Type I-like intervening sequences are found in the rDNA of the nematode *Ascaris lumbricoides*

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**ABSTRACT**

The intervening sequences in the large ribosomal RNA gene of *Ascaris lumbricoides* var. *suum* show many similarities to the type I insertions, previously found only in some insect species. They include structural features, but also a presumed transcriptional inactivity in vivo: No transcript of the rDNA intervening sequence in *A. lumbricoides* could be detected in Northern and dot blot hybridizations. However, the primary structure of the Pol I promoter region is well conserved in interrupted and uninterrupted genes. Moreover, genes with an intervening sequence are correctly initiated in a whole-cell in vitro extract from *Ascaris oogonia*. Hence, the presence of the intervening sequence alone does not seem to account for a transcriptional inhibition in rRNA genes. As with the type I insertions of insect rDNA, some copies of the *A. lumbricoides* intervening sequence are also present in locations outside the rDNA cluster. About 50% of the extraribosomal copies are found in a repetitive sequence of the genome, and additional copies are inserted in unique sequences. These striking analogies to type I insertions are discussed, and lead to the conclusion that the two phenomena are undoubtedly related. This is the first report proving the presence of a type I-like insertion element outside of the class Insecta.

**INTRODUCTION**

Intervening sequences (IVS) in the large ribosomal RNA gene were first reported in *Drosophila melanogaster* (1-4). Two non-homologous insertion elements, the so-called type I and type II insertions, have been described. Type II insertions are found in about 15% of the rDNA repeats on both the X and Y chromosomes (5). They are located about 60 bp upstream of the site of type I insertions, and unlike the type I insertions they are not flanked by direct repeats from duplicated rDNA sequences, and no extragenic copies of the IVS are detected (6, 7). Type I insertions are found in about 60% of the rRNA genes on the X chromosome (5). The genomic organization of rDNA units interrupted by type I-like

elements varies between different species: While they are clustered in both D. hydei (8) and Calliphora erythrocephala (9), they are interspersed with noninterrupted rDNA in D. melanogaster (3, 5). Some copies of the IVS also exist outside the rDNA cluster in Drosophila (10-12). All interrupted rRNA genes in the insect species investigated so far are generally not transcribed (for review, see 13).

Originally, these type I elements seemed to be an evolutionary speciality of dipteran flies (reviewed in 13). With the discovery of similar intervening sequences in the rDNA of the nematode Ascaris lumbricoides (14) and the lepidopteran Bombyx mori (15, 16), it appeared that the phenomenon could be more widespread. There are striking organizational similarities between the Ascaris intervening sequence and the type I D. melanogaster element (14). This is rather surprising, because Arthropoda and Nemathelminthes are phylogenetically quite distant. It was therefore of interest to know how these two elements are related. To answer this question we made detailed structural analyses of A. lumbricoides rDNA intervening sequences and searched for a functional resemblance to insect type I insertions. We have investigated the transcriptional activity, both in vivo and in vitro, and the genomic organization of IVS⁺ rDNA of Ascaris lumbricoides, and we also report the presence of some extraribosomal copies of the intervening sequence.

MATERIALS AND METHODS

Isolation of nucleic acids, electrophoresis, Southern and Northern blot hybridizations

DNA and RNA were isolated from different tissues of A. lumbricoides as reported earlier (17, 18). Nick-translation of DNA and Southern blot hybridization was carried out as described in 19. For the Northern blot experiments, samples were denatured with glyoxal (20), following the modification described in 21. Electrophoresis, transfer to nitrocellulose and hybridization were done according to 22.

Construction of genomic DNA libraries

For the construction of a cosmid library, DNA from oogonia of A. lumbricoides was partially digested with Sau3AI and fractionated
on a 5-24% NaCl gradient (4 1/2 h at 152'000 g). Fractions between 30 and 50 kb were pooled and ligated to cosmid V34 (gift from B. Hohn), which had been linearized with BamHI and dephosphorylated. After in vitro packaging of the DNA (23) and infection of E. coli strain DH 1, the resulting clones were screened with a 32P labelled probe from the rDNA intervening sequence. Positive cosmid clones were isolated by the SDS-lysis method described in 19.

