) is present only in  
162 RQ-producing species. The genes used for each species are listed in Supplemental Table 3. The gene structures  
163 shown are based on genome annotations but in many cases include manual reannotations — in all such cases,  
164 the manual annotations are confirmed with RNA-seq data.  
165

166 We aligned the two mutually exclusive exons across helminth species and compared them to the similar  
167 regions of their host COQ-2 sequences, and of other eukaryotes that cannot make RQ (*S. cerevisiae*, *S. pombe*)  
168 (Figure 4). We find that the *coq-2a*-specific exon is similar to the pan-eukaryotic COQ-2 sequence whereas the  
169 *coq-2e*-specific exon is distinct in all species that make RQ. We examined the alignments of the a-specific and e-  
170 specific exons and identified two residues that are strictly conserved in pan-eukaryotic COQ-2 sequences, Phe204  
171 and Ala243 (*C. elegans* numbering), that are switched to a Leu and a Ser residue in all COQ2-e-specific exons

172 that we examined (Figure 4). These residues sit very close to the substrates in the active site of the enzyme (Figure  
173 2C) and we note that mutation of the equivalent Ala243 residue dramatically affects the ability of human COQ2  
174 to make UQ (Desbats et al., 2016). Altogether these results suggest that animals that make both UQ and RQ make  
175 two forms of COQ-2 — one looks similar to that in all other eukaryotic species, whereas the other has a single  
176 exon that appears to be specific for species that make RQ. To test whether these two COQ-2 isoforms have distinct  
177 roles in UQ and RQ synthesis, we turned to *C. elegans*.



**Figure 4.** Conserved changes between a- and e-form exons across RQ-producing phyla. Amino acid sequences of COQ-2 orthologues were aligned using Clustal Omega (Madeira et al., 2019). The sequences of exons homologous to exon 6a/e in *C. elegans*, as well as the flanking 5 amino acid sequences were used to generate the alignment. Sequences of the mutually exclusive exons are shaded in red (a-form) or blue (e-form). Two residue changes between the a- and e- forms are highlighted (Phe to Leu, Ala to Ser) and are invariant across diverse species that make RQ. The COQ-2 orthologues and exons used for each species are listed in Supplemental Table 3.

186 **RQ synthesis requires the *coq-2e* isoform**

187 We found that helminths make two major isoforms of *coq-2* whereas their hosts only make a single isoform. To  
188 test the requirement for each of the two major helminth isoforms of *coq-2* for RQ synthesis, we used CRISPR  
189 engineering to generate *C. elegans* mutant strains that either lack *coq-2* exon 6a (*coq-2(syb1715)*) or *coq-2* exon  
190 6e (*coq-2(syb1721)*) — we refer to these as *coq-2Δ6a* and *coq-2Δ6e* respectively from here on (see Figure 5A  
191 and Supplemental Table 1 for details of engineering). We find that the *coq-2Δ6e* strain makes essentially no  
192 detectable RQ but has higher levels of UQ, whereas *coq-2Δ6a* has greatly reduced UQ levels but higher RQ levels  
193 (Figure 5B, Supplemental Table 2). We conclude that the *coq-2e* isoform, that includes the helminth-specific exon  
194 6e, is required for RQ synthesis.

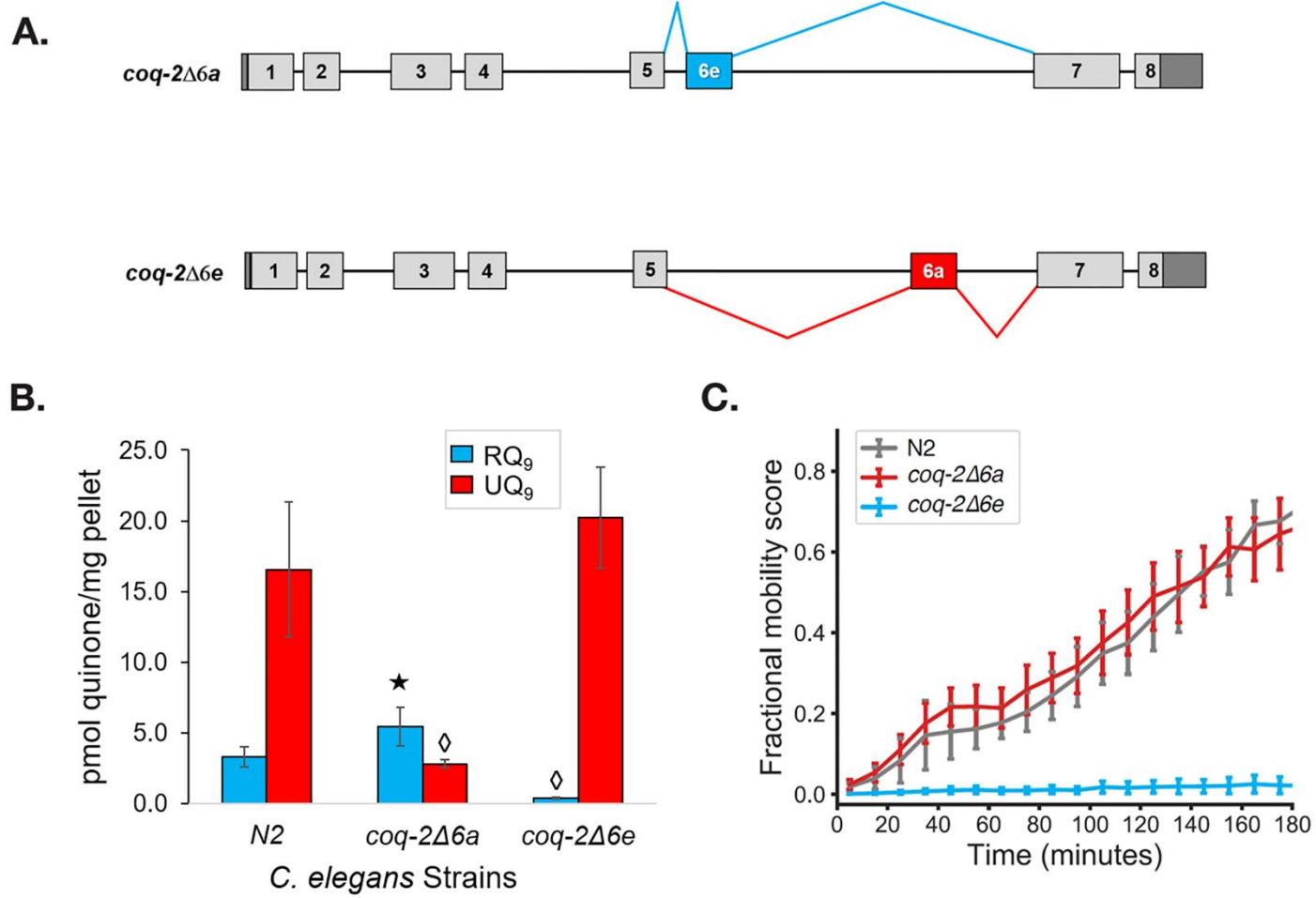
195 To further examine whether COQ-2e is required for RQ synthesis and thus for RQ-dependent metabolism, we  
196 tested whether the *coq-2Δ6a* and *coq-2Δ6e* strains could survive long-term exposure to potassium cyanide (KCN)  
197 (Figure 5C). We previously showed that when *C. elegans* is exposed to KCN it switches to RQ-dependent  
198 metabolism and that while wild-type worms can survive a 15 h exposure to KCN, *C. elegans* strains that do not  
199 make RQ cannot survive. We found that while the *coq-2Δ6a* strain (that can make RQ) survives 15 h of KCN  
200 exposure as well as wild-type animals, the *coq-2Δ6e* strain that makes no RQ does not survive, confirming the  
201 functional relevance of the *coq-2e* isoform as being critical for RQ synthesis.

