Chapter 2

SCHISTOSOME RETROTRANSPOSONS

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1. INTRODUCTION

Eukaryotic genomes generally contain substantial amounts of repetitive sequences, many of which are mobile genetic elements (e.g., Lander *et al.,* 2001; Holt *et al.,* 2002). These mobile sequences have played fundamental roles in the evolution of the human and other eukaryotic genomes (Charlesworth *et al.,* 1994; Deininger and Batzer, 2002), and are among the most powerful endogenous human mutagens (Kazazian, 1999; Dewannieux *et al.,* 2003). Although less is known about the schistosome genome, recent findings suggest that up to half of the entire schistosome genome may be comprised of repetitive sequences, and much of this repetitive complement will be comprised of mobile genetic elements (see Brindley *et al.,* 2003). Here we review a series of mobile genetic elements from the schistosome genome, focusing on schistosome retrotransposable sequences. The identity, structure, phylogenetic relationships, and contribution of these elements to genome size in schistosomes are described, and we address their probable role in schistosome evolution and potential utility in introducing transgenes into schistosomes and other applications.

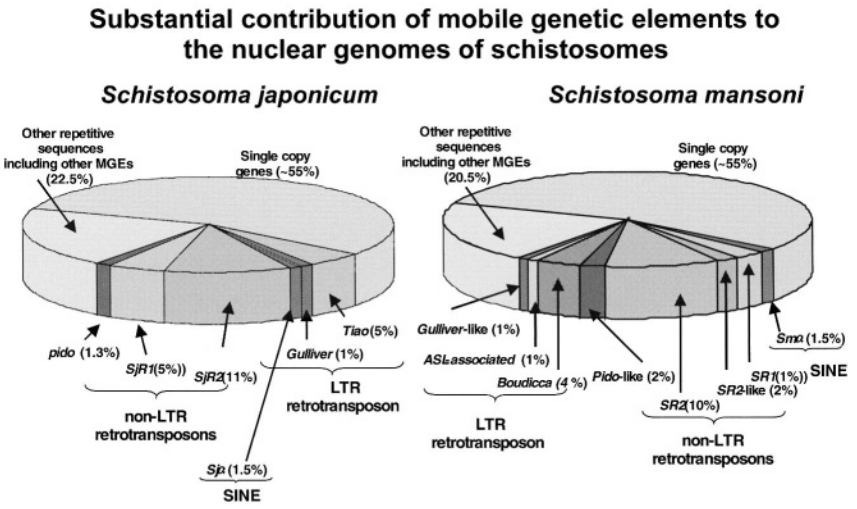

Figure 2-1. Predicted contribution of mobile genetic elements to the nuclear genomes of *Schistosoma japonicum* and *S. mansoni* are represented in pie charts, predicted from published and gene database sources (Adapted from Brindley et al., 2003, with permission).

2. THE SCHISTOSOME GENOME

Schistosomes have a comparatively large genome, estimated at ~270 megabase pairs for the haploid genome of *Schistosoma mansoni,* arrayed on seven pairs of autosomes and one pair of sex chromosomes (Simpson *et al.,* 1982). For comparison, the schistosome genome is about the same size as that of the puffer fish, *Fugu rubripes,* two to three times the size of that of the angiosperm, *Arabidopsis thaliana,* or the free-living nematode, *Caenorhabditis elegans,* ten times the size of the *Plasmodium falciparum* genome, and about one tenth the size of the human genome. The other major schistosome species parasitizing humans probably have a genome of similar size to that of *S. mansoni,* based on their karyotypes (Hirai *et al.,* 2000). Though none of the schistosome genomes have been sequenced in their entirety, several hundred thousand schistosome expressed sequence tags (ESTs) and genome survey sequences have been lodged in GenBank, probably covering the entire transcriptome and indicating that there are ~14,000 genes in *S. mansoni* (Verjovski-Almeida *et al.,* 2003; Hu *et al.,* 2003). The mobile genetic elements (MGEs) of the schistosome genome include SINE-like elements, non-long terminal repeat (non-LTR)

retrotransposons and LTR retrotransposons (Figs. 2-1-2-4), and appear to make up at least one quarter of the schistosome genome.

