U6 RNA is an abundant, capped small nuclear RNA (snRNA) associated with hnRNP particles (Reddy, R., and Busch, H. (1983) Prog. Nucleic Acid Res. Mol. Biol. 30, 127–162). Small nuclear ribonucleoprotein particles containing U4 and U6 RNAs are required components for splicing particles containing U4 and U6 RNAs (Berget and Robberson, 1986; Black and Steitz, 1986). In this study the Drosophila U6 RNA genes have been isolated and characterized. The Drosophila genome contains three U6 snRNA genes which are clustered in a 2-kilobase-pairs long DNA fragment. The U6 RNA coding regions are 100% homologous in all three genes, but the flanking sequences diverged significantly from each other. A possible secondary structure model for the Drosophila U4/U6 RNA complex is presented.

Consistent with our previous observation that U6 RNA is a RNA polymerase III product (Reddy, R., Henning, D., Das, G., Harless, M., and Wright, D. (1987) J. Biol. Chem. 262, 75–81), all three genes contain a region homologous to the conserved intragenic regulatory region and a cluster of T residues on the 3'-end, characteristic of genes transcribed by RNA polymerase III. A TATA box was found between nucleotides -23 and -31, and a stretch of 28 nucleotides from -43 to -71 was conserved in the 5' flanking region of all three U6 RNA genes. The Drosophila U6 RNA genes were transcribed in vitro by Drosophila nuclear extracts but were not transcribed by Novikoff hepatoma or HeLa cell extracts. Similarly, a mouse U6 RNA gene was transcribed in Novikoff hepatoma or HeLa cell extracts but not in Drosophila nuclear extracts. These results suggest that species-specific factor(s) are involved in the transcription of U6 snRNA genes.

U6 RNA is one of the six abundant, capped small nuclear RNAs present in all eukaryotic cells examined. It has been characterized from human, rodent, amphibian, and insect cells (Zieve and Pennman, 1976; Letterer et al., 1995; Epstein et al., 1996; Harada et al., 1990; Sri-Widada et al., 1993; Ireland et al., 1993; Reddy and Busch, 1993). In human and rat cells, U4 RNA and U6 RNA are present in 1:1 ratio to form part of a single small nuclear ribonucleoprotein particle as a result of intermolecular base pairing (Hashimoto and Steitz, 1984; Bringmann et al., 1994). In yeast, U4 and U6 RNAs appear to be present in a single contiguous transcript (Tollervey et al., 1983). U6 RNA was found to be associated with hnRNP particles and was proposed to be involved in packaging hnRNP particles (Zieve and Pennman, 1981); U4/U6 RNPs assemble as part of spliceosomes in vitro (Grabowski and Sharp, 1986). Recently, it was shown that U4/U6 RNP complex is required for the splicing of pre-mRNAs (Berget and Robberson, 1986; Black and Steitz, 1986).

While many studies have focused on the genes coding for m3G cap-containing U1 to U5 RNAs (Roop et al., 1981; Murphy et al., 1982; Marzluff et al., 1983; Mattaj and Zeller, 1983; Stroke and Weiner, 1985), very few studies are available on genes coding for U6 RNA, which contains a different cap structure (Epstein et al., 1980). Oshshima et al., (1981) have characterized several pseudogenes and one true gene for U6 RNA from the mouse genome. The conserved promoter sequences in the 5'-flanking region of most U-snRNA genes were absent from the 5'-flanking region of the mouse U6 RNA gene (Oshshima et al., 1981). Whereas RNA polymerase II was found to be involved in the synthesis of the U1 to U5 snRNAs (reviewed in Busch et al., 1982), U6 snRNA is synthesized by RNA polymerase III (Reddy et al., 1986; Kunkel et al., 1986). The number of U6 RNA genes in Drosophila has been estimated to be between one and three and these genes have been localized to a single cytological region, 96A (Saluzz et al., 1983).

The present study forms part of our attempt to elucidate the sequences and factors involved in the synthesis of U6 RNA. The results showed that U6 RNA genes are clustered in Drosophila genome and species-specific transcription factor(s) are involved in the synthesis of U6 RNA.

MATERIALS AND METHODS

Reagents—Enzymes RNA ligase and polynucleotide kinase were obtained from Pharmacia P-L Biochemicals; DNA ligase was obtained from International Biotechnologies, Inc., New Haven, CT; restriction enzymes were obtained from New England Biolabs; radioisotopes were from New England Nuclear, Amersham Corp., or ICN, Irvine, CA. Vectors pSP-65 and pUC-12 were purchased from Promega Biotec, Madison, WI and Bethesda Research Laboratories, respectively.