202

203 **Regulation of the alternative splicing of *coq-2* in helminths**

204 Helminths make two isoforms of COQ-2 — COQ-2a resembles the pan-eukaryotic consensus and cannot make  
205 RQ, whereas COQ-2e includes an exon that is only found in species that make RQ and COQ-2e is required for  
206 RQ synthesis. Changing the levels of *coq-2a* and *coq-2e* splice forms could thus regulate the switch from UQ  
207 synthesis in the aerobic environment outside the host to RQ synthesis in the host gut. We thus examined RNA-  
208 seq data to see whether parasites switch from between these isoforms as they switch between these environments.

209

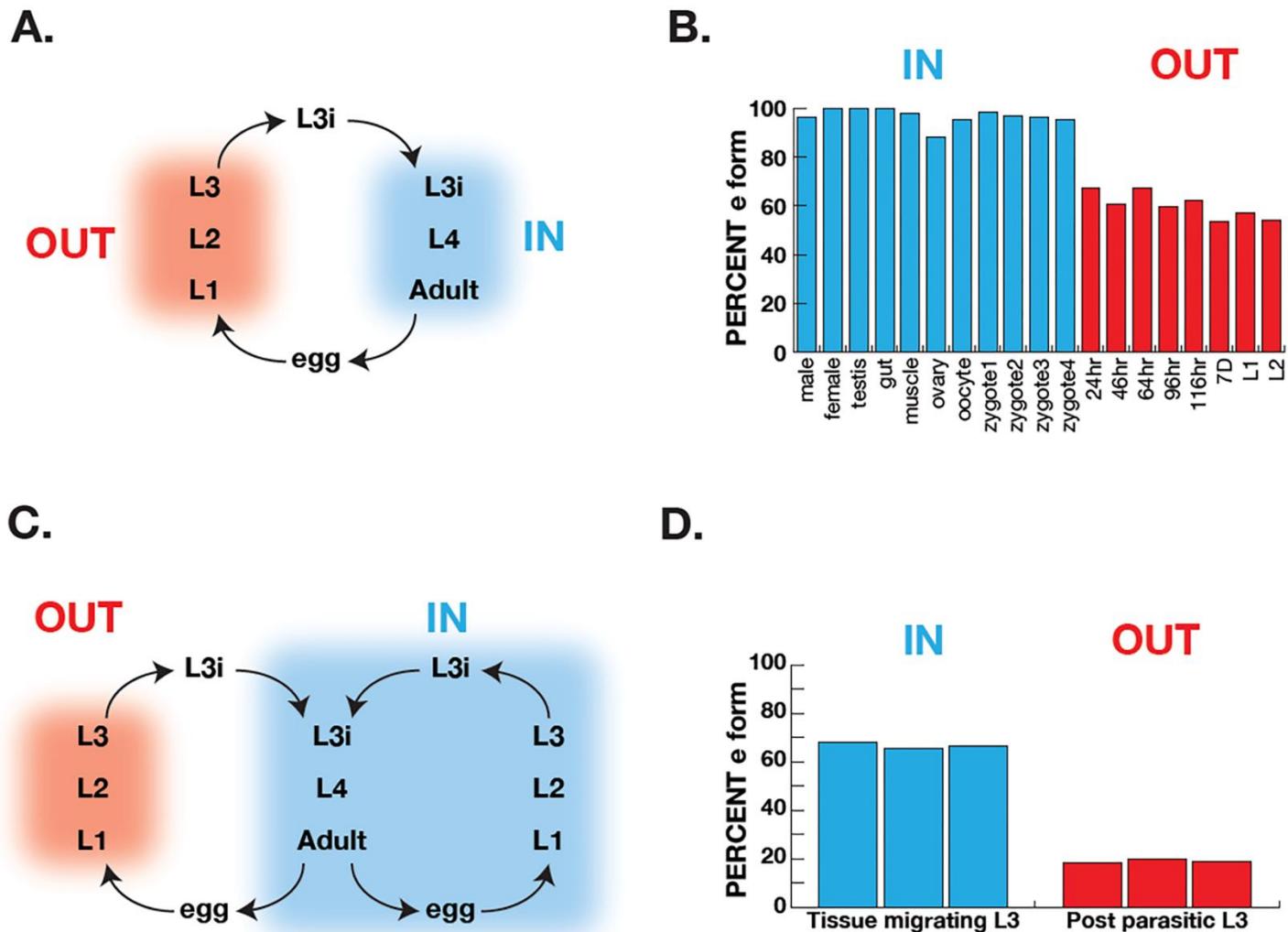


**Figure 5.** The *C. elegans* *coq-2* edited strains and effects of exon 6a and 6e deletions on quinone biosynthesis. (A) Mutant strains were generated in *C. elegans* by deletion of exon 6a (*coq-2Δ6a*) or exon 6e (*coq-2Δ6e*). (B) Deletion of exon 6a from the *coq-2* gene significantly increased the level of RQ<sub>9</sub> ( $p = 0.013$ ) and significantly decreased UQ<sub>9</sub> ( $p < 0.001$ ) compared to the N2 control. In contrast, the deletion of exon 6e decreased RQ<sub>9</sub> to a negligible level ( $p < 0.001$ ) and slightly increased the level of UQ<sub>9</sub> ( $p = 0.130$ ) compared to N2. Statistically significant increases and decreases with respect to N2 levels are denoted with ★ and ◇, respectively; error bars reflect standard deviation where  $N = 4$ . (C) Deletion of *coq-2* exon 6e affects the ability of worms to survive extended KCN treatment. Wild-type (N2) and *coq-2* mutant L1 worms were exposed to 200  $\mu$ M KCN for 15 h. KCN was then diluted 6-fold and worm movement was measured over 3 h to track recovery from KCN exposure (see Materials and Methods). Worms without exon 6e could not survive extended treatment with KCN while deletion of exon 6a had little effect on KCN survival. Curves show the mean of 4 biological replicates and error bars are standard error.

*Ascaris suum* has a relatively simple life cycle (schematic Fig 6A) and is the pig equivalent of *Ascaris lumbricoides* which infects ~900 M humans. Eggs are laid in the host and emerge via defecation and the L1-L3

225 larval stages develop within the egg outside the host. The L3 infective larval stage then enters the host via  
226 ingestion into the digestive tract. These then leave the digestive tract and make their way to the lungs where they  
227 develop into L4 larvae and, finally, the L4 re-enter the digest tract and move to the small intestine where adults  
228 develop.. The adults remain in the anaerobic environment for the remainder of their life. The free-living larval  
229 stages have relatively low RQ (~ 35% of total quinones), whereas in adults, RQ is ~ 100% of total quinones  