3. CATEGORIES OF TRANSPOSABLE ELEMENTS AND MODES OF TRANSPOSITION

MGEs are grouped in two major categories, Class I and Class II (Finnegan, 1992). Class I elements transpose through a RNA intermediate whereas Class II elements transpose directly as DNA. Class I comprises (a) the long terminal repeat (LTR) retrotransposons and the retroviruses, (b) the non-LTR retrotransposons, and (c) the short interspersed nuclear elements (SINEs). Class I elements occur in taxa as diverse as fungi and mammals, and are mobilized by replicative processes that generate numerous daughter copies and facilitate insertion into the host genome, thereby directly expanding the size of the host genome. Class II elements are termed 'transposons', and include groups from prokaryotes and eukaryotes.

LTR retrotransposons resemble retroviruses in their structure and intracellular life cycles. These elements are typically 5 - 10 kb in length. Their general structure consists of two open reading frames (ORFs) flanked by long direct terminal repeats of ~200-600 bp in length (Fig. 2-2). Some, such as *gypsy* from *Drosophila melanogaster, Osvaldo* from *Drosophila buzzatii,* and *Tas* from *Ascaris lumbricoides,* include a third ORF, *env,* encoding the envelope protein characteristic of retroviruses (Fig. 2-2). Retroviruses probably evolved from LTR retrotransposons, mediated by the acquisition of envelope proteins that facilitated extracellular existence and horizontal transmission between cells and species (Malik *et al.,* 2000). The LTRs play a pivotal role in initiating transcription and in transposition. The first ORF, *gag,* encodes a polyprotein precursor that is later processed to yield the structural proteins making up the virion core. Of these, the nucleocapsid protein associates directly with the RNA, and exhibits a characteristic cysteine/histidine motif, which appears to function as a zinc finger domain. The second ORF, *pol,* encodes a polyprotein with discrete protease, reverse transcriptase (RT), RNaseH, and integrase enzyme domains. The *pol* domain order varies between the two major *gypsy/Ty3* and *Copia/Ty1* clades of LTR retrotransposons. In retroviruses and LTR retrotransposons with an *env* gene, the envelope protein associates with the cell membrane, which envelops the virion core, allowing the viral particle to bud off from the host cell. Envelope facilitates infection via attachment to specific cell surface receptors. Thus, in addition to vertical transmission in the germ line, LTR retrotransposons with *envelope* genes are capable of extracellular existence and horizontal transmission.

