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Bombyx mori 28S ribosomal genes contain insertion elements similar to the Type I and II elements of *Drosophila melanogaster*

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We have examined the 28S ribosomal genes of the silkmoth, *Bombyx mori*, for the presence of insertion sequences. Two types of insertion sequences were found, each ~5 kb in length, which do not share sequence homology. Comparison of the nucleotide sequences of the junction regions with the uninserted gene reveals that one type of insertion has resulted in a 14 bp duplication of the 28S coding region at the insertion site. The location of this insertion and the 14 bp duplication are identical to that found in the Type I ribosomal insertion element of *Drosophila melanogaster*. The second type of insertion element is located at a site corresponding to ~75 bp upstream of the first type. The location of this insertion, the variability detected at its 5′ junction, and a short region of sequence homology at its 3′ junction suggest that it is related to the Type II element of *D. melanogaster*. This is the first example of a Type II-like rDNA insertion outside of sibling species of *D. melanogaster*, and the first example of a Type I-like rDNA insertion outside of the higher Diptera.

Key words: insertion elements/ribosomal genes/silkmoth

Introduction

The 28S ribosomal RNA genes of *Drosophila melanogaster* contain two non-homologous types of insertion elements (Dawid *et al.*, 1978; Wellauer and Dawid, 1978). 'Type I' insertions are found in ~60% of the rDNA units of the X chromosome, while 'Type II' insertions are found in ~15% of the rDNA units on both the X and Y chromosomes (Tartof and Dawid, 1976; Wellauer *et al.*, 1978; Roiha and Glover, 1980). Type II insertions are located within the 28S gene ~75 bp upstream of the site of Type I insertions (Roiha *et al.*, 1981; Dawid and Rebbert, 1981). rDNA units containing these insertions are inefficiently transcribed (Long and Dawid, 1979). While the *D. melanogaster* rDNA gene family is capable of rapid turnover (Ritossa, 1968; Coen *et al.*, 1982b; di Cicco and Glover, 1983), the organism is unable to eliminate these insertions from its rDNA repeats. It has been suggested that the elements continually reinsert themselves into the 28S genes in a manner similar to transposable elements (Roiha and Glover, 1981; Coen *et al.*, 1982a).

Several other Dipteran species contain rDNA units with insertions similar to that of *D. melanogaster*. DNA from Type I insertions has been used as a hybridization probe to demonstrate that Type I-like inserts are present in all examined sibling species of *melanogaster* (Coen *et al.*, 1982a; Roiha *et al.*, 1983). Insertion elements have also been detected in the rDNA units of a distant *Drosophila* species, *D. virilis* (Barnett and Rae, 1979). These inserts exhibit sequence homology to the Type I inserts, and are located at a position identical to that of *melanogaster* (Rae *et al.*, 1980). A fraction of the rDNA units of the sand fly, *Calliphora erythrocephala*, contain rDNA insertions (Beckingham and White, 1980). These inserts are also located at a position identical to the *Drosophila* Type I inserts, and contain sequences at their 3′ end which are homologous to the Type I insert of *D. virilis* (Smith and Beckingham, 1984). Thus, Type I inserts are believed to be widely distributed among higher Dipterans. Type II inserts, on the other hand, have only been detected in certain sibling species of *melanogaster* (Coen *et al.*, 1982a; Roiha *et al.*, 1983). Sequence information concerning the exact position of the Type II inserts is only available for *melanogaster*.

In a previous report (Lecanidou *et al.*, 1984), we have shown that two types of insertion sequences exist in a minor fraction of the rDNA repeats of the Lepidopteran, *Bombyx mori*. One insert is within the 28S coding region, and one is within the non-transcribed spacer region of the rDNA unit. Here we show that there are actually two types of insertion elements within the 28S coding region. The nucleotide sequences of the junction regions indicate that they should be classified as Type I-like and Type II-like, due to their remarkable similarities to the *D. melanogaster* insertion elements.