230 (Takamiya et al., 1993). We used RNA-seq data (Wang et al., 2011, Wang et al., 2012) to analyse *coq-2* isoforms  
231 in free-living stages and in the adults to examine whether there was switch from *coq-2a* to *coq-2e* as the parasites  
232 switch from low RQ aerobic-respiring embryos and larvae to high RQ anaerobic adults. We see a clear switch:  
233 ~60% of *coq-2* transcripts are *coq-2e* in the free-living, aerobic stages but >90% is the RQ-synthesising *coq-2e*  
234 form in adults (Figure 6B). Increased *coq-2e* levels thus correlate with increased RQ levels.

235 The analysis in *Ascaris* is complicated by the life cycle: while we are comparing *coq-2* splicing in two distinct  
236 environments, this is also necessarily a comparison between developing embryos and larvae and adults. It is  
237 possible that the changes in splicing we see are not environmentally-induced by the switch from normoxia to  
238 anaerobic conditions, but is simply a developmentally programmed switch. To address this, we turned to  
239 *Strongyloides stercoralis*. The life cycle of *S. stercoralis* (schematic Figure 6C) is broadly similar to *Ascaris* —  
240 L1-L3 stages are free-living, the L3 infective stage infects hosts, and L4 larvae and adults develop and live in the  
241 host. However, they have an alternative life cycle where instead of L1-L3 developing outside the host, the eggs  
242 can hatch in the host and the entire life cycle takes place inside the host. This allows us to compare the same larval  
243 stage in two conditions — here we compare L3 animals that developed inside the host anaerobic environment  
244 with L3 animals that developed outside the host. The difference is clear: <20% of *coq-2* transcripts are *coq-2e* in  
245 the free-living L3s but >60% is the RQ-synthesizing *coq-2e* form in L3s that developed inside the host (Figure  
246 6D) (Stoltzfus et al., 2012).  
247



**Figure 6.** Correlation of COQ-2 splicing with change from aerobic to anaerobic life stages. (A) Schematic of life cycle of *A. suum*. ‘OUT’ denotes aerobically-respiring free-living stages; ‘IN’ indicates stages living inside the host intestine. (B) Graph indicates the percentage of all COQ-2 transcripts that include the RQ-specific exon (‘Percent e form’) in a number of life cycle stages, sexes, and tissues. Timings for embryogenesis show the time post-fertilisation in hours. (C) Schematic of life cycle of *Strongyloides stercoralis*. ‘OUT’ denotes aerobically-respiring free-living stages; ‘IN’ indicates stages living inside the host. Note that egg, L1, L2 and L3 can either develop inside or outside the host. (D) Graph indicates the percentage of all COQ-2 transcripts that include the RQ-specific exon (‘Percent e form’) in L3 larvae that either developed outside the host (‘OUT’) or inside the host (‘IN’). Data derive from three individual replicates.