Novikoff hepatoma U6 RNA was prepared as described earlier (Reddy et al., 1981) and the RNA was 3'-end-labeled with [32P]pCp by RNA ligase (Englund et al., 1980) and used for hybridizations. The specific radioactivity of the RNA was about 1 X 10^6 counts/µg RNA. For the Southern blot analysis of Drosophila genomic DNA and ADU6 cloned DNA, a nick-translated 20-base pair DNA fragment containing part of U6 gene from pD61-2 or a 3'-end-labeled U6 RNA was used as probe.

Isolation and Sequencing of Clones Containing U6 RNA Sequences—A Drosophila genomic library in λ phage EMBL 4 was

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) J06963.1.

1 The abbreviations used are: RNP, ribonucleoprotein; snRNA, small nuclear RNA; SDS, sodium dodecyl sulfate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; kb, kilobase pairs.

Structure, Organization, and Transcription of Drosophila U6 Small Nuclear RNA Genes*
Transcription of Drosophila U6 Small Nuclear RNA Genes

screened by the method of Benton and Davis (1977). The genomic library, constructed by inserting \textit{Drosophila melanogaster} Canton S DNA, partially digested with Sau3A endonuclease, in the BamHI site of the \textit{AEML} 4 vector, was kindly provided by Dr. Vincent Pirrotta, Cell Biology Department, Baylor College of Medicine, Houston, TX. Hybridizations were done in 50% formamide, 80 mM Tris (pH 7.5), 600 mM NaCl, 4 mM EDTA, 1.5 \times \text{Denhardt's reagent}, 0.1% SDS, and 5 \mu{g}/ml of yeast tRNA. Whenever RNA was used as a probe, instead of tRNA, 0.1 mg/ml unlabeled sonicated calf thymus DNA was used during prehybridizations and hybridizations. The nick-translucose filters were hybridized for 24 h either at 42 or 25 °C, washed in 3 \times \text{SSC} and 1.5 \times \text{SSC} containing 0.1% SDS for 5 min at 42 °C, air dried, and autoradiographed using XAR-5 film and Du Pont Lightning Plus screens at -70 °C. 5' end labeling using polynucleotide kinase was according to Maxam and Gilbert (1980), and sequencing was carried out according to the modified Maxam and Gilbert protocol (Benci et al., 1984; Rubin and Schmid, 1980).

Dot Hybridizations—DNA dot hybridizations were carried out as described by Kafatos et al. (1979). The 32P-labeled Novikoff hepatoma whole cell 4-8s RNAs that hybridized to XDU6, pDU6-1, pDU6-2, and pDU6-3 clones, were eluted into water at 100 °C for 5 min, precipitated in the presence of carrier yeast tRNA, and electrophoresed on polyacrylamide gels as described earlier (Reddy et al., 1981).

Transcription of U6 Genes in Vitro—Cell-free nuclear extracts, prepared from \textit{Drosophila} Kc0 cells (Parker and Topol, 1984), were kindly provided by Dr. Carl S. Parker, Division of Chemistry, California Institute of Technology, Pasadena, CA. Cell-free extracts from Novikoff hepatoma cells and HeLa cells were prepared according to Manley et al. (1980).

Plasmid DNAs (1 pmol) containing U6 RNA genes were incubated at either 25 °C with \textit{Drosophila} nuclear extract or at 30 °C with Novikoff hepatoma or HeLa cell extracts for 1 h. The reaction mixtures (final volume 25 \mu{l}) contained 30% (v/v) cell extract, 0.5 mM each ATP, UTP, and CTP, 0.1 mM GTP, and 20 \mu{Ci} of [\text{w-32P}]GTP (3000 Ci/mmol) 16 mM creatine phosphate, 5 mM MgCl2, 100 mM KCl, 30 mM HEPES-KOH (pH 8.0). Reactions were initiated by the addition of cell extract to an otherwise complete reaction mixture..

The reactions were terminated by the addition of an equal volume of a solution containing 0.3% SDS, 0.14 M sodium chloride, and 50 mM sodium acetate (pH 5.0), followed by phenol extraction and precipitation with ethanol. The RNA samples were analyzed on 10% polyacrylamide gels followed by autoradiography.

Analysis of the U6 RNA Transcripts—The labeled RNA transcripts were localized on the gel by autoradiography and U6 RNA was identified with reference to labeled Novikoff hepatoma total 4-8 S RNA. The RNAs were eluted from the polyacrylamide gel slices and were digested with T1-RNase and fingerprinted as described by Brownlee et al. (1968).