Results

Identification of genomic clones containing two types of 28S gene insertions

A number of genomic clones containing *B. mori* rDNA units have been isolated from a partial *Eco*RI charon 4 library (Eickbush and Kafatos, 1982). The major rDNA repeat in *B. mori* is 10.6 kb in length, with a single *Eco*RI restriction site near the 3′ terminus of the 18S coding region (Manning *et al.*, 1978). Thirty-six of these clones were examined for the presence of interrupted rDNA units by screening for the presence of *Eco*RI fragments other than 10.6 kb. Of this set, thirty clones contained the predominant uninserted rDNA units. The restriction maps of four of the six clones that contain DNA insertions are shown in Figure 1 in comparison to the uninterrupted rDNA unit, B108. The insertions in the two clones not shown are within the non-transcribed spacer region of the rDNA repeat near the 3′ end of the 28S gene and are not discussed further in this report.

Clone B108, containing an uninterrupted repeat, begins and ends at the *Eco*RI site within consecutive 18S genes. Below B108 are three examples of genomic clones containing one type of insertion element within the 28S coding region. B131 contains the entire insertion, which is 5.0 kb in length, while B98 and B74 end at an *Eco*RI site within the insertion element. The restriction maps of the insertion elements from these three clones are identical over the regions that can be compared. The left portion of B74 is a typical uninserted rDNA repeat, while the left portion of B98 contains a second DNA insertion near the 3′ end of the 28S gene. Although the numbers are not large, the ease with which these two clones were found suggests that repeats with this type of 28S insertion are interspersed along the chromosome with uninserted repeats and with repeats containing inser-

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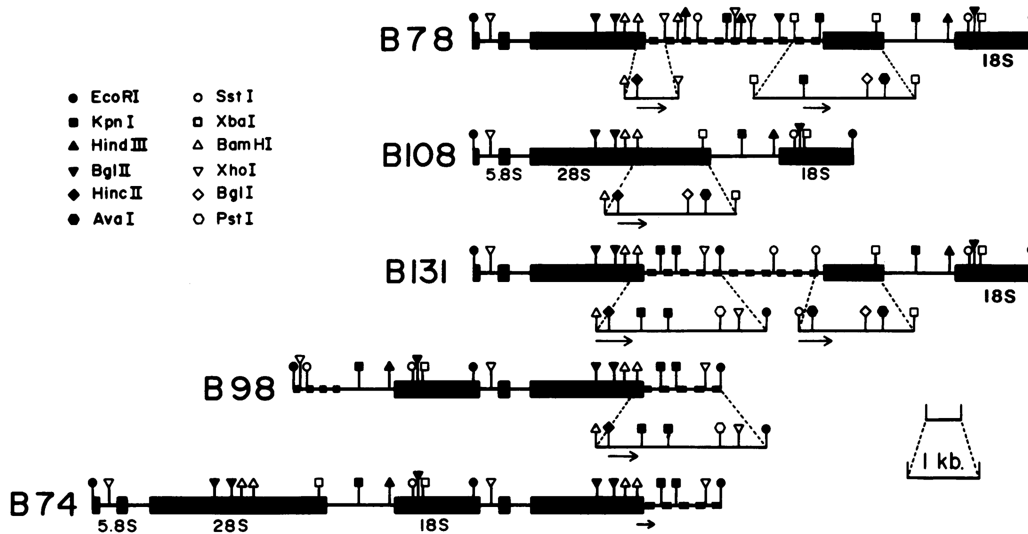