In summary, our data show that eukaryotic species that make RQ regulate the choice between making UQ and RQ by alternative splicing of the polypropenyltransferase COQ-2. A switch between two mutually exclusive exons changes the core of the COQ-2 enzyme and switches it from generating UQ precursors to RQ precursors.

261 This alternative splicing event is only seen in species that make RQ and the switch correlates with the change  
262 from aerobic to anaerobic metabolism in parasitic helminths.

263

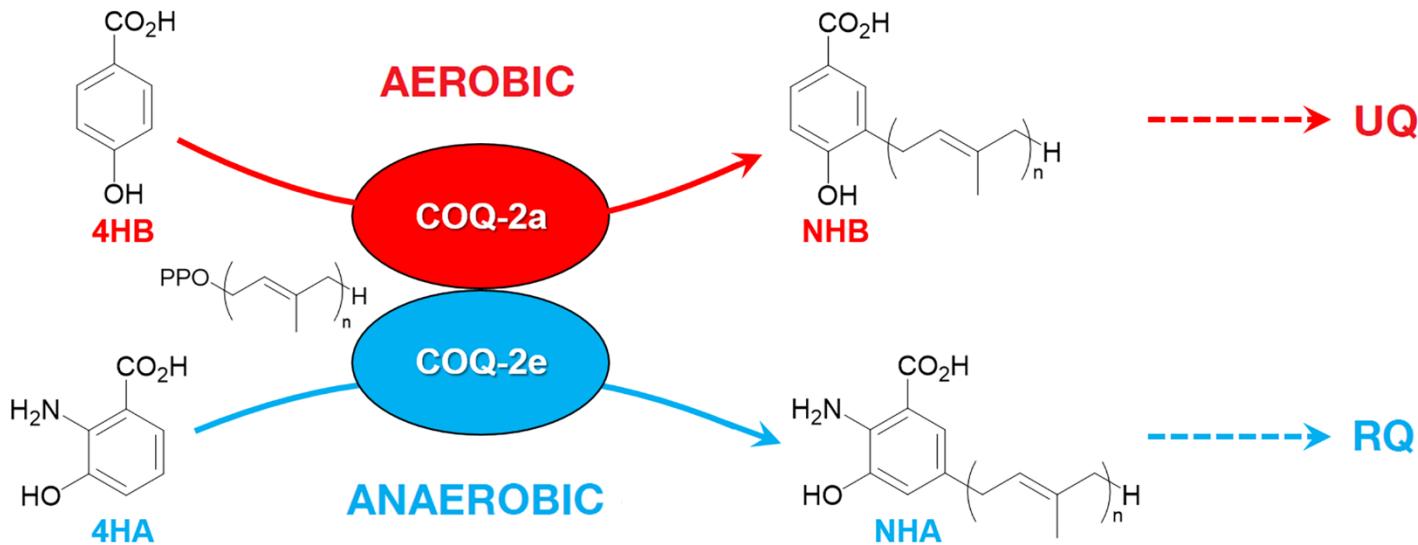
264 **Discussion**

265 Organisms are continually challenged by changes in their environments and they must be able to respond for them  
266 to survive. Hypoxia is one such challenge and animals have evolved diverse strategies to alter their metabolism  
267 to cope with low oxygen levels. For example, humans rapidly switch to anaerobic glycolysis, generating lactate;  
268 goldfish on the other hand can adapt to hypoxia by fermenting carbohydrates to generate ethanol (Shoubridge,  
269 Hochachka, 1980). In this paper, we focus on how helminths can survive in anaerobic conditions by switching  
270 from ubiquinone (UQ)-dependent aerobic metabolism to rhodoquinone (RQ)-dependent anaerobic metabolism.  
271 The ability to use RQ is highly restricted amongst animal species — only helminths, molluscs, and annelids are  
272 known to make and use RQ. This is a key adaptation since it allows them to rewire their mitochondrial electron  
273 transport chain (ETC) to use a variety of terminal electron acceptors in the place of oxygen. They can therefore  
274 still use Complex I to pump protons and generate the proton motive force need to power the F0F1ATPase in the  
275 absence of oxygen. This allows them to survive without oxygen for long periods — they are thus facultative  
276 anaerobes. This ability is critical for parasitic helminths which survive for long periods in the anaerobic  
277 environment of the human gut. Since the host does not make RQ or use RQ-dependent metabolism if we could  
278 interfere with RQ synthesis, this would be an excellent way to target the parasite and leave the host untouched.

279 We previously showed that the key decision on whether to make UQ to power aerobic metabolism or RQ to  
280 make anaerobic metabolism is dictated by the choice of substrate of the polyprenyltransferase COQ-2 (Roberts  
281 Buceta et al., 2019, Del Borrello et al., 2019). COQ-2 must switch from using 4HB to make UQ in aerobic  
282 conditions to 3HA to make RQ in anaerobic conditions. Here we reveal the simple mechanism for that switch in  
283 substrate specificity in helminths: they use the mutually exclusive alternative splicing of two internal exons to  
284 remodel the core of COQ-2. Inclusion of exon 6a results in the COQ-2a enzyme that can make UQ but not RQ;

285 switching 6a for the alternative exon 6e yields COQ-2e which principally makes RQ (Figure 7). All eukaryotes  
286 make a homologue of COQ-2a — only the species known to make RQ (helminths, annelids, and molluscs) have  
287 genomes encoding the RQ-specific exon 6e and this exon is introduced by alternative splicing in a similar  
288 mutually exclusive splicing event in all these phyla. These different lineages thus have the same solution to the  
289 problem of substrate switching in COQ-2 — to have two distinct forms of COQ-2 due to alternative splicing —  
290 and all do it with the same structural switch in COQ-2.