RESULTS

Isolation of U6 Gene Containing Recombinant Phages—\textit{Drosophila} genomic library was screened with 3' end-labeled rat U6 RNA genes. 6 pg each of \textit{Drosophila} genomic DNA for U6 RNA genes. 6 pg each of \textit{Drosophila} genomic DNA was digested with different restriction endonucleases and fractionated on a 1% agarose gel, transferred to nitrocellulose paper and hybridized. The probe used was a nick-translated DNA fragment, obtained by digesting pDU6-2 with MnlI and HaeIII restriction enzymes and corresponds to -10 to 59 nucleotides of U6-2 DNA (see Fig. 3). The hybridization was carried out at 42 °C for 16 h, washed with 3 \times \text{SSC}, and 1.5 \times \text{SSC} containing 0.1% SDS, and subjected to autoradiography. The restriction enzymes used to digest the \textit{LDU6} DNA are indicated on top of each lane. The numbers on the sides are molecular weight markers expressed in kilobase pairs.

The \textit{Drosophila} Genome Contains Three U6 RNA Genes—Southern hybridization of \textit{Drosophila} genomic DNA, using conditions and probe identical to those employed in analyzing \textit{LDU6} DNA, showed that the size and number of DNA fragments were identical to those observed with \textit{LDU6} DNA (compare Fig. 1B with Fig. 1A). For example, EcoRI enzyme yielded three bands of 0.6 kb, 1.3 kb, and 4 kb with genomic DNA (Fig. 1B, lane 1) and the same size bands were observed in \textit{LDU6} clone (Fig. 1A, lane 1). Similarly, BamHI, HindIII, and BamHI + PstI yielded a single 4.5-kb, 9-kb, and 4-kb band, respectively, with \textit{Drosophila} genomic DNA (Fig. 1B, lanes 2, 3, and 4). The same results were obtained when \textit{LDU6} clone was analyzed with these restriction enzymes (Fig. 1A, lanes 2, 3, and 4). These results indicate that all the U6 genes present in \textit{Drosophila} are present in the \textit{LDU6} clone.

Subcloning, Mapping, and Sequencing of Three U6 RNA Genes—Three EcoRI fragments 4 kb, 1.3 kb, and 0.6 kb in size, which hybridized to U6 RNA (Fig. 1A), were subcloned. The 4-kb fragment subcloned in pSP65 was designated pDU6-1; the 0.6-kb fragment and 1.3-kb fragment subcloned in pUC-12 were designated pDU6-2 and pDU6-3, respectively. Sequencing the subclones revealed each EcoRI fragment contained one U6 RNA gene. Transcription and sequence analysis showed that a trRNA gene is present in pDU6-1 clone.
besides the U6 RNA gene (Das et al., 1986). By extensive restriction nuclease mapping of XDUG as well as the three subclones separately, the orientation of the three U6 RNA genes was determined. The organization as well as the orientation and the strategy used to sequence the DNA are shown in Fig. 2.

Characterization of Drosophila U6 RNA Genes—The nucleotide sequence corresponding to the U6 RNA and the flanking regions were determined for the three U6 RNA genes (Fig. 3). The sequence of DNA corresponding to the U6 RNA is shown as nucleotides 1-107. The coding regions of all three Drosophila U6 RNA genes were identical to each other. The mouse U6 RNA sequence (Fig. 3) has only five differences from the Drosophila U6 DNA. Comparison of the flanking regions showed that the nucleotide sequences in the flanking regions diverged significantly between the three Drosophila U6 genes as well as between Drosophila and the mouse U6 RNA gene. However, some regions of homology could be found in the 5'-flanking sequences of the three Drosophila U6 RNA genes. There were three nucleotides (TTG) conserved on the 3' -side past the coding region and only two nucleotides, (TC), common before the initiation nucleotide. When U6-1, U6-2, and U6-3 genes were compared, the 5'-flanking region from -43 to -71 was 60% homologous; the 5'-portion of this conserved region, corresponding to nucleotides -59 to -71, was 92% homologous. A TATA box-like sequence was found in the 5'-flanking region of all three Drosophila U6 RNA genes and the mouse U6 RNA gene. These TATA-like boxes were found between -23 and -31 nucleotides (Fig. 3). DU6-1 contained TATATATAG sequence at -31 to -23; DU6-2 contained TTTATATAG at -30 to -22; DU6-3 contained TATATATAG sequence at -30 to -22; and mouse U6 RNA gene contained TATAATAAT sequence at -31 to -22. These results show that the DNA sequence corresponding to U6 RNA is conserved in Drosophila U6 RNA genes but the flanking regions appear to be divergent, except for some conserved upstream sequences (Fig. 3).