Fig. 1. Restriction maps comparing *B. mori* ribosomal repeats with and without DNA insertions. Each clone is drawn in a 5′ to 3′ direction with respect to the rRNA transcripts. All clones contain fragments of a partial *Eco*RI digestion of silkmoth DNA cloned into the vector charon 4 (Eickbush and Kafatos, 1982). Thickened (boxed) regions correspond to the 18S, 5.8S and 28S RNA coding regions. The exact 5′ and 3′ ends of the primary transcript are not known. *B. mori* 28S rRNA, as in all insect species examined (Shine and Dalgarno, 1973), undergoes an additional cleavage dividing the molecule into fragments termed γ and β. The location of this cleavage site near the middle of the 28S gene is not presented in the figure. Insertion sequences are indicated by a horizontal line with small boxes. Regions of the genomic clones containing the insertion junctions (expanded regions below the charon 4 clones) were subcloned onto plasmid vectors, and restriction-mapped with a variety of additional enzymes (only *Ava*I, *Pst*I, *Hinc*II, and *Bgl*I are shown) to define further the sites of insertion. Arrows below the maps correspond to the direction and extent of the sequence analysis.

```
                                25                        50                        75                        100
                                 *                         *                         *                         *
B108   GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCA
B78    GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTAAGGTAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCA

                                125          138
                                 *            *
B108   TGAATGGATTAACGAGATTCCCACTGTCCCTATCTACT
B78    TGAATGGATTAACGAGATTCCCACTGTCCCTATCTACTTGACTTCGCCGTCGGCCTTGGTCGAGGACAGACGTGCGTTCCGTTATTTCTTTATTTTCCGT

B78    CATTTAAGTGTATTGTGTTTCTATTGGTGTATCGGACCCTCTCGTTTCGGCTTGAGGTTTAAGTCATAAGACGCCGCGGCCATCTTGCTGTGTGAGCGGT

B78    GTGACGAGTGCGAAGGCGGAGTTTAGCTCGACGTGGAGTCGGCCCCTCTCGCTTCCTCTTGGGTGCCGGTCCATATAGGTCGGTGTCCATATTGGATTGC

B78    GTGTGAGACGGCCGATTTGCGTGAGGGCGGACCCATTTAGGTCTCGTGACAGTGACACTAGTGTGCGATCAGTGACGTTTTATAATTTGCTG........

B78    .........APPROXIMATELY 4.5 KB.................GGTACCTTGGTGCCGTGAAGTTCATGCTTCGGTCCTAATAACCGCAAGGTTGG

                                                          150                       175                       200
                                                           *                         *                         *
B108                                         ATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGGAGAATCAGCGGGGAAAGAAGACCCTG
B78    TGGGACCATGGGAGGTGGTGGGAATGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGGAGAATCAGCGGGGAAAGAAGACCCTG

                                225                       250                       275                       300
                                 *                         *                         *                         *
B108   TTGAGCTTGACTCTAGTCTGGCATTGTAAGGAGACATGAGAGGTGTAGCATAAGTGGGAGATCGTTTCGCGCGATCGTCGCTGAAAAACCACTACTTTCA
B78    TTGAGCTTGACTCTAGTCTGGCATTGTAAGGAGACATGAGAGGTGTAGCATAAGTGGGAGATCGTTTCGCGCGATCGTCGCTGAAAAACCACTACTTTCA

                                325                       350                       375                       400
                                 *                         *                         *                         *
B108   TTGTTTCATTACTTACTCGGTTGGGCGGACACGGTGCGCGTCGATAATATCGGCGGGCGCACGGTGTTTCGTTCCAAGCGTGCAGAGTGGTGACGTGGCG
B78    TTGTTTCATTACTTACTCGGTTGGGCGGACACGGTGCGCGTCGATAATATCGGCGGGCGCACGGTGTTTCGTTCCAAGCGTGCAGAGTGGTGACGTGGCG
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Fig. 2. Nucleotide sequences flanking the junction sites for the B78 insertion. The uninserted rDNA sequence from B108 is presented as the top line, and the B78 sequence is shown immediately below. Nucleotides are numbered from the *Hinc*II site used in the sequence determination. Nucleotides within the insertion element or the 14 bp duplication are not numbered. Underlined nucleotides correspond to duplications of the coding region in the insertion. Regions of the insertion represented by dots have not been sequenced.

tions within their non-transcribed spacer.

The rDNA repeat represented by B78 contains a DNA insertion with a length and position similar to that in B131. However, the restriction map of this insertion is entirely different from that in B131, indicating that it represents a second class of insertion elements in the 28S coding region. B78 is our only example of this type of insertion element. No *Eco*RI cleavage sites are present within the insertion in B78; therefore, the rDNA units containing this insertion are located on 16.6 kb fragments after *Eco*RI digestion of genomic DNA. In our previous report (Lecanidou *et al.*, 1984) a 16–17 kb *Eco*RI fragment had been detected in genomic blots (Figure 3, lane B), but no corresponding genomic clone had been isolated. rDNA units of the type found in B78 will also generate the 9.0 kb *Hind*III fragment seen in genomic blots, but not previously isolated as part of a genomic clone (Lecanidou *et al.*, 1984; Figure 3, Lane C). All the predominant restriction fragments detected by genomic blots are now represented by genomic clones, thus all abundant, large insertion elements within the rDNA repeats of *B. mori* have been identified.