291



292 **Figure 7.** Discrimination between RQ and UQ biosynthesis in *C. elegans*. There are two variants of exon 6 in the  
293 *C. elegans* *coq-2* gene (6a and 6e) which undergo mutually exclusive alternative splicing leading to COQ-2a and  
294 COQ-2e isoforms, respectively. Synthesis of UQ originates from 4-hydroxybenzoic acid (4HB) and prenylation  
295 is catalyzed by COQ-2a (and marginally by COQ-2e) to form 4-hydroxy-3-nonaprenylbenzoic acid (NHB). In  
296 contrast, RQ is most likely synthesized from 3HA, and prenylation is facilitated by COQ-2e to form NHA. Several  
297 additional steps are required to convert NHB to UQ and NHA to RQ, respectively.  
298

299 The alternative splicing of COQ-2 in all lineages that make RQ draws focus to COQ-2 as a potential  
300 anthelmintic target. COQ-2e is required for RQ generation and has a distinct sequence to the pan-eukaryotic  
301 COQ-2a. The switch between COQ-2a to COQ-2e causes a change in the core of the COQ-2 active site and all  
302 species that make RQ have a pair of conserved residues in COQ-2e. We suggest that small molecule inhibitors  
303 that selectively target COQ-2e and not the host COQ-2 enzyme could be potent anthelmintics and our groups are

304 initiating these screens at this point. We also note that it is rare to see such a clear and profound change in enzyme  
305 specificity due to a single splice event affecting the core of an enzyme catalytic site. There are other examples,  
306 most notably a switch in the substrate specificity of the cytochrome P450 CYP4F3 from LTB4 to arachidonic  
307 acid (Christmas et al., 1999, Christmas et al., 2001). This switch is tissue specific rather than environmentally  
308 induced, however, and there are few other examples to our knowledge. The regulation of COQ-2 specificity by  
309 alternative splicing is thus a beautiful and rare example of this type of enzyme regulation by alternative splicing.  
310 We note that while alternative splicing of COQ-2 appears to be the key regulated step in determining RQ or UQ  
311 biosynthesis, we see no such splicing regulation for other enzymes that are required for both RQ and UQ synthesis  
312 downstream of COQ-2 (Roberts Buceta et al., 2019) e.g. there are no known splice variants of COQ-3 and COQ-  
313 5, which are quinone methylases downstream of COQ-2. This suggests that while COQ-2 can clearly discriminate  
314 between substrates that have/lack a 2-amino group, COQ-3 and COQ-5 would be more promiscuous than COQ-  
315 2 and act on both RQ and UQ precursors.

316 Finally, the mechanism of alternative splicing to regulate the synthesis of UQ or RQ may explain some part  
317 of the evolution of RQ synthesis in animals and its phylogenetic distribution. One of the enduring mysteries of  
318 RQ synthesis is that it only occurs in very restricted animal phyla. RQ synthesis could either be the ancestral state  
319 with widespread loss across most animal species or else RQ synthesis could have arisen independently in  
320 helminths, annelids, and molluscs. We believe that the alternative splicing switch from UQ to RQ synthesis  
321 favours this independent evolution model. First, we note that there are only two key conserved residues in the  
322 RQ-specific exons — this minor sequence change could easily have arisen independently in multiple lineages.  
323 Second, there is a clear precedent for a mutually exclusive splicing event that has arisen independently in  
324 helminths, annelids, and molluscs in the gene *mrp-1* (Yue et al., 2017). This encodes a transporter that is required  
325 for the uptake of vitamin B12 into mitochondria. We note that B12 is critical for aspects of RQ-dependent  
326 metabolism. The B12-dependent propionate breakdown pathway (Bulcha et al., 2019) may be required under  
327 production of high levels of succinate (under anaerobiosis); succinate can be converted into propionyl-CoA and  
328 further used to synthesise branched-chain fatty acids such as 2-methyl butyrate and 2-methyl valerate. This

329 synthesis of branched-chain fatty acids is an RQ-dependent reductive process: RQ carries electrons to ETFDH  
330 which is driven in reverse to fatty acid oxidation (Muller et al., 2012). The *mrp-1* gene undergoes alternative  
331 splicing in these species and just like in *coq-2*, *mrp-1* has mutually exclusive exons of exactly the same length.  
332 Crucially, *mrp-1* mutually-exclusive alternative splicing has arisen independently in each lineage in which it is  
333 seen. We suggest that the reason that RQ synthesis is only seen in helminths, annelids, and molluscs is that these  
334 phyla have likewise independently evolved a gene structure of *coq-2* that allows them to switch from a UQ-  
335 specific form to a RQ-specific form by mutually-exclusive alternative splicing. Our data thus show that alternative  
336 splicing of COQ-2 provides a simple switch from UQ synthesis to RQ synthesis but may also explain how RQ  
337 synthesis has arisen independently in multiple distinct animal lineages.

