piRNA-like small RNAs target transposable elements in a clade IV parasitic nematode

Mona Suleiman[1], Asuka Konosu[2], Ben Murcott, Mehmet Dayi[2], Rebecca Pawluk[1] , Akemi Yoshida[2], Taisei Kikuchi[2], Vicky Hunt[1]

1 Department of Biology and Biochemistry, University of Bath, Calverton Down, Bath, BA2 YA7, UK
2 Department of Infectious Diseases, Faculty of Medicine, University of Miyazaki, Japan 889-1692

Email: ms3056@bath.ac.uk

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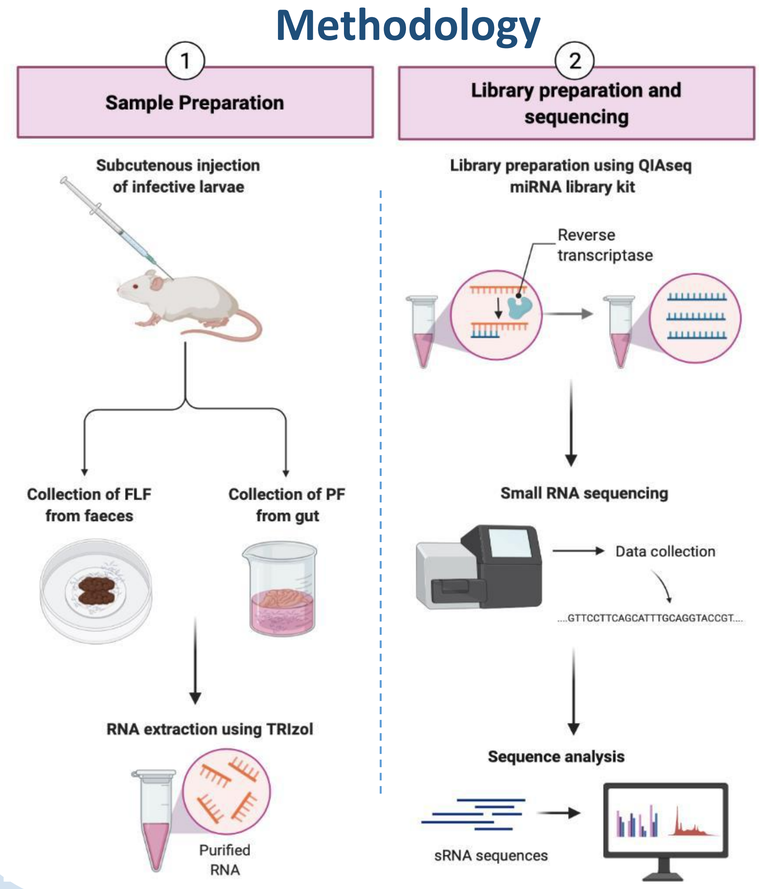
Background

Small RNAs (sRNA) are short non-coding RNAs that are important for the regulation of at least 30% of genes in humans via post-transcriptional gene silencing[1,2]. They are associated with chromatin structure, mRNA translation and the regulation of transposable element (TE) activity[2]. Three main sRNA classes have been described in eukaryotes; microRNAs (miRNAs), small-interfering RNAs (siRNAs), and piwi-interacting RNA (piRNAs)[1]. The majority of sRNA research has been carried out in the Clade V free-living nematode *Caenorhabditis elegans* which possess all three classes of sRNAs. Recent studies have shown that sRNA pathways are highly diverged in nematodes and *C. elegans* does not closely represent the sRNAs used by more distantly related nematodes, including parasitic species[3,4]. For example, the PIWI pathway involved in the production of piRNAs is important in regulating TE activity and has been well characterised in *C. elegans* but has been lost in nematodes outside of the Clade V nematodes, including *Strongyloides* spp.[4,5]. It is still not clear how nematodes outside of Clade V compensate for the loss of piRNAs and regulate TE activity, especially in parasitic species, and if TE regulation by sRNA is different during parasitism.