```
                                   25                       50        60
                                   *                        *         *
B108  GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTAAGG
B98   GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTA                         GGGGCGATACGCAT
B74   GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTAA                        --------------
B131  GTCAACGTGAAGAAATTCAAGCAAGCGCGGGTAAACGGCGGGAGTAACTATGACTCTCTTAAGCGGGAGTAACTATGACTCTCTTA--------------

B98   AATTTTAATTTTTCGATTCAAATCCAGTCGTCTTAATCTGGTGACCAGTGGCGCGGTCACCAGTATAGTGCACAGGACGTGAATGGCTCCGAGGCTGGCG
B74   -----------CC-----G---------------------------------------------------------------------------------
B131  ----------------------------------------------------------------------------------------------------

B98   GAGTCACTCACTATAAGTGTGAGAGACGATGTCCTGTGCCAAGTATACGTCCAACCCTAACGGGTTAAGTGAAATTAGTTGCTCATAACAGGGACGGTGT
B74   ----------------------------------------------------------------------------------------------------
B131  ---------------------------------------------------------------------------------------------.......

B98   ACCTGTTTGCTCGTGGCTGGCTATCGAATGGACGGGACCAATACACCCCCCTGTTAGTAATGGGGTAAGAGAGAGCGGTCTGAAACTATGGCCGAGATCA
B74   ----------------------------------------------------------------------------------------------------
B131  ....................................................................................................

B98   CGACGCCCCACTCCTACCCATAACCTG
B74   ---........................
B131  ................................APPROXIMATELY 4.4 KB.......................GAGCTCGCTCCCTTGGCTTCCCTTAT

B131  ATATTTTAACATCAGAAACAGACATTAAACATCTACTGATCCAATTTCGCCGGCGTACGGCCACGATCGGGAGGGTGGGAATCTCGGGGGTCTTCCGATC

                                                                           75                       100
                                                                           *                        *
B108                                                                  TAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCA
B131  CTAATCCATGATGATTACGACCTGAGTCACTAAAGACGATGGCATGATGATCCGGCGATGAAAATAGCCAAATGCCTCGTCATCTAATTAGTGACGCGCA

                                   125                      150                      175                      200
                                   *                        *                        *                        *
B108  TGAATGGATTAACGAGATTCCCACTGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGGAGAATCAGCGGGGAAAGAAGACCCTG
B131  TGAATGGATTAACGAGATTCCCACTGTCCCTATCTACTATCTAGCGAAACCACAGCCAAGGGAACGGGCTTGGGAGAATCAGCGGGGAAAGAAGACCCTG

                                   225                      250                      275                      300
                                   *                        *                        *                        *
B108  TTGAGCTTGACTCTAGTCTGGCATTGTAAGGAGACATGAGAGGTGTAGCATAAGTGGGAGATCGTTTCGCGCGATCGTCGCTGAAAAACCACTACTTTCA
B131  TTGAGCTTGACTCTAGTCTGGCATTGTAAGGAGACATGAGAGGTGTAGCATAAGTGGGAGATCGTTTCGCGCGATCGTCGCTGAAAAACCACTACTTTCA