338

---

339 **Materials and methods**

340

341 **Sequence identification and analysis**

342 To identify COQ-2 sequences from lineages known to synthesize RQ we searched and analyzed from genomes and  
343 transcriptomes platyhelminths (*Schistosoma mansoni*, *Fasciola hepatica* and *Schmidtea mediterranea*), nematodes  
344 (*Ascaris suum*, *Brugia malayi*, *Haemonchus contortus*, *Trichuris muris*, *Strongyloides stercoralis*, *Strongyloides*  
345 *ratti*, *Ancylostoma ceylanicum* and *C. elegans*), mollusc (*Crassostrea virginica*, and *Biomphalaria glabrata*) and  
346 annelid (*Capitella teleta*). Sequences were retrieved from <https://parasite.wormbase.org> (WBPS14), *S.*  
347 *mediterranea* database (<http://smedgd.neuro.utah.edu>), NCBI protein and nucleotide databases and UniProt  
348 (<https://www.uniprot.org>). Human, mice and *Saccharomyces cerevisiae* COQ-2, eukaryotic lineages known to be  
349 unable to synthesize RQ, were also identified for comparison. Searches were performed initially with BLASTP  
350 (protein databases) using human and *C. elegans* COQ-2 sequences as queries. Additionally, TBLASTN searches  
351 were performed using genomic sequences and cDNAs databases. This served to confirm the annotated protein  
352 sequences and to identify the non-annotated ones. Identified sequences were confirmed by best reciprocal hits in

353 BLAST. Multiple sequence alignments were made with MUSCLE 3.8 (Chojnacki et al., 2017). Gaps were  
354 manually refined after alignment inspection.

355

356 **RNA-seq analysis of mutually exclusive exons**

357 To confirm if *coq-2* exons are spliced in a mutually exclusive manner in other helminths, molluscs and annelids,  
358 we analyzed existing RNA-seq data for evidence of alternative splicing (listed in Supp. Table 3). Whippet  
359 (v0.11) (Sterne-Weiler et al., 2018) was used to analyze RNA-seq data for quantification of AS events. To  
360 create a splicing index of exon-exon junctions in Whippet, genome annotations were taken from WormBase  
361 Parasite (WBPS14) and Ensembl Metazoa (Release 45). To identify novel exons and splice sites, reads were  
362 first aligned to the genome using HISAT2 (Kim, Langmead & Salzberg, 2015). The BAM file generated was  
363 then used to supplement existing genome annotations to create a splicing index of known and predicted exon-  
364 exon junctions in Whippet (using the --bam --bam-both-novel settings). Where required, TBLASTN data was  
365 also used to guide manual re-annotation of the *coq-2* gene. Quantification of AS events was then performed by  
366 running whippet quant at default settings. This analysis was repeated for all species listed in Figure 5. A  
367 summary of *coq-2* exons with reads that mapped to alternative exon-exon junctions are listed in Supplemental  
368 Table 3. We also identified cases where both *coq-2* exons were either included or skipped. However, since  
369 these are likely to be non-productive transcripts due to a pre-mature termination codon, we expressed exon  
370 usage as the proportion of events where either only the ‘a’ or the ‘e’ form is included.

371

372 **Structural analysis**

373 Multiple sequence alignment was performed using Clustal Omega (Madeira et al., 2019). The substrate-bound  
374 structure of a UbiA homolog from *A. pernix* (PDB: 4OD5) was displayed on Chimera (Pettersen et al., 2004)  
375 and the *C. elegans* sequence was threaded by homology using Modeller (Sali, Blundell, 1993, Webb, Sali,  
376 2016).

377

378 ***Caenorhabditis elegans* strains and culture conditions**

379 The *C. elegans* wild-type Bristol strain (N2) was obtained from the Caenorhabditis Genetics Center (CGC,  
380 University of Minnesota, USA), which is supported by the National Institutes of Health-Office of Research  
381 Infrastructure Programs. The *C. elegans* mutant strains in *coq-2* exon 6A (PHX1715, *coq-2(syb1715)*) and *coq-2*  
382 exon 6E (PHX1721, *coq-2(syb1721)*) were generated by Suny Biotech Co., Ltd (Fuzhou City, China) using  
383 CRISPR/Cas9 system. The precise deletion of both mutant strains (134 bp) was verified by DNA sequencing the  
384 flanking region of exons 6a and 6e. The wild-type sequence, the deleted sequence in each strain, the sgRNAs, and  
385 primers used are listed in Supplemental Table 1.

386 The general methods used for culturing and maintenance of *C. elegans* are described in (Brenner, 1974). All  
387 chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA). The *E. coli* OP50 strain, used as *C.*  
388 *elegans* food, was also received from CGC.

389

390 **Lipid extraction and LC-MS quantitation**

391 Lipid extractions of *C. elegans* N2 and mutant strains were performed on ~100 mg worm pellets after adding 1000  
392 pmol UQ<sub>3</sub> internal standard (11). LC-MS samples were prepared from lipid extracts and diluted 1:100 (Bernert, et  
393 al., 2019). Standards were extracted using the same method as for worm samples at the following concentrations:  
394 UQ<sub>3</sub> (10 pmol/10  $\mu$ L injection), RQ<sub>9</sub> (0.75, 1.5, 3.0, 4.5, or 6.0 pmol/10  $\mu$ L injection), and UQ<sub>9</sub> (3.75, 7.5, 15.0,  
395 22.5, or 30.0 pmol/10  $\mu$ L injection). The Q<sub>3</sub> standard was synthesized at Gonzaga University (Campbell et al.,  
396 2019), the RQ<sub>9</sub> standard was isolated by preparative chromatography from *A. suum* lipid extracts (Roberts Buceta  
397 et al., 2019) and the UQ<sub>9</sub> standard was purchased (Sigma-Aldrich, St. Louis, MO). The general LC-MS conditions  
398 and parameters were previously reported (Bernert et al., 2019, Campbell et al., 2019). Samples were analyzed in

399 quadruplicate and the pmol quinone was determined from the standard curve and corrected for recovery of internal  
400 standard. Samples were normalized by mg pellet mass.