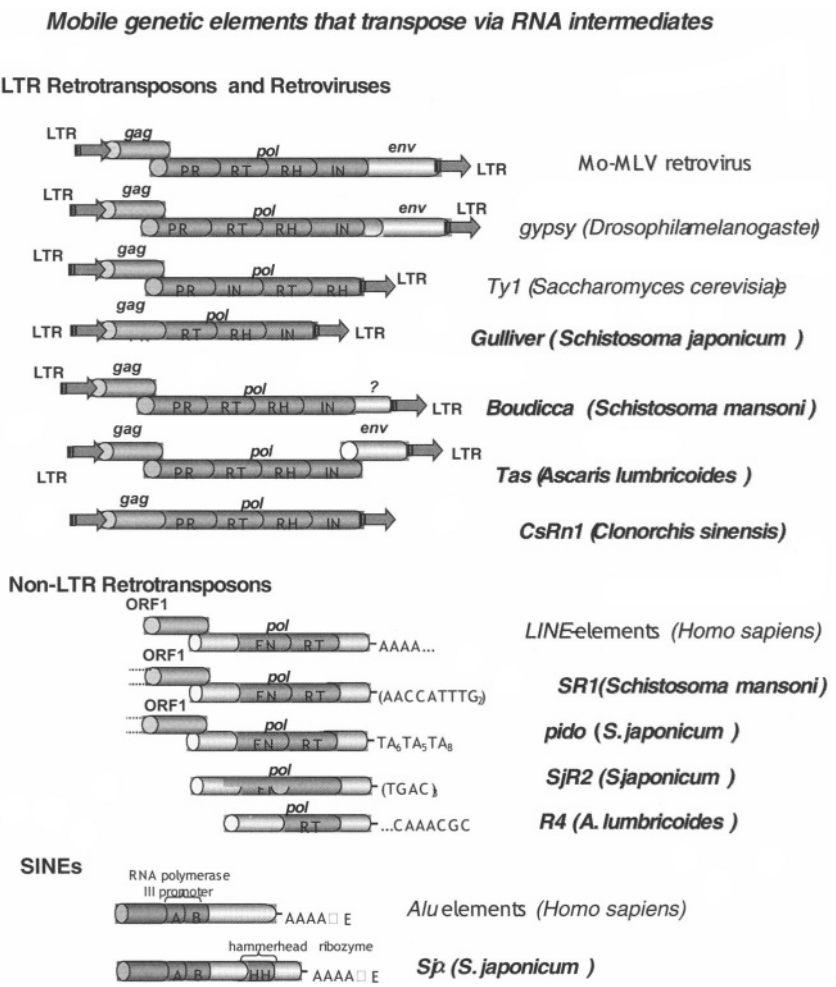

Figure 2-2. Schematic representation of the structure of representative retrotransposable elements from schistosomes and other parasitic helminths (denoted in bold text) and other hosts. Abbreviations: gag, group associated antigen; pol, polyprotein; env, envelope; PR, protease, RT, reverse transcriptase, RH, RNaseH, IN, integrase; EN, endonuclease; LTR, long terminal repeat (Adapted from Brindley et al., 2003, with permission).

Non-LTR retrotransposons are usually ~ 4 - 6 kb in length, generally have two ORFs, often have A-rich 3'-termini, and are transmitted vertically. The Long Interspersed Nuclear Elements (LINEs) of humans are well known members. Full length LINE1 is bicistronic: the product of the first ORF has RNA binding function, although this is not well characterized, whereas the second ORF encodes a polyprotein with RT and apurinic endonuclease (APE) activities. Of 11 clades of non-LTR retrotransposons recognized,

several also have an RNaseH domain within the polyprotein (Malik *et al.,* 1999). The movement of these elements involves binding of the ORF2 product to both the full-length retrotransposon RNA transcript and the host chromosome at the integration target site. Nicking of the host chromosome by APE activity of the ORF2 product provides an initiation start site, a 3'-hydroxyl group, to prime reverse transcription of the retrotransposon RNA. Target site duplication, a footprint of retrotransposition, is a by-product of integration of non-LTR retrotransposons into the host chromosome. Most copies of non-LTR retrotransposons are 5'-truncated, the consequence of premature termination of reverse transcription (see Deininger and Batzer, 2002).

SINEs are short (< 600 bp in length), non-autonomous MGEs, with poly A-rich 3'-termini (like most non-LTR retrotransposons) (Fig. 2-2). Most SINEs have a composite structure comprising a 5' tRNA-related region followed by a tRNA-unrelated region. *Alu*'s, however, which are the best characterized SINEs and which are known only from humans and some other primates, have evolved from the 7SL RNA gene (Deininger and Batzer, 2002). SINEs do not encode any proteins of their own, but rely on RT from other sources for their mobilization. Non-LTR retrotransposons provide the RT activity that drives retrotransposition of SINEs (Kajikawa and Okada, 2002; Dewannieux *et al.,* 2003). Transcription of SINEs is driven by RNA polymerase III followed by reverse transcription and integration into the new site of the genome. SINE transcripts appear to be incorporated into the ribonucleoprotein particles that are the transposition intermediates for non-LTR retrotransposons, from where they are transported into the nucleus, reverse transcribed, and integrated into the host chromosome. SINEs are transmitted vertically.

Transposons constitute the Class II MGEs. They move by a 'cut and paste' process that is generally independent of host-specific factors. Transposons range in size from ~ 1.3 to 3.0 kb and are bounded by terminal inverted repeat (IR) sequences that are recognized by the transposase enzyme, the only protein encoded by the transposon. Transposase mediates excision of the transposon and its re-insertion into the host genome. Transposons can move horizontally between species, as well as being propagated through the germ line. In evolutionary terms, transposons require horizontal movement between species for their long-term survival. (This attribute has been pivotal in harnessing of transposons such as *mariner* as vectors for transgenesis.) Because transposase is *trans*-acting, cutting and pasting both older and younger copies of the transposon, and because older copies tend to have accumulated deleterious mutations, efficiency of transposition declines over time until extinction.