Here, we have investigated the role of sRNAs in the endogenous regulation of genes and TEs in the nematode *Strongyloides ratti*, a well-established laboratory model of nematode parasitism[2,4]. The life cycle of *S. ratti* includes genetically identical parasitic (PF) and free-living (FLF) adult female stages allowing direct comparison between these two adult life cycle stages to uncover genetic features associated with parasitism.

Methodology



Result I: Expression of sRNAs in *S .ratti*

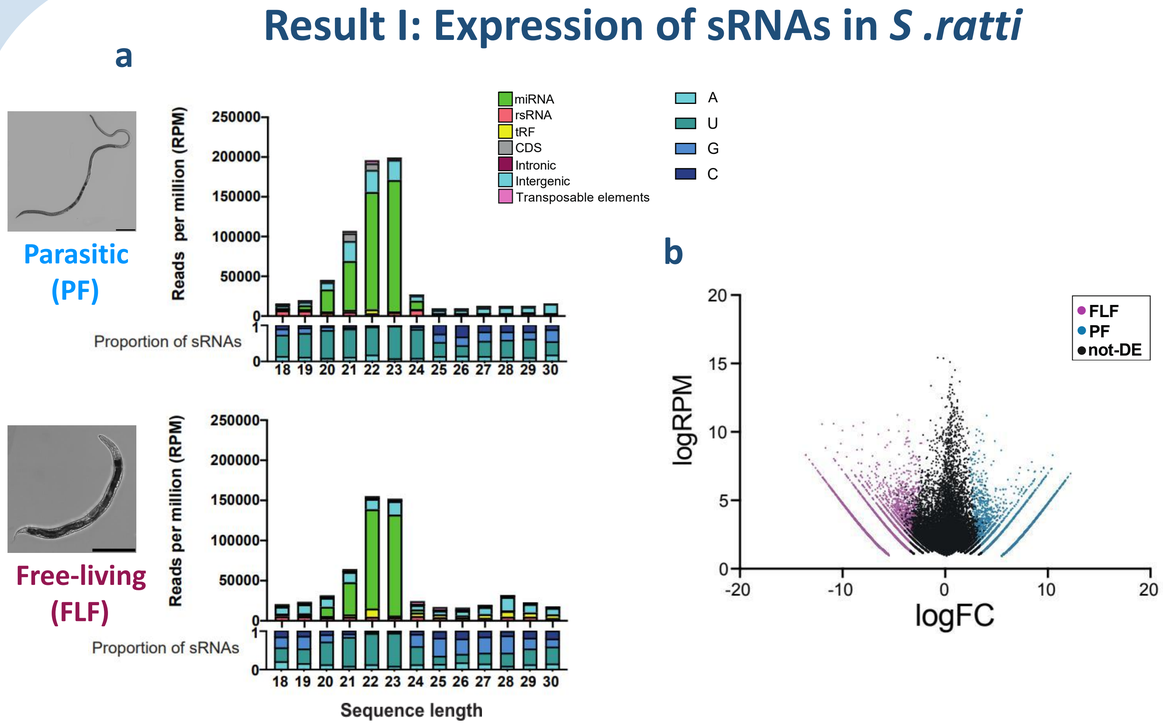


Figure 1: (a) The Unitas[6] pipeline was customised for a non-model organism and used to annotate and identify the different classes of sRNA, their origin and their first 5' nucleotide (nt) in PF and FLF. The most abundantly expressed class of sRNAs identified was miRNAs with lengths of 21 - 23 nt followed by sRNAs originating from intergenic regions ranging between 21 – 24 mostly beginning with uracil (u). Interestingly, 21-22 nt sRNAs originating from CDS and TEs were expressed at higher levels in the PF than the FLF. **(b)** edgeR[7] was used to identify subsets of sRNAs upregulated in both the PF (red) and FLF (blue) (FDR < 0.01; logCPM = log counts per Million, logFC = log fold change).