401  
402 **Image-based KCN recovery assay**

403 The KCN recovery assay was performed as previously described (Spensley et al., 2018, Del Borrello et al.,  
404 2019). Briefly, L1 worms were isolated by filtration through an 11  $\mu$ m nylon mesh filter (Millipore:  
405 S5EJ008M04). Approximately 100 L1 worms in M9 were dispensed to each well of a 96 well plate and an  
406 equal volume of potassium cyanide (KCN) (Sigma-Aldrich St. Louis, MO) solution was then added to a final  
407 concentration of 200  $\mu$ M KCN. Upon KCN addition, plates were immediately sealed and incubated at room  
408 temperature for 15 h on a rocking platform. After 15 h, the KCN was diluted 6-fold by addition of M9 buffer.  
409 Plates were immediately imaged on a Nikon Ti Eclipse microscope every 10 min for 3 h. Fractional mobility  
410 scores (FMS) were then calculated using a custom image analysis pipeline (Spensley et al., 2018). For each  
411 strain, FMS scores for the KCN-treated wells were normalized to the M9-only control wells at the first  
412 timepoint. Three technical replicates were carried out in each experiment and the final FMS scores taken from  
413 the mean of four biological replicates.

414  
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419  
420

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425

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560

561 **Supplementary Documentation**

**SUPPLEMENTAL TABLE 1: *C. elegans* strains**

**A. Strain PHX1715 (*coq-2(syb1715)*), deletion mutant in *coq-2* exon 6A**

1) *coq-2* wild-type sequence of exon 6A (capital letters) and 30bp flanking sequence (red):

atctcttacactctcttatcatagtagcag GACTCACGTTCAATTGGGGCGCTCTTGGATGGTGTGCGCTGAAAGGT  
GATTGTCGTCTAGTGCACCGTTGGATGTATGCAGCTGCACTTCAATGGACACTGATCTACGACA  
CTATCTATGCACATCAG gtggcctatttgggattggactttt

2) Deleted sequence (-134 bp):

GACTCACGTTCAATTGGGGCGCTCTTGGATGGTGTGCGCTGAAAGGTGATTGTCGTCTAGTGC  
ACCGTTTGGATGTATGCAGCTGCACTTCAATGGACACTGATCTACGACACTATCTATGCACATCAG

3) sgRNA target sites used for strains PHX1715 (PAM motives underlined):

sg1 : acagGACTCACGTTCAATTGGGG  
sg2 : TGTATGCAGCTGCACTTCAATGG  
sg3 : GCACATCAGgtggcctatttggg

4) PCR and sequencing primers:

GUC01-ko-f: AAATAAGGTTTCCCGCCAG

GUC01-ko-r: GTCGACTTGACACCGATCAT

**B. Strain PHX1721 (*coq-2(syb1721)*), deletion mutant in *coq-2* exon 6E**

1) *coq-2* wild-type sequence of exon 6E (capital letters) and 30bp flanking sequence (red):

acacctagtctgtcgtttgcattgcag GTGCCACCCCTAAACTGGAGTGTGCTGATAGCGTGGGCAGAATTGGGCCA  
TTTCAATGATTGGCATCTTTGCCACTCTACACTGCCACCACCTGCACACGGTCATCTACGACA  
CTATTATAGTCATCAG gttagagctaagaaggcaggaaaaagtat

2) Deleted sequence (-134 bp):

GTCGCCACCCCTAAACTGGAGTGTGCTGATAGCGTGGGCAGAATTGGGCCATTCAATGATTGGCAT  
CTTTTGCCACTCTACACTGCCACCACCTGCACACGGTCATCTACGACACTATTATAGTCATCAG

3) sgRNA target sites used for strain PHX1721 (PAM motives underlined):

sg1 : CCACCCCTAAACTGGAGTGTGCTG  
sg2 : CCTGCACACGGTCATCTACGACA

4) PCR and sequencing primers:

GUC02-ko-f: CAAAAACAAGTACGGTTCCC

GUC02-ko-r: CGCAGCACGGTATGAGACTTT

**SUPPLEMENTAL TABLE 2.** Statistical analysis of RQ<sub>9</sub> and Q<sub>9</sub> levels in *coq-2* mutant strains

Strain	Avg pmol RQ <sub>9</sub> /mg pellet	p value (N = 4)	Avg pmol Q <sub>9</sub> /mg pellet	p value (N = 4)
N2	3.30 ± 0.74		16.55 ± 4.76	
<i>coq-2</i> _Δ6A	5.43 ± 1.35	0.016	2.81 ± 0.27	< 0.001
<i>coq-2</i> _Δ6E	0.40 ± 0.08	< 0.001	20.23 ± 3.54	0.130

**SUPPLEMENTAL TABLE 3.** Genomic coordinates of known and predicted mutually exclusive *coq-2* a/e exons (see attached Excel file).

Supplemental Table 3. Genomic coordinates of known and predicted mutually exclusive coq-2 a/e exons