4. SCHISTOSOME RETROTRANSPOSABLE ELEMENTS

4.1 *Boudicca*

Boudicca is a ~6 kb long terminal repeat retrotransposon from *S. mansoni.* Two 328bp LTRs flank a coding region consisting of open reading frames representing the *gag* and *po1* polyproteins, 5' and 3' untranslated regions, and, at least in the well characterized copy, an additional unknown third open reading frame (Copeland *et al.,* 2003). *Boudicca* is a high copy number element, estimated at 2,000 to 3,000 copies per haploid genome, and is actively transcribed in adult worms, cercariae, and sporocysts. One of the only members of the *Kabuki/CsRn1* clade of gypsy-type LTR retrotransposons, *Boudicca's* closest relatives are *PwRn1* from *Paragonimus westermani, CsRn1* from *Clonorchis sinensis,* and *Kabuki* from the silkworm, *Bombyx mori.* This clade, closely related to the errantiviruses, is differentiated not only on the primary sequence level but also by a unique Cys-His box structure, CHCC instead of the more common retroviral CCHC Cys-His box (Bae *et al.,* 2002). Currently, we are investigating the potential of *Boudicca* as a transgenesis vector for schistosomes.

4.2 *Gulliver*

The LTR retrotransposon *Gulliver,* ~4.8 kb in length, is present in multiple copies in the genome of *S. japonicum* (Figs. 2-1, 2-2) (Laha *et al.,* 2001). Southern blot analysis indicates that it is also present in the genome of *S. mansoni.* The LTRs of *Gulliver* are 259 bp in length and include RNA polymerase II promoter sequences, a CAAT signal and a TATA box. *Gulliver* exhibits features characteristic of a functional LTR retrotransposon including two read through (termination) ORFs encoding retroviral gag and pol proteins of 312 and 1,071 amino acid residues, respectively. The *gag* ORF encodes motifs conserved in nucleic acid binding proteins, while the *pol* ORF encodes conserved domains of aspartic protease, reverse transcriptase (RT), RNaseH and integrase, in that order, a pol pattern conserved in the *gypsy* lineage of LTR retrotransposons. Its structure is similar to that of *gypsy,* although *Gulliver's,* closest relatives include *mag* from *B. mori* and *Blastopia* from *D. melanogaster* (Figs. 2-2, 2-3).