                                   325                      350                      375                      400
                                   *                        *                        *                        *
B108  TTGTTTCATTACTTACTCGGTTGGGCGGACACGGTGCGCGTCGATAATATCGGCGGGCGCACGGTGTTTCGTTCCAAGCGTGCAGAGTGGTGACGTGGCG
B131  TTGTTTCATTACTTACTCGGTTGGGCGGACACGGTGCGCGTCGATAATATCGGCGGGCGCACGGTGTTTCGTTCCAAGCGTGCAGAGTGGT.........
```

Fig. 3. Nucleotide sequences flanking the junction sites for B131, B98 and B74. As in Figure 2, the uninserted rDNA sequence from B108 is presented as the top line with nucleotides numbered from the *Hinc*II site. The various insertion sequences are shown below the B108 sequence. Underlined nucleotides in B131 correspond to a 23 bp duplication of the coding region in the insert. Nucleotides missing from certain repeats are indicated by blank spaces. Regions in the B74 and B131 insertions represented by dashed lines agree with the B98 sequence except where indicated. Regions represented by dots have not been sequenced.

Sequence analysis of the insertion junctions

The two types of inserts detected in the 28S coding region of *B. mori* are in the same general location as the insertion elements characterized in *D. melanogaster* (Roiha *et al.*, 1981; Dawid and Rebbert, 1981). To determine the exact location of the insertions in *B. mori*, and what effect these inserts may have on the coding sequences themselves, we determined the nucleotide sequences of the insertion element/28S coding junctions. The junction regions of B78, B131, and B98, and the corresponding uninserted region of B108, were first subcloned (expanded regions below the genomic clones in Figure 1) and restriction mapped in greater detail to define the junction sites more narrowly. The junctions were sequenced by the strategy shown in Figure 1. The insertion region from B74 was not subcloned, but placed directly into a M13 sequencing vector (Vieira and Messing, 1982) from the charon 4 clone.

Figure 2 presents the sequence of the junction regions from B78 in comparison to the uninserted region from B108. The insertion is located 138 bp downstream of the *Hinc*II site used in the sequence analysis, and is flanked by a 14 bp target site duplication (underlined nucleotides). The 28S coding region of the interrupted gene in B78 is identical with the uninterrupted gene (B108) over the 400 bp that have been sequenced.

Figure 3 presents the sequences of the junction regions from clones B131, B98 and B74, along with the corresponding uninserted regions from B108. Based on the sequences at the 5′ junction of the insertions, all three examples of this insert interrupt the coding region approximately 75 bp upstream of the site of B78 insertions. Each example of this type of insertion, however, has a somewhat different sequence at its 5′ border with the coding region. B98 is missing the A at position 62 while this base is found in B74 and B131, and B131 has a 24-bp duplication of the region immediately preceding the insert (bases 38 – 61). The exact border of the insertion is actually somewhat ambiguous. The sequences in Figure 3 were aligned by assuming that all deletions or duplications have occurred at the precise coding/insert junctions. It is possible that the two G residues shown as the first two bases of the insert may actually correspond to bases 63 and 64 of the coding region. In this arrangement, which has been used in the comparison with the *D. melanogaster* sequences in Figure 4, the deletion in B98 and the duplication in B131 have occurred two bases 5′ of the insertion site. The insertion sequences themselves are almost identical. B74 contains three nucleotide differences from B98 and B131, located ~30 bp downstream of the insertion site. The 3′ junction of the insert is only present in B131. The sequence of this junction reveals that no significant deletion or target site duplication is present. As in the case of B78, the 28S coding regions of the interrupted genes are identical with the uninterrupted gene over the regions sequenced.