For the isolation of extraribosomal copies of the IVS, intestinal DNA from A. lumbricoides was partially digested and fractionated as mentioned above. Fractions between 15 and 20 kb were ligated into the BamHI site of λEMBL4 vector DNA. In vitro packaging, infection of host cells and isolation of clones were done by standard laboratory methods (19).

DNA sequencing
Appropriate fragments from the region surrounding the transcription initiation site and from extraribosomal IVS junctions were cloned into pUC 18/19 vectors. For sequencing, the double-stranded plasmids were heat-denatured before annealing to the universal primer (24), and the dideoxy chain termination method (25) was used following the protocol of the Amersham handbook.

In vitro transcription
The rDNA templates from clones with and without an intervening sequence were transcribed in a whole-cell in vitro extract from A. lumbricoides oogonia, as described (26). After transcription, the RNA was purified and electrophoresed on 5% polyacrylamide gels as reported (26).

RESULTS
Genomic organization of IVS+ rDNA and nucleotide sequence of the promoter region

In Ascaris lumbricoides, two main forms of uninterrupted rDNA can be distinguished by the different sizes of the nontranscribed spacers (18). These two forms are clustered separately on a single autosomal locus (27). Only rRNA genes of the long size class are interrupted by an intervening sequence in the 26S region (14). The question, whether or not IVS+ genes are organized in tandem arrays, can be answered by cleavage of Ascaris DNA
with *ApaI* or *KpnI*. Both enzymes have a single recognition site within the intervening sequence of the rDNA. Double digestion of genomic DNA with *ApaI* and *KpnI* created a 460 bp long fragment, with an intensity corresponding to 12–15 copies (Figure 1; copy standards not shown). Because about 5% or 15 copies of the genomic rRNA genes are interrupted by a 4.5 kb long IVS (14), most if not all were cleaved by *ApaI* and *KpnI*. The copies of the IVS in a different genomic environment (see below) may have been the origin of the longer fragments, present in the single copy range in the *ApaI* & *KpnI* track of Figure 1.

Digestion of genomic DNA with *ApaI* or *KpnI* would generate fragments of the cistron length of 13.3 kb only if at least two IVS+ genes are directly adjacent. However, *BamHI* will cleave all IVS+ rRNA genes into 13.3 kb long fragments, irrespective of
Fig. 2. Schematic diagram and restriction map of an interrupted rDNA repeat, as present in the cosmid clone C1. The 8.2 kb long HindIII fragment was subcloned in the pUC18 vector. From this subclone, the fragments HindIII/ClaI (~500 bp), ClaI/HincII (~200 bp) and HincII/XbaI (~450 bp) were isolated, ligated with pUC18 and pUC19 vectors and sequenced. The transcription initiation site (TS) is located within the ClaI/HincII subclone.

their location, because the BamHI site is present in the 26S coding region of all rRNA genes. As shown in Figure 1, digestion with BamHI, but not with ApaI or KpnI, led to fragments of 13.3 kb length. These results indicate that the rRNA genes bearing an IVS are not clustered, but rather, are intermingled with the uninterrupted species of the long rDNA form. The fragments of about 8 kb, visible in the BamHI track, probably arose from cleavage of the most abundant extraribosomal IVS copies (cf. map in Figure 5).

In order to analyze the structure of the transcription initiation site, we have screened a cosmid DNA library from A. lumbricoides oogonia for clones containing IVS+ rDNA. From one clone (called C1), an 8.2 kb long HindIII fragment, extending from the nontranscribed spacer up into the center part of the IVS in the 26S coding gene (cf. Figure 2), was subcloned. The region homologous to the Pol I promoter of IVS- rDNA repeats (cf. 26) was sequenced and compared to the corresponding region of the uninterrupted form in the clone pAlr8 (Figure 3).