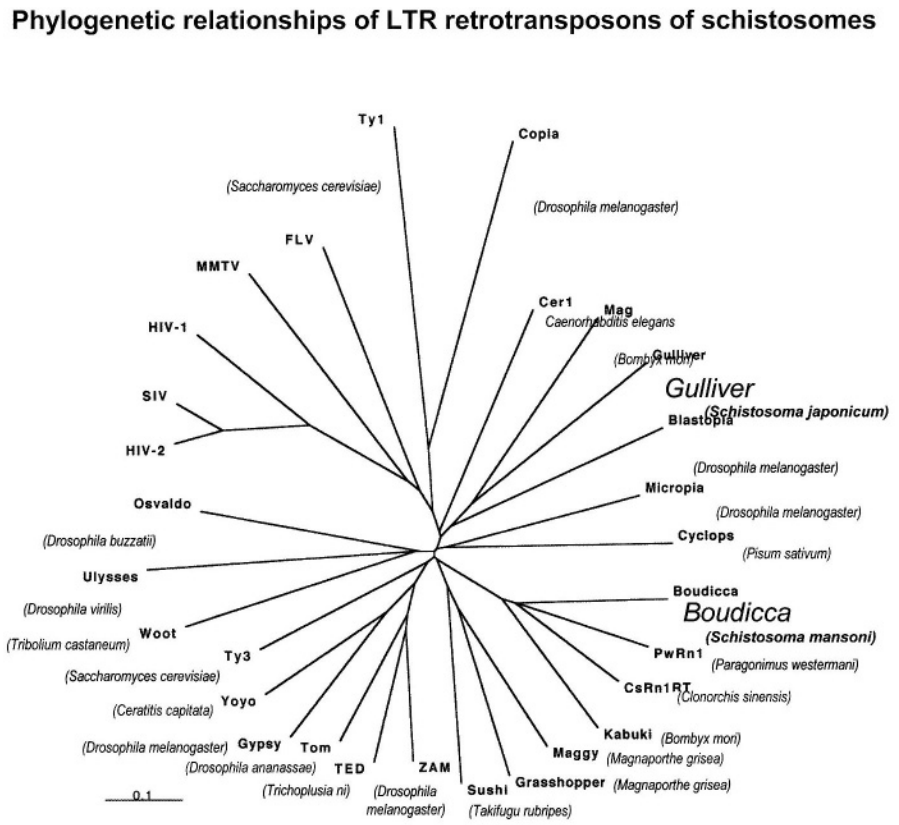

Figure 2-3. Phylogenetic tree comparing the relationships among the schistosome LTR retrotransposons Boudicca, Gulliver and other LTR retrotransposons and retroviruses. Reverse transcriptase regions were aligned and tree files were created using MacVector and ClustalX software, and unrooted output trees were created using Treeview software. Accession numbers: Blastopia: CAA81643, Boudicca: AY308018-AY308026, Cer1: AAA50456, Copia: OFFFCP, CsRn1: AAK07486, Cyclops: AAL06415, HIV-1: P04585, HIV-2: AAA76841, FLV: NP_047255, Grasshopper: AAA21442, Gulliver: AF243513, Gypsy: GNFFG1, Kabuki: BAA92689, Mag: S08405, Maggy: AAA33420, Micropia: S02021, MMTV: GNMVMM, Osvaldo: CAB39733, PwRn1: AY237162, SIV: AAA47606, Sushi: AAC33526, TED: AAA92249, Tom: CAA80824, Ty1: P47100, Ty3: AAA35184, Ulysses: CAA39967, Woot: AAC47271, Yoyo: T43046, Zam: CAA04050.

4.3 Other LTR retrotransposons

Tiao, the first LTR type retrotransposon to be reported from any of the human schistosomes, is a high copy number (about 10,000) LTR retrotransposon found in *S. japonicum* (Genbank AF073334) (Fan and Brindley, 1998). *Tiao* is related to the *Pao*-like retrotransposons *Kamikaze,*

Yamato, and *Pao* from *B. mori,* and *Ninja, BEL,* and *Max* from *Drosophila melanogaster.* In *S. mansoni,* fragments of additional LTR type retrotransposons distinct from *Boudicca* have also been identified. Within the locus of the *S. mansoni* gene encoding adenylosuccinate lyase, intron 6 contains two 500 bp direct LTRs flanking a 1.9 kb sequence with homology to LTR retrotransposon type gag and pol (Foulk *et al.,* 2002). In addition, several other LTR type retrotransposons related to *Pao, Mag,* and *Osvaldo* are present in the genome of *S. mansoni,* awaiting characterization (Copeland and Brindley, unpublished).

4.4 The non-LTR retrotransposon *SR1*

SR1 is a non-LTR retrotransposon and was the first autonomous retrotransposon to be reported from schistosomes (Drew and Brindley, 1997). Orthologous versions occur in *S. mansoni (SR1)* and *S. japonicum (SjR1),* with a copy number estimated in the range of 200 to 2,000 (Fig. 2-1). *SR1* is a *CR1*-like element (Figs. 2-2, 2-4), although the full-length element (predicted to be ~ 4 kb in length) has not been totally characterized. *SR1* elements possess atypical 3' termini consisting of the tandem repeat $(AACCATTTG)_2$ which are similar in structure to the imperfect tandem repeat of the 3' termini of *CR1* from chickens and other vertebrates.