Discussion

The region of the 28S rRNA gene containing the two types of insertion elements described in this report is highly conserved

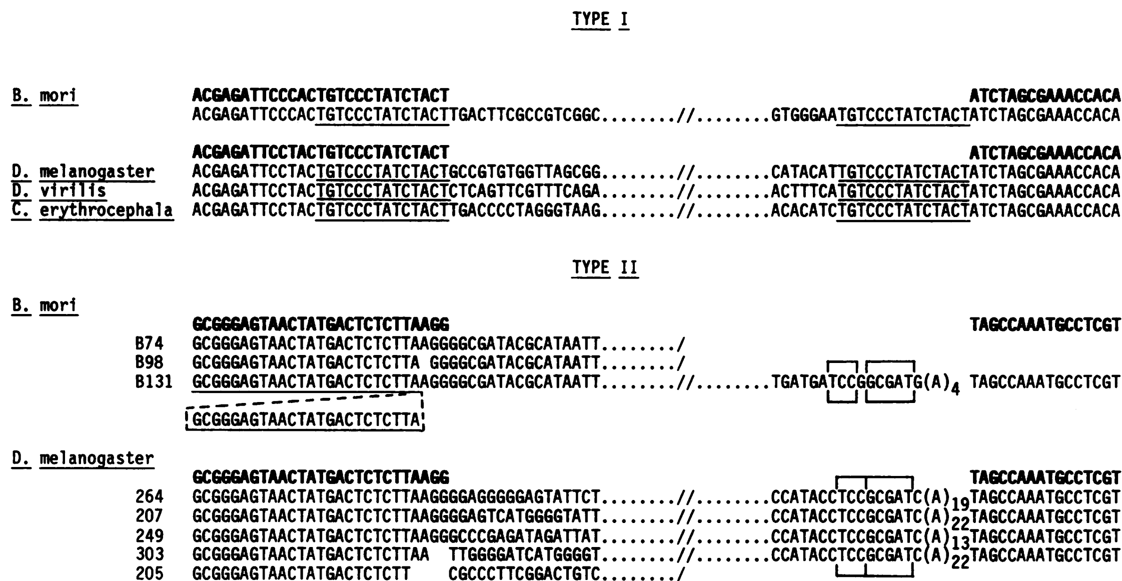

Fig. 4. Comparison of the junction sequences of *B. mori* with the Type I and II inserts of Diptera. The uninserted 28S coding sequence for each comparison is the top line in all cases and is presented in bold type. This sequence in the Type I comparison is identical in the three Dipteran species and is only presented once. Sequence data from other species are those of: *D. melanogaster*, Type I (Dawid and Rebbert, 1981; clone e52); Type II (Dawid and Rebbert, 1981; Roiha *et al.*, 1981, clones used are numbered at left); *D. virilis* (Rae, 1981); and *Calliphora erythrocephala* (Smith and Beckingham, 1984). Gene regions duplicated in the insertions are underlined. Dots indicate regions of variable length not shown. Double slashes; both junctions of the insert are present in the clone and have been sequenced. Single slashes; only one junction sequence is present in the clone. Multiple A residues at the 3′ border of the Type II inserts are represented by subscripts. Spaces correspond to coding sequences missing from the insert junctions. The 24 additional nucleotides present in B131 are displaced and positioned below the flanking gene region to emphasize that these sequences represent an exact duplication of the gene region. Boxed nucleotides at the 3′ border of the Type II sequence corresponds to identical sequences between *B. mori* and *D. melanogaster* insertion sequences.

between *B. mori* and several Dipteran species. For example, only one nucleotide difference is found between *B. mori* and *D. melanogaster* in the 140 bp immediately surrounding the two insertion sites (nucleotides 34 – 173 in Figures 2 and 3). Nucleotide 122 is a C in *B. mori* and a T in *D. melanogaster*. This high sequence homology surrounding the insertion sites enables one to align unambiguously the sequences between species. As shown in Figure 4, the position of the B78 insertion and the 14 bp target site duplication in *B. mori* are identical to those reported for a type I insert in *D. melanogaster* (Dawid and Rebbert, 1981), *D. virilis* (Rae *et al.*, 1980), and *C. erythrocephala* (Smith and Beckingham, 1984). It should be noted that the sequence presented for *D. melanogaster* is representative of only a fraction of the Type I insertions (Dawid and Rebbert, 1981). In the major 5.0 kb Type I variant of *D. melanogaster* (not shown), the 14 bp duplication and an additional 9 bp upstream are absent from the 5′ side of the insertion (Roiha *et al.*, 1981; Rae, 1981).

No sequence homology can be found by computer assisted matrix analysis (Pustell and Kafatos, 1982) between the sequenced regions of the inserts in B78 and the identical regions in the three Dipteran species. We also cannot detect cross-hybridization between several internal segments of the Type I insert DNA of *D. melanogaster* and B78 (data not shown). This lack of sequence homology between *B. mori* and Dipteran Type I sequences is consistent with the findings of Barnett and Rae (1979), that the Type I insert of *D. virilis* does not cross-hybridize to *B. mori* genomic DNA.