The sequenced parts of the interrupted and uninterrupted rDNA comprise about 600 bp of the nontranscribed spacer (NTS), the entire external transcribed spacer (ETS) of 415 bp, and the first 162 bp of the 18S rRNA gene. The amount of sequence varia-
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Fig. 5. Nucleotide sequence of the region surrounding the putative transcription initiation site (TS) of IVS<sup>+</sup> rDNA. The reference sequence of IVS<sup>-</sup> rDNA is taken from the clone pAl8, representing the main size class rDNA of 8.8 kb (26). Nucleotides are numbered with respect to the initiation site. The boxed sequence is part of the 18S rRNA gene. Sequence variations of IVS<sup>+</sup> vs. IVS<sup>-</sup> rDNA (base substitutions, insertions and deletions) are shaded.

It is important to note that the nucleotide sequence of the region surrounding the putative transcription initiation site (TS) of IVS<sup>+</sup> rDNA is nearly identical in the NTS (1.7%) and the ETS (1.6%). Of the coding regions, only the first 162 bp of the 18S rRNA gene have been analyzed, and a single point-mutation is found at position +464. However, the region which most probably contains the Pol I promoter (26) and surrounds the transcription initiation site (TS in Figures 2 and 3) from posi-
Fig. 4. (A) Northern blot of A. lumbricoides RNAs from different developmental stages (see below). The filter was prehybridized for 20 h in 40% formamide, 0.1% SDS, 0.1% Na-pyrophosphate at 45°C, and hybridized for 24 h in the same buffer with a nick-translated DNA probe from the left end of the rDNA intervening sequence. (B) Dot blot of A. lumbricoides RNA. Hybridization conditions were the same as in A. (C) Hybridization of the same filter as in B, but with an rDNA probe. RNA was prepared from: Oocytes (Oc 10 μg), oogonia (Og 5 μg), spermatids (Sp 5 μg) and larvae (La 10 μg). S represents 33 pg of the 2.2 kb long hybridisation probe. (D) In vitro transcription of cloned fragments from IVS+ and IVS− rDNA. 0.16 pmol of each fragment were transcribed with 12.5 μl of extract in a total volume of 25 μl. After purification, the resulting RNA was run on a 5% polyacrylamide gel and autoradiographed after drying. The used templates were either a HindIII/XbaI or a HindIII/EcoRI fragment, resulting in specific transcripts of 570 or 1850 nucleotides (indicated by arrows). The following templates were used: a) The HindIII/XbaI fragment from pAIR8 (uninterrupted rDNA), b) Fragment HindIII/XbaI from pC1H (interrupted rDNA), c) Fragment HindIII/EcoRI from pC1H, d) Fragments HindIII/EcoRI from pC1H and HindIII/XbaI from pAIR8.
tions -116 to +176, is free of any base heterogeneities (numbers indicate position with respect to the TS).

**Transcriptional analysis of IVS\(^+\) rDNA**

Because the primary structure of the promoter region in IVS\(^+\) genes is identical to the corresponding region of uninterrupted genes, it should allow a correct initiation of transcription. In order to detect possible *in vivo* transcripts of the interrupted rDNA, a Northern blot with total RNAs from different developmental stages of *A. lumbricoides* was hybridized with a DNA probe derived from the IVS (Figure 4A). Whereas the internal 2.2 kb DNA standard (33 pg of the hybridization probe) on the same filter gave a clear signal, no hybridization was detectable in any of the RNA tracks (5-10 \(\mu\)g of total RNA; cf. Figure legend). Similar, negative results were obtained when dot blots of *Ascaris* RNA were hybridized with the same probe (Figure 4B), but control hybridization with an rDNA probe produced strong signals (Figure 4C). Assuming that the rRNA accounts for at least 50\% of the total cellular RNA, equal transcriptional activity of uninterrupted (95\% of all rDNA repeats) and interrupted genes (5\%) should produce about 250 ng of IVS-containing RNA per 10 \(\mu\)g of total RNA. Our hybridization experiments show that the amount of transcripts of the intervening sequence does not exceed 66 pg per 10 \(\mu\)g of total RNA. Hence, if they exist *in vivo* at all, they would be dramatically underrepresented.