4.5 *SR2*

SR2 is a non-LTR retrotransposon of ~3.9 kb in length with a single ORF encoding APE and RT. Orthologous versions appear to occur in *S. mansoni (SR2)* and *S. japonicum (SjR2),* with copy numbers estimated in the range of 1,000 to 10,000 (Drew *et al.,* 1999; Laha *et al.,* 2002a). *SR2* elements are related to *RTE-1* from *C. elegans.* The ORF is bounded by 5'- and 3'-terminal untranslated regions and, at its 3'-terminus, *SjR2* bears a short $(TGAC)_3$ repeat (Figs. 2-1, 2-2, 2-4). Active, recombinant RT of *SjR2* has been produced in insect cells (Laha *et al.,* 2002a).

4.6 *pido*

This is the third non-LTR retrotransposon characterized from the genome of *S. japonicum* (Laha *et al.,* 2002b). Although the full-length element has yet to be characterized, a consensus sequence of 3564 bp of the truncated *pido* element has been assembled from several genomic fragments that contained *pido*-hybridizing sequences. The sequence encodes part of the first ORF, the entire second ORF and, at its 3'-terminus, a tandemly repetitive,

A-rich ($TA_6TA_5TA_8$) tail (Fig. 2-2). ORF1 of *pido* encodes a nucleic acid binding protein and ORF2 encodes a retroviral-like polyprotein with APE and RT domains. mRNA encoding the RT of *pido* was detected by reverse transcription-PCR in the egg, miracidium and adult developmental stages of *S. japonicum,* indicating that the RT domain is transcribed and suggesting that *pido* is replicating actively and mobile within the *S. japonicum* genome. At least 1,000 partial copies of *pido,* which is not closely related to either SR1 or SR2 (Figs. 2-1, 2-4), are dispersed throughout the genome of *S. japonicum.*

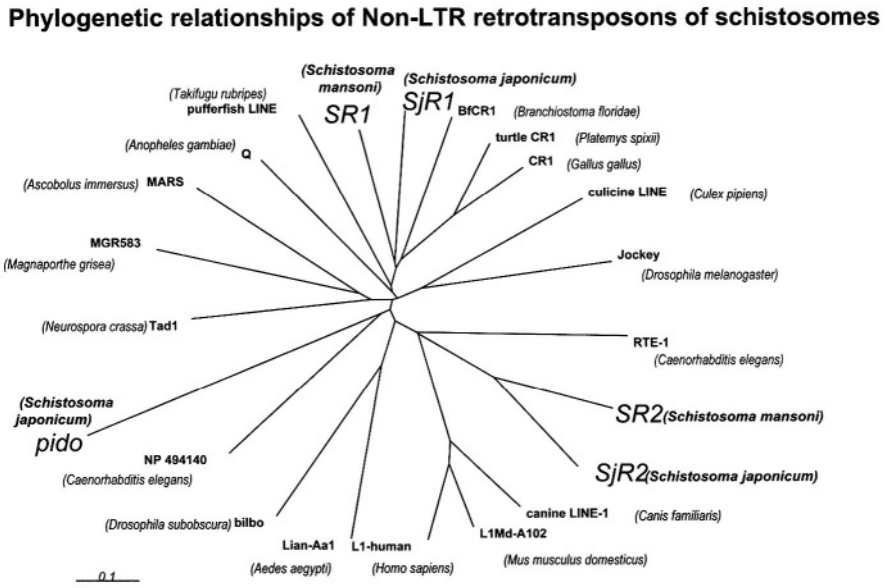

Figure 2-4. Phylogenetic tree comparing the relationships among the schistosome non-LTR retrotransposons SR1, SR2 and pido and other non-LTR retrotransposons. Reverse transcriptase regions were aligned and tree files were created using MacVector and ClustalX software, and unrooted output trees were created using Treeview software. Accession numbers: BfCR1: AAL40415, Bilbo: AAB92389, canine LINE-1: BAA25253, Cr1: AAC60281, culicine LINE: AAA28291, Jockey: P21328, L1-human: AAD04635, LlMd-A102: AAL17970, Lian-Aa1: T30319, MARS: CAA67543, MGR583: AAB71689, NP 494140: NP_494140, Pido: AY034006, pufferfish LINE: AAD19348, Q: T43020, RTE-1: S68633, SjR1: AAC62955, SjR2: AAK14815, SR1: AAC06264, SR2: AAC24982, Tad1: AAA21781, turtle CR1: BAA88337.