Result II: 21-22Us are parasite specific and target TEs

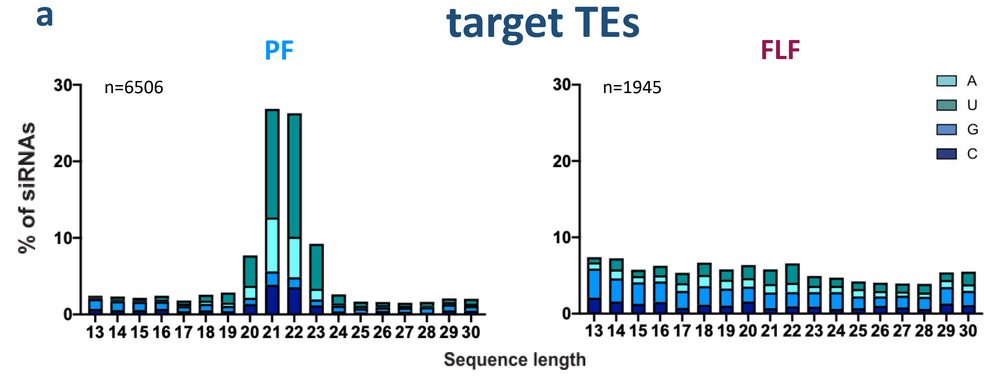
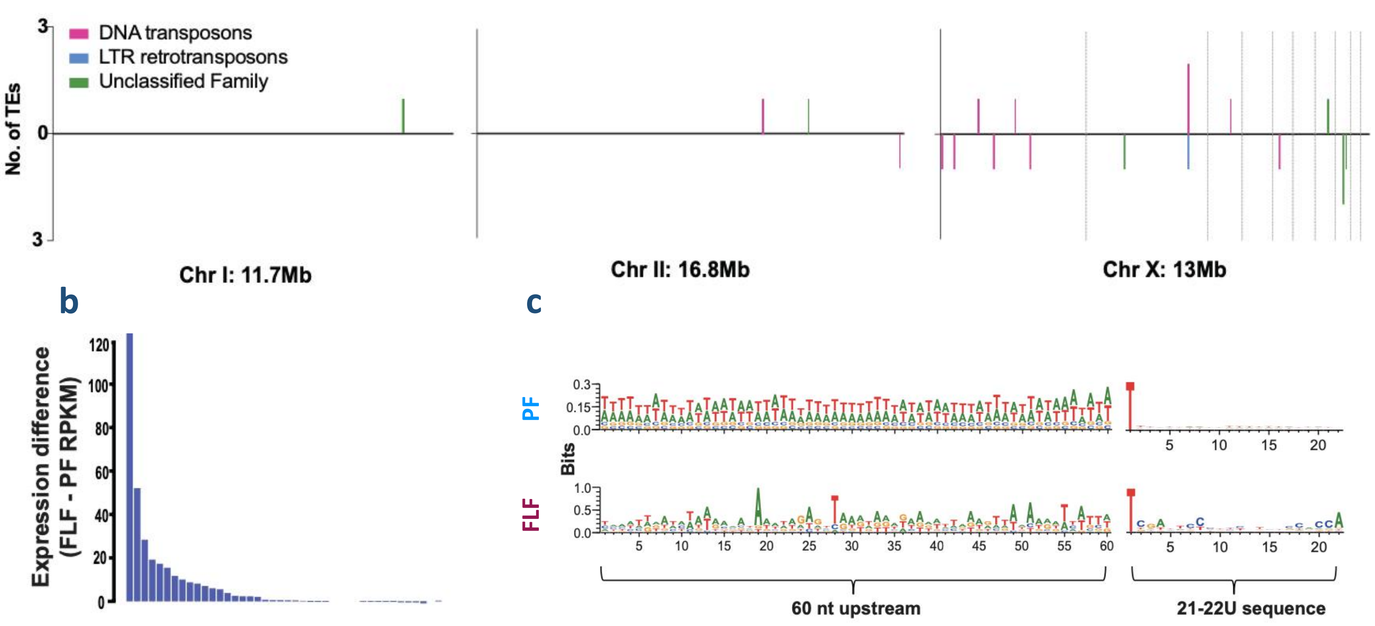