Two explanations for the absence of IVS RNA are conceivable: 1) initiation cannot occur on these genes, or 2) the transcripts are unstable and rapidly degraded. To test the first hypothesis, we have performed *in vitro* transcription experiments with restriction fragments of IVS\(^+\) rDNA as templates. The HindIII subclone pCH (cf. Figure 2), truncated with different restriction enzymes, was assayed in a whole-cell *in vitro* extract from *A. lumbricoides* oogonia (26). Figure 4D shows, besides some non-specific end-to-end transcripts and RNA breakdown products, that the different fragments from IVS\(^+\) rDNA are correctly transcribed. The transcription started at the same site, whether fragments of the uninterrupted or of the interrupted rDNA clones were used.

The quantitative aspects of transcription initiation were tested by competition experiments with templates derived from the
Fig. 5. Schematic representation of the genomic organisation of two clones (perl3 and perl5), containing each an rDNA intervening sequence inserted into an extraribosomal region, and hybridisation of subcloned fragments to DNA from Ascaris oocytes. The thick black bars represent the IVS-homologous sequences. The four different hybridisation probes (labelled A, B, C and D) are indicated, and the corresponding genomic blots are shown. Probe A contains no IVS-homologous sequence, probe B is from the boundary region and contains both IVS homologous and adjacent non-homologous sequences, probe C is an internal fragment from the IVS, and probe D is a subcloned unique sequence which includes a small portion of the IVS. For further explanations see text.

IVS$^+$ as well as the IVS$^-$ rDNA. In order to distinguish the transcription products, we used templates of different lengths (cf. legend of Figure 4). Each one of these different templates ini-
tiated transcription correctly and efficiently (Figure 4D, lanes a, b and c). The HindIII/XbaI fragments of both forms gave rise to a specific transcript, 570 nucleotides in length, whereas a transcript of 1850 nucleotides was produced from the HindIII/EcoRI fragments. Co-transcription of equimolar amounts of the long template from the interrupted clone and the short template from the uninterrupted clone (Figure 4D, lane d) resulted in production of both the 1850 nucleotides long RNA and the 570 nucleotides long RNA. These results revealed that the IVS+ rDNA promoter element contains all structural features necessary for a correct initiation of transcription, and that the binding of putative transcription factors and/or polymerase I under the in vitro conditions used was at least not weaker than on IVS− rDNA.

Structure and genomic organization of extraribosomal copies of the IVS

By analogy to the situation in *D. melanogaster*, we expected to find some chromosomal copies of the intervening sequence outside the rDNA cluster. We therefore performed a double-screening of an *Ascaris* genomic DNA library with both an rDNA probe as well as an internal probe from the IVS. Clones hybridizing to the second but not to the first probe were chosen for further analysis. All clones hybridizing to both ends of the rDNA IVS turned out to carry an IVS inserted in a repetitive DNA family of the *Ascaris* genome. From a representative of these clones (perI3), a *BamHI*-*XbaI* fragment lying next to the IVS-homologous sequence was subcloned and used as hybridization probe with genomic *Ascaris* DNA (Figure 5, probe and blot A). The fragment lengths corresponding to the chromosomal copies of clone perI3 are given on the margin of the figure. These fragments are represented 6-8 times in the genome (copy standards not shown). Additional bands visible in each track may be due to "empty sites" or to restriction enzyme site polymorphisms.

The DNA probe B in Figure 5 was a subcloned *EcoRI* fragment from perI3, containing both IVS homologous and non-homologous sequences. This probe hybridized to ribosomal and extraribosomal IVS fragments of the *Ascaris* genome (Figure 5, blot B). Fragments hybridizing to it and derived from the 13.3 kb rDNA repeating unit were 2.9 kb long upon cleavage with *EcoRI*, 13.3 kb with
BamHI, 8.2 kb with XbaI, and 4.6 kb with BamHI/XbaI. The fragments corresponding to genomic copies of the extraribosomal IVS in clone perI3, on the other hand, were 1.8 kb, 8.0 kb, 8.0 kb and 6.7 kb long, respectively. The intensities of the signals of the ribosomal and extraribosomal fragments in each track were about 3:1, corresponding again to 6-8 copies for the perI3 genomic fragments.