4.7 Sm•-, Sj•-like SINEs

SINE-like retroposons known as Sm , Sj , Sh etc occur in high copy number, 7000-10,000 copies, in the genomes of schistosomes (Spotila *et al.,* 1989; Drew and Brindley, 1995; Ferbeyre *et al.,* 1998; Laha *et al.,* 2000). The sequence of the consensus Sm –like SINE includes the hallmark features of SINE-like elements including a promoter region for RNA polymerase III, a region of identity of the bovine $tRNA^{arg}$ gene, an AT-rich stretch at its 3'-terminus, a short length of 330 bp or less, and short direct repeat sequences flanking the insertion site (Figs. 2-1, 2-2). Sm encodes an active ribozyme bearing a hammerhead domain; Ferbeyre *et al.* (1998) suggested a role for these self-catalytic RNAs in control of retrotransposon expansion. Some Sm elements appear to be linked to the female (W) sex chromosome (Drew and Brindley, 1995). Based on the interaction of *Alu* elements and other SINES with LINEs (Dewannieux *et al.,* 2003), the RT activities of *pido, SR2* and/or *SR1* are likely utilized by the Sm –like elements for replication and dispersal.

5. (RETRO)TRANSPOSONS FOR SCHISTOSOME TRANSGENESIS?

The ability to introduce transgenes into genomes of pathogenic organisms has revolutionized molecular approaches to microbial diseases. Whereas this revolution has included protozoan parasites, and indeed mosquito and other insect vectors, so far it has largely bypassed medical helminthology because neither cell lines nor transgenesis systems have been developed for parasitic worms. Consequently, molecular research in schistosomes and schistosomiasis, and indeed other helminthoses, has been structurally disadvantaged in comparison with other microbes (Boyle and Yoshino, 2003). Since MGEs are valued as vectors for genetic transformation of other invertebrate genomes (e.g., O'Brochta *et al.,* 2003), likewise they hold promise for genetic transformation of schistosomes. Many free-living taxa are malleable to transformation with transposons. Because transposons often exhibit minimal requirements in terms of host cell factors for mobility, they can mobilize in genomes of diverse species (Guerios-Filho and Beverley, 1997; O'Brochta *et al.,* 2003). There are no reports of transformation of schistosomes or indeed other parasitic helminths with transposons, although this should be feasible now that conditions for transgenesis are being elucidated (Davis *et al.,* 1999; Wippersteg *et al.,* 2002; Heyers *et al.,* 2003). Isolation of endogenous forms of *mariner*-like elements from schistosomes, if indeed they are present in the schistosome genome,

might facilitate development of target species-specific constructs, (*mariner*-like elements occur in planarians [Garcia-Fernàndez *et al.,* 1995].)

Retroviruses such as Moloney murine leukemia virus (Mo-MLV) (Fig. 2-2) have been widely used for transgenesis studies, including human gene therapy research. Although retroviruses exhibit narrow host ranges, pseudotyping Mo-MLV with the envelope glycoprotein (G) of the vesicular stomatitis virus (VSV) expands its host range (Yee *et al.,* 1994). It is also feasible that Mo-MLV constructs pseudotyped with envelope proteins from endogenous retroviruses, for example the envelope of *Tas* from *A. lumbricoides* (Fig. 2-2), would enhance the parasite species cell-specificity of these constructs, and circumvent host cell-specific entry blocks in *Ascaris* and related nematodes. This approach appears feasible for schistosomes once envelope genes from endogenous retrovirus-like retrotransposons from schistosomes are characterized. A desirable transgenesis vector would be one that already integrates into the schistosome genome as part of the vector's natural life cycle. The active search for and characterization of endogenous MGEs in schistosomes have already yielded several possible candidates (Figs. 2-1-2-4). It seems feasible that schistosome retrotransposons such as *Boudicca* and *Gulliver* can be adapted directly as transgenesis vectors given similar success with other mobile elements (Garraway *et al.,* 1997; Ivics *et al.,* 1997; Dewannieux *et al.,* 2003). We are pursuing this and similar lines of investigation in our laboratories, towards the goal of development of a tractable system for schistosome transgenesis.

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