Figure 4 also shows an alignment of the DNA insertions in B74, B98 and B131 with the Type II insertions of *D. melanogaster*. Several key features of these *B. mori* insertions are similar to the Type II inserts of *D. melanogaster*. First, as defined by their 3′ junctions, the locations of the insertions within the coding region are identical. Second, in both species the 5′ junctions are less precisely defined. Third, a short region of sequence homology exists at the 3′ border of the insert. This homology includes a short run of As at the extreme end, and a 5 bp identity upstream of these As which can be extended another 3 bp by a single base deletion in *B. mori* (boxed bases). No additional sequence homology can be found beyond this short region.

We conclude that a fraction of the *B. mori* 28S ribosomal genes contain inserts which bear remarkable similarity to the Type I and II inserts detected in *D. melanogaster*. While species specific differences exist, the exact identity of the insertion sites, the effects these insertions have had on the coding sequences at these sites, and in the case of the Type II inserts, sequence homology at the 3′ junction in one of these inserts, suggest a common origin for the insertion elements in these two species. Unless there has been a transfer of sequence information between species, both Type I and II inserts entered the class Insecta before divergence of the Dipteran and Lepidopteran orders, and may account for many of the ribosomal insertion sequences detected in certain insect species but not yet characterized (reviewed in Beckingham, 1982).

The ability of the *D. melanogaster* insertion elements to remain within a fraction of the 28S genes has been suggested to be a function of their continual reinsertion into the rDNA repeats (Roiha and Glover, 1981; Coen *et al.*, 1982a). Such a model for their preservation would suggest that they be classified as transposable elements or retroviruses which are sequence-specific in their sites of insertion. Type I elements are found only within rDNA repeats and within small segments of the 28S gene at the chromacentral heterochromatin (Kidd and Glover, 1980; Roiha *et al.*, 1981). Type II elements have only been reported within rDNA repeats. Our discovery of similar insertion elements in *B. mori* suggests that the mechanism and the sequence specifici-

ty of the insertion events have been maintained even though the nucleotide sequence of the elements has substantially changed. We are currently investigating the distribution of the *B. mori* insertions outside of the rDNA repeats and are attempting to identify regions within the insertions that exhibit weak sequence homology with the *D. melanogaster* elements.

Materials and methods

All genomic clones were obtained from a charon 4 library of partial *Eco*RI digested fragments of *B. mori* DNA (Eickbush and Kafatos, 1982). Regions surrounding the insertion junctions were subcloned into the vector pUC13. Based upon detailed restriction maps of these subclones, double-digested restriction fragments were cloned into the M13 vectors mp18 and mp19 (Messing and Vieira, 1982), and the fragments sequenced by the dideoxy chain termination technique (Sanger *et al.*, 1977). The general sequencing strategy for each junction site is shown in Figure 1.

Acknowledgments

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