Hybridization with an internal EcoRI fragment from the IVS gave a prominent signal of 2.2 kb length in the EcoRI track (Figure 5, probe and blot C). Because both the ribosomal and extraribosomal copies of the IVS are cleaved with EcoRI at the same sites, they cannot be distinguished. In all other tracks, fragment lengths of intra- and extraribosomal IVS copies corresponded to those observed with probe B in Figure 5. Additional bands in blots B and C of Figure 5 resulted from less abundant copies of the IVS, both within and outside of the rDNA cluster (cf. also 14).

The intensities of all hybridization signals in Figure 5 indicates that there were not more than 15-20 copies of extraribosomal intervening sequences in the Ascaris genome. Therefore, the 6-8 chromosomal copies homologous to clone perI3 (Figure 5) represent about 50% of all extraribosomal IVS’s. To study extraribosomal IVS organizational forms represented in lower abundance in the Ascaris genome, we isolated and analyzed another clone, perI5, containing an extraribosomal IVS in a chromosomal environment different from clone perI3. A subcloned BamHI-SalI fragment, including a 0.5 kb long IVS-homologous sequence, was hybridized to genomic Ascaris DNA (probe and blot D in Figure 5). The intensities of the hybridization signals for the 12.5 kb long fragments in the SalI track and the 3.0 kb long fragments in the BamHI-SalI track demonstrated that this fragment belongs to the single copy DNA of the genome (copy standards not shown).

DNA sequences from extraribosomal IVS boundaries

The cloned representatives of extraribosomal IVS copies showed a considerable variation in length and were all shorter than the ribosomal IVS analyzed first. The two extraribosomal IVS’s from clones perI3 and perI5, along with the ribosomal IVS from clone pAlr20 (14), are depicted in Figure 6A. The IVS from
Fig. 6. (A) Schematic representation of an rDNA IVS and homologous extraribosomal copies. The rDNA IVS from clone pAlr20 is 4.5 kb in length, the IVS from the clone per13 is about 3.2 kb long, and the one from per15 about 0.5 kb. (B) Nucleotide sequences covering the left- and right-hand junctions of intervening sequences with adjacent rDNA or non-rDNA regions of clones pAlr20 (ribosomal IVS), per13 and per15 (extraribosomal IVS's). All IVS-homologous sequences are framed, and only differences in the nucleotide sequence relative to the ribosomal IVS are indicated. Single base deletions are represented by the symbol (\(\cdot\)). The 15 bp long direct repeats flanking the rDNA IVS are indicated by asterisks.

per13, representing the most abundant extra-cistronic form, is about 3.2 kb long, the other one, from per15, belonging to the single copy genomic DNA, measures only about 0.5 kb.

To assemble more detailed information about their organization, we have sequenced the boundary regions of the extraribosomal IVS's of both clones. The data were compared to the corresponding sequence of the ribosomal IVS (Figure 6B). This study revealed that, with respect to the ribosomal copy, the IVS from per13 lacks 63 bp of the left border, and the one from per15 is truncated by about 520 bp at the same end. On the right hand end, the two extraribosomal copies are considerably shorter than the ribosomal IVS, because the borders lay 1.2 kb (per13) and 3.5 kb (per15), respectively, further upstream of the end of the rDNA IVS (see Figure 6B). As far as sequenced, the three IVS copies are nearly identical, and the transitions between IVS homologous
Fig. 7. Sequence comparison of the different intervening sequence boundaries in clones pAlr20, pAlr22, pAlr23 (ribosomal IVS's), perl5 and perlS (extraribosomal IVS's). Intervening sequences are framed by interrupted lines. The boxed regions represent the direct repeats flanking the ribosomal insertion in pAlr20 and its homologous locus in the uninterrupted rDNA (at top of the figure), as well as the internal copies within the IVS and the flanking repeats of the shorter ribosomal and extraribosomal forms. Sequence homologies to the rDNA direct repeats of pAlr20 are indicated by bold characters, base deletions by (.), and inserted bases are written below the main line. Sequences of pAlr22, pAlr23 and of the uninterrupted rDNA, and part of the sequences of pAlr20 are from 14.

and non-homologous adjacent sequences are sharp and unambiguous.