Species	Gene	Exon-type	Chromosome/Scaffold	Location	Strand	Annotated	Length	Version	Phylum	RNA-seq study analyzed
<i>Trichinella spiralis</i>	T01_13908	e	scaffold26s	87773-87909	+	Novel splice site	137	WBPS14	Nematoda	SRP014316
<i>Trichinella spiralis</i>	T01_13908	a	scaffold26s	89164-89297	+	Yes	134	WBPS14	Nematoda	SRP014316
<i>Trichuris muris</i>	WBGene00293864 (TMUE_2000006714)	e	TMUE_LG2	12613169-12613308	+	Yes	140	WBPS14	Nematoda	ERP002000
<i>Trichuris muris</i>	WBGene00293864 (TMUE_2000006714)	a	TMUE_LG2	12613537-12613670	+	Novel exon	134	WBPS14	Nematoda	ERP002000
<i>Ascaris suum</i>	AgB05_g070	e	AgB05	1194260-1194390	+	Yes	131	WBPS14	Nematoda	SRP005511,SRP013573
<i>Ascaris suum</i>	AgB05_g070	a	AgB05	1198569-1198702	+	Yes	134	WBPS14	Nematoda	SRP005511,SRP013573
<i>Brugia malayi</i>	WBGene00255591 (Bm16942)	e	Bm_v4_Chr1_scaffold_001	6349844-6349974	-	Yes	131	WBPS14	Nematoda	ERP000948
<i>Brugia malayi</i>	WBGene00255591 (Bm16942)	a	Bm_v4_Chr1_scaffold_001	6348862-6348992	-	Yes	131	WBPS14	Nematoda	ERP000948
<i>Strongyloides ratti</i>	WBGene00260212 (SRAE_2000002300)	e	SRAE_chr2	66293-66432	-	Novel splice site	140	WBPS14	Nematoda	ERP002187
<i>Strongyloides ratti</i>	WBGene00260212 (SRAE_2000002300)	a	SRAE_chr2	66040-66173	-	Yes	134	WBPS14	Nematoda	ERP002187
<i>Strongyloides stercoralis</i>	SSTP_0000143000	e	SSTP_scaffold0000001	1888297-1888436	+	Yes	140	WBPS14	Nematoda	ERP001556
<i>Strongyloides stercoralis</i>	SSTP_0000143000	a	SSTP_scaffold0000001	1888554-1888687	+	Novel exon	134	WBPS14	Nematoda	ERP001556
<i>Strongyloides papillosus</i>	SPAL_0000295400	e	SPAL_scaffold0000002	401438-401577	-	Novel splice site	140	WBPS14	Nematoda	ERP016188
<i>Strongyloides papillosus</i>	SPAL_0000295400	a	SPAL_scaffold0000002	401165-401298	-	Yes	134	WBPS14	Nematoda	ERP016188
<i>Ancylostoma ceylanicum</i>	maker-ANCCEYDFT_Contig505-augustus-gene-0.53	e	ANCCEYDFT_Contig505	81986-82119	-	Novel exon	134	WBPS14	Nematoda	SRP058598
<i>Ancylostoma ceylanicum</i>	maker-ANCCEYDFT_Contig505-augustus-gene-0.53	a	ANCCEYDFT_Contig505	80277-80407	-	Novel splice site	131	WBPS14	Nematoda	SRP058598
<i>Caenorhabditis elegans</i>	WBGene00000762	e	III	6937600-6937733	+	Yes	134	WBPS14	Nematoda	
<i>Caenorhabditis elegans</i>	WBGene00000762	a	III	6938412-6938545	+	Yes	134	WBPS14	Nematoda	
<i>Haemonchus contortus</i>	HCON_00082210	e	hcontortus_chr3_Celeg_IT_arrow_pilon	2402222-24022355	-	Novel exon	134	WBPS14	Nematoda	ERP002173,SRP026668
<i>Haemonchus contortus</i>	HCON_00082210	a	hcontortus_chr3_Celeg_IT_arrow_pilon	24017677-24017807	-	Yes	131	WBPS14	Nematoda	ERP002173,SRP026668
<i>Nippostrongylus brasiliensis</i>	NBR_0001085001	e	NBR_scaffold0000650	60610-60743	-	Novel splice site	134	WBPS14	Nematoda	ERP023010
<i>Nippostrongylus brasiliensis</i>	NBR_0001085001	a	NBR_scaffold0000650	58731-58864	-	Yes	134	WBPS14	Nematoda	ERP023010
<i>Fasciola hepatica</i>	maker-scaffold10x_13_pilon-augustus-gene-0.26	e	scaffold10x_13_pilon	2309470-2309603	+	Yes	134	WBPS14	Platyhelminthes	ERP006566
<i>Fasciola hepatica</i>	maker-scaffold10x_13_pilon-augustus-gene-0.26	a	scaffold10x_13_pilon	2309839-2309972	+	Yes	134	WBPS14	Platyhelminthes	ERP006566
<i>Schistosoma mansoni</i>	Smp_347140	e	SM_V7_4	21491638-21491771	+	Yes	134	WBPS14	Platyhelminthes	ERP000427
<i>Schistosoma mansoni</i>	Smp_347140	a	SM_V7_4	21491970-21492103	+	Yes	134	WBPS14	Platyhelminthes	ERP000427
<i>Schmidtea mediterranea</i>	SMESG000018280	e	dd_Smes_g4_166	691338-691471	+	Yes	134	WBPS14	Platyhelminthes	
<i>Schmidtea mediterranea</i>	SMESG000018280	a	dd_Smes_g4_166	696985-697118	+	Yes	134	WBPS14	Platyhelminthes	
<i>Crassotrea gigas</i>	CGI_10026525	e	scaffold156	818738-818868	-	Novel splice site	131	Ensembl Metazoa 45	Mollusca	SRP014559
<i>Crassotrea gigas</i>	CGI_10026525	a	scaffold156	817079-817212	-	Yes	134	Ensembl Metazoa 45	Mollusca	SRP014559
<i>Biomphalaria glabrata</i>	BGLB019291	e	LG17_random_Scaffold1016	30223-30353	+	Yes	131	Ensembl Metazoa 46	Mollusca	SRP067658
<i>Biomphalaria glabrata</i>	BGLB019291	a	LG17_random_Scaffold1016	31998-32131	+	Yes	134	Ensembl Metazoa 46	Mollusca	SRP067658
<i>Capitella teleta</i>	CapteG154146	e	CAPTEscaffold_83	114793-114923	+	Novel exon	131	Ensembl Metazoa 45	Annelida	PRJNA379706
<i>Capitella teleta</i>	CapteG154146	a	CAPTEscaffold_83	115489-115622	+	Yes	134	Ensembl Metazoa 45	Annelida	PRJNA379706