Drosophila U6 RNA Genes Are Transcribed in Vitro by Drosophila Nuclear Extracts—Recently we have shown that RNA polymerase III is involved in the synthesis of U6 RNA (Reddy et al., 1983). Fig. 4 shows the in vitro transcription of U6 RNA genes using different extracts. The transcripts were analyzed on a 10% acrylamide, 7 M urea gel. The Drosophila U6 gene (DU6-2) was not transcribed in Novikoff hepatoma cell extracts or in HeLa cell extracts (Fig. 4, lanes 7 and 8). Both DU6-2 and DU6-3 genes were transcribed in Drosophila nuclear extract (Fig. 4, lanes 5 and 6). The predominant transcript (Fig. 4, lane 2) of a tRNA<sup>**</sup> gene, present in the pDU6-1 clone (besides the DU6-1 gene; see Fig. 2 and Das et al., 1986), made the direct analysis of DU6-1 transcript difficult. However, pDU6-3 clone hybridized to the U6 RNA transcript synthesized from pDU6-1 clone by Drosophila nuclear extract (Fig. 4, lane 14), but not to the transcripts synthesized by Novikoff hepatoma cell extracts (Fig. 4, lane 15). These results indicate that DU6-1 gene is transcribed in vitro but is masked by the abundant precursor tRNA<sup>**</sup> transcript.

The mouse U6 RNA gene was transcribed both by Novikoff hepatoma and HeLa cell extracts (Fig. 4, lanes 10 and 11) but not by Drosophila nuclear extract (Fig. 4, lane 9). The tRNA<sup>**</sup> gene present in the pDU6-1 clone was transcribed and processed by Drosophila, Novikoff hepatoma, and HeLa extracts (Fig. 4, lanes 2, 3, and 4). Thus, U6 RNA genes require a homologous or nearly homologous extract to transcribe while the tRNA<sup>**</sup> gene can be transcribed in heterologous transcription systems.

Characterization of Drosophila U6 RNA Transcripts Synthesized in Vitro—The U6 RNA synthesized from DU6-2 and DU6-3 clones was fingerprinted after digestion with T<sub>r</sub>-RNase. Fig. 5 shows the fingerprint of Drosophila U6 RNA synthesized from DU6-2 gene in vitro. The transcript of DU6-3 gene gave the identical fingerprint (data not shown). The oligonucleotide pattern obtained was completely consistent with the Drosophila U6 gene sequence (see Fig. 3). The T<sub>r</sub>-RNase oligonucleotides were numbered according to Epstein et al. (1980) used for rat U6 RNA. The oligonucleotides 1-9, 11, 13, 14, 15, and 16 were the same as in rat U6 RNA. Oligonucleotides 10, 17, and 20 had altered mobilities because of nucleotide changes in this region of the Drosophila U6 RNA gene. Oligonucleotide T-18 was a nonanucleotide in rat U6 RNA but was represented as a pentanucleotide AUUAG and tetranucleotide CAUG because of the absence of post-transcriptional methylation of sugar moieties in vitro. The oligonucleotide 19' may correspond to the 3'-end of the U6 RNA synthesized in vitro. Since the nucleotide sequence of Drosophila U6 RNA is not known, it was not possible to determine the accuracy of termination in vitro. However, the U6 RNA synthesized in vitro initiated properly with pppG at nucleotide 1, and this pppG was converted in vitro into XpppG cap structure (oligonucleotide 5, Fig. 5).

These data showed that U6 RNA transcribed from Drosophila U6 genes was properly initiated resulting in an RNA of the right size. (Figs. 4 and 5). The 4 S-sized RNA, as well as the larger transcripts coming from pDU6-1 clone (Fig. 4, lanes 2-4) were fingerprinted and were found to be structurally related (results not shown). Because of the presence of these predominant tRNA<sup>**</sup> transcripts, the U6 RNA transcript from pDU6-1 could not be analyzed by fingerprinting.

U4 Small Nuclear RNA Genes Are Not Immediately Adjacent to the U6 RNA Genes—The XDUG DNA and pDU6-1, pDU6-2, and pDU6-3 DNAs were immobilized on nitrocellulose paper and hybridized with labeled 4-8 S RNA of Novikoff hepatoma cells. The RNAs that hybridized to the DNA were
This study shows that the *Drosophila* genome contains a total of three genes that can code for U6 snRNA. These three genes are clustered in 2-kb long DNA and are present in head to tail orientation. The DNA sequences corresponding to the U6 RNA are identical to each other but the flanking sequences are far less conserved. In addition, these genes are accurately transcribed in *vitro* using *Drosophila* nuclear extracts.