Imperfect direct repeats flank the extra-cistronic IVS's, which are homologous in sequence to the flanking repeats of rDNA insertions. Furthermore, the direct repeats bordering the extraribosomal copies were found at homologous positions inside the ribosomal IVS (Figure 7). A similar observation has been made for shorter forms of the ribosomal IVS (14). Sequence homologies to the direct repeats flanking the rDNA therefore seem to be scattered throughout the IVS, and we suggest that shorter IVS forms (both intra- and extraribosomal) may have arisen by recombinational events at these sites.

DISCUSSION

The rRNA genes of A. lumbricoides exist in two main organizational forms which are both transcribed (26). A third form, carrying an intervening sequence in the 26S rRNA gene, accounts for about 5% of the total genomic rDNA (14). Assuming equal transcriptional efficiency of IVS+ and IVS− genes, approximately 5% of the nuclear rRNA should contain sequences homologous to IVS's. In our hybridization experiments, however, no transcript of IVS homologous sequences could be detected. Any IVS-homologous RNA is therefore not expected to be present in more than 66 pg
per 10 μg of total RNA or 0.6 x 10^{-3} % (lowest level of detection in our hybridization experiments). This indicates that transcripts from interrupted rRNA genes, if there are any at all, are strongly underrepresented.

Alterations in the primary structure of the Pol I promoter region are not the cause of the absence of in vivo transcripts. The sequences surrounding the transcription initiation site (from positions -116 upstream to position +176 downstream of the initiation site) are strictly conserved in IVS+ rRNA genes. These sequences include the region shown to be sufficient for faithful in vitro transcription of non-interrupted rDNA repeats (26). It therefore is not surprising that transcription of IVS+ genes is correctly initiated in an Ascaris specific in vitro extract. Furthermore, the entire 8.2 kb long HindIII fragment, carrying the first half of the intervening sequence (cf. Figure 2), was transcribed without interruption in the in vitro assay (data not shown).

The absence of in vivo transcripts may be explained by one of the following two mechanisms: i) in vivo transcription, unlike in vitro transcription, is somehow repressed and does not initiate at all, or ii) initiation of transcription may occur correctly, but the resulting RNA is unstable or rapidly spliced, followed by fast degradation of the IVS-homologous RNA. Our results do not allow us to discriminate between these possibilities, but similar observations have already been made for the type I insertions of Diptera. In Drosophila, only a few transcripts of interrupted genes have been identified (28, 29), and they have been shown to be abortive and rapidly degraded (30). As in A. lumbricoides, the nucleotide sequence of the promoter region is identical in IVS+ and IVS− genes (31) and isolated fragments covering the transcription initiation site of interrupted genes are correctly initiated in vitro (32). This has led to the speculation that transcription could be repressed in vivo by a specific chromatin conformation of IVS+ genes (31). By analogy, this could also be true for the interrupted genes in A. lumbricoides. Such a mechanism would act on single genes rather than upon long blocks of rDNA repeats, because genes with and without insertions are intermingled both in D. melanogaster (3,
Another similar feature shared by the rDNA intervening sequences of *Drosophila* and *Ascaris* is the occurrence of homologous copies within non-ribosomal locations. In *D. melanogaster*, most of the extraribosomal copies are either tandemly repeated or interspersed with repetitive elements (10, 11). We have not found any evidence for tandem arrays of the *A. lumbricoides* IVS, but a high proportion of the extra-rDNA elements are located within a family of repetitive sequences. Other IVS-like elements are inserted in unique sequences of the *Ascaris* genome. Detailed analyses of two cloned extraribosomal elements showed that they are flanked by imperfect direct repeats, which are homologous in sequence to the direct repeats of the rDNA IVS. Sequences homologous to these repeats are also present enclosed within the IVS, at exactly the sites corresponding to the endpoints of the extraribosomal copies. The shorter IVS copies within the rDNA cluster of *A. lumbricoides* show the same organization (14). Interestingly, a similar observation has been made for the different length variants of the type I insertions in the rDNA of *D. melanogaster* (33). Moreover, by comparing the sequence of an extraribosomal IVS in the *D. melanogaster* genome (12) to the corresponding sequence of the rDNA IVS (7), we found a short homology to the direct repeat of the rDNA IVS. This homology was present within the IVS at the site where the homology to the extra-cistronic copy ends. In summary, we conclude that shorter forms of ribosomal and extraribosomal IVS's always end at a site closely related to the duplicated sequences which flank the rDNA IVS. The ancestral sequence may therefore be one inserted in the highly conserved region within the large rRNA gene subunit, possibly by integration of a transposable element. Shorter forms may have derived from this sequence by unequal crossing over, as postulated for *D. melanogaster* (33). In *A. lumbricoides*, this would have taken place early in evolution because hybridization of genomic DNA from single individuals revealed no differences in the distribution of intra- and extraribosomal intervening sequences (unpublished observation).