Figure 2: (a) Analysis of the length and first nt at the 5' site revealed that 21-22 nt long sRNAs starting with U (21-22Us) were the most highly expressed sRNAs upregulated in PF in comparison to the FLF, which showed no bias. **(b)** Based on perfect sequence complementarity, 21-22Us upregulated in the PF targeted the coding sequence followed by the 3'UTR and 5'UTR. 21-22Us directly target and regulate TEs, specifically the DNA transposon TcMariner and the unannotated rnd_1 class family.

Result III: Parasitism-associated 21-22Us resemble piRNAs



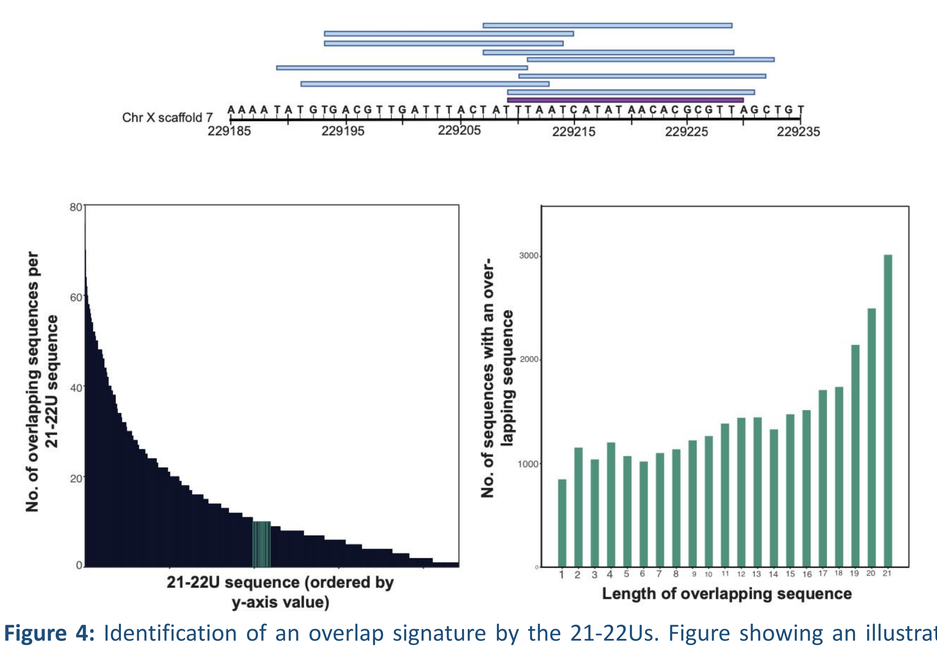


Figure 3: (a) Distribution of 21-22Us targeted TEs across the genome which consists of two autosomes (chromosomes I and chromosome II) and the X chromosome, made up of 10 scaffolds. The TEs are predominately clustered on the X-chromosome. **(b)** Expression of 21-22U targeted genes related to TEs are higher in the FLF than the PF, indicating that 21-22Us are repressing genes expressed in the PF. **(c)** AT richness in the sequence upstream of *S. ratti* 21-22Us comparable to *C. elegans* piRNAs without a piRNA-associated motif.

Figure 4: Identification of an overlap signature by the 21-22Us. Figure showing an illustration example of with a 21Us originating from chromosome X, scaffold 7 and all overlapping 21-22U strands. Bottom left figure showing the number of 21-22Us that overlap with themselves (88.78%). Bottom right figure showing the overlap lengths of siRNAs against the 21-22Us. This is similar to the patterns observed for piRNAs in *C. elegans.*

Conclusion

- Distinct subset of 21-22U sRNAs are specifically upregulated in the PF and hypothesised to have a role in parasitism or features associated with the parasitic life style.
- *S. ratti* 21-22Us share many similarities with the piRNA class of sRNAs, a pathway which is assumed to have been lost in nematodes outside of clade V.

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