The type I insertions interrupting the large rRNA genes of different insect species and the intervening sequences of *A.*
lumbricoides show the following structural and functional analogies: 1) Intervening sequences in the rDNA are flanked by direct repeats, present only once in the uninterrupted rDNA (34, 14, 35, reviewed in 13). 2) The main size class of the IVS is between 4.5 and 6 kb, and, though to a lesser amount, different length variants are found within the rDNA cluster, always having the same 3' end (right hand) and a variable 5' end (left hand) (6, 33, 7, 34, 14). 3) About 50% of the genomic copies of the IVS are found within the rRNA genes, the other half being distributed in a semi-random manner outside of the rDNA cluster (for insects, 12, reviewed in 13). 4) The shorter copies of the rDNA IVS as well as the extraribosomal copies are flanked by the same direct repeat as the longest IVS. These shorter copies always end at a site where the IVS has some sequence homology to the direct repeat (see also 33). 5) Interrupted genes are either not transcribed at all or not efficiently transcribed in vivo, but are correctly initiated in vitro. Moreover, no transcripts of extraribosomal copies of the IVS can be found (for Drosophila, see 13 for review, 32).

However, in one aspect, the rDNA IVS of A. lumbricoides is different from the type I insertion in D. melanogaster: Its location is 34 bp further upstream (14), but still within the same region of the rRNA gene, which is highly conserved between different species. This region of the rDNA seems to be permissive for the insertion of foreign DNA, because all known IVS's of the large ribosomal gene are found therein (cf. 13). Based on the secondary structure model proposed for the 28S rRNA of Xenopus laevis (36), we found that both the Ascaris and the insect type I IVS's are inserted in a loop, immediately after the last nucleotide of a stem-structure (stem No. 56 for Drosophila, and No. 67 for Ascaris; numeration of stems after 36). Moreover, intron 2 of Physarum is located at the opposite position of the Ascaris IVS, within the loop of the same secondary structure, immediately adjacent to the first nucleotide of the complementary strand in stem 67. As a result of the different insertion sites in A. lumbricoides and in the Diptera, the respective flanking sequences are divergent. Consequently, the homologous copies of the direct repeats which are found inside the IVS of both D. melano-
gaster and A. lumbricoides differ in sequence. Therefore, a scattering of the direct repeat within the IVS seems to be a consequence of the insertion into an intact rRNA gene, rather than a requirement for insertion.

On the basis of the common functional and structural features of IVS's in insects and A. lumbricoides, we suggest an extension of the term "type I-like IVS" to this nematode intervening sequence. We believe that the basic mechanisms for insertion, inactivation and distribution of these elements within the genomes have been maintained throughout the evolutionary separation of the two classes Insecta and Nematoda. If the IVS arose by insertion of a transposon-like element into an intact rRNA gene, then this has probably occurred independently in the two cases, given the evolutionary distance between the two phyla, and the shift of the insertion site. Thus, the observed phenomenon seems to be more widespread and fundamental than originally thought. This is the first example of such a type I-like element outside the class Insecta, but it would not be surprising if similar elements, satisfying the conditions cited above, were to be found in the large rRNA coding genes of other organisms.

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