**XD6 U6 DNA Contains True U6 RNA Genes**—The U6 RNA genes characterized in this study are real genes because: 1) they are accurately transcribed and capped in *vitro* to produce U6 RNA and, 2) *Drosophila* genome has three U6 RNA genes and these are contained in XD6. All three genes contain identical nucleotide sequence corresponding to U6 RNA coding region (Fig. 3). Therefore, one or more of these genes must code for U6 RNA in *Drosophila* cells.

Recent studies have shown that mouse and human U6 RNA genes are transcribed by RNA polymerase III and the U6 RNA synthesized in *vitro* was properly capped (Reddy et al., 1986; Kunkel et al., 1986). The analysis of U6 RNA synthesized from *Drosophila* U6 genes also showed that the U6 RNA was initiated at nucleotide 1 (pppG) and was also capped in *vitro* (Fig. 5).

**U6 RNA Is Highly Conserved in Evolution**—The homology between rat and *Drosophila* U6 snRNAs is >95%. Thus U6 RNA is the most conserved among all U series of small nuclear RNAs reported so far (see Table 1). This high degree of conservation may be necessary for the intermolecular interaction between U6 and U4 small nuclear RNAs. The presence of an intragenic control region in U6 RNA genes characteristic of Class III genes, could also contribute to the high degree of conservation. The identical coding regions of the *Drosophila* U6 RNA genes suggest the involvement of gene duplication in their evolution whereas the divergence in flanking regions could be attributed to genetic drift.

**A Consensus Class III Intragenic Regulatory Sequence and a Transcription Termination Signal Are Present in U6 RNA Genes**—A stretch of intragenic DNA segment of the U6 gene (48-72) has a high degree of homology with certain intragenic control regions of other genes transcribed by polymerase III. The consensus sequence PurGPyrNNAPurPyr-GG (where Pur is purine and Pyr is pyrimidine) for box A of eukaryotic tRNA (Ciliberto et al., 1983) is highly conserved in both *Drosophila* and mouse U6 genes (nucleotides 48-59). Nucleotides 48-72 of U6 genes have a high degree of homology with certain intragenic DNA segments of the U6 gene. This high degree of conservation may be necessary for the interaction between U6 and U4 small nuclear RNAs. The presence of an intragenic control region in U6 RNA genes characteristic of Class III genes, could also contribute to the high degree of conservation. The identical coding regions of the *Drosophila* U6 RNA genes suggest the involvement of gene duplication in their evolution whereas the divergence in flanking regions could be attributed to genetic drift.
1981; Ciliberto et al., 1983). This termination signal is also found in all the U6 RNA genes.

Conserved Sequences Are Found Flanking the DU6 RNA Genes—Although the regulatory sequences are intragenic for genes transcribed by RNA polymerase III (Bogenhagen et al., 1980; Sakonju et al., 1980; Fowlkes and Shenk, 1980; Ciliberto et al., 1983), in some cases 5'-flanking sequences have been reported to modulate their transcription (DeFranco et al., 1980; Ullu and Weiner, 1985). In the case of Drosophila tRNA gene, sequences affecting factor binding to 5'-flanking regions (Schaak et al., 1984). An upstream TATA box has been shown to be required for in vitro transcription of Neurospora 5S RNA genes (Tyler, 1986). Therefore, it is possible that the flanking regions of U6 RNA genes contain sequences important for transcription. It is notable that the -20 to -30 region of the three U6 RNA genes, and a mouse U6 RNA gene (Fig. 3; Ohshima et al., 1981), contain a TATA box. The significance of this TATA box, which is characteristic of RNA polymerase II promoter (Goldberg, 1979; Gannon et al., 1979), in a gene transcribed by RNA polymerase III, is not known. In addition to the TATA box, another conserved region is found in the 5'-flanking region of the Drosophila U6 gene, transcribed using Drosophila nuclear extract, Novikoff hepatoma cell extract, and HeLa cell extract, respectively. The RNAs labeled after gene, transcribed using rat U6 RNA. The primed numbers indicate oligonucleotides with different mobilities due to sequence differences between Drosophila and rat U6 RNAs.

FIG. 6. Analysis of small RNAs homologous to ADU6 DNA and U6-1, U6-2, and U6-3 genes. 5 μg each of ADU6 DNA, pDU6-1 DNA, pDU6-2 DNA, or pDU6-3 DNA was immobilized on nitrocellulose dots and hybridized in a solution of 50% formamide, 80 mM Tris (pH 7.5), 600 mM NaCl, 4 mM EDTA, 1.5 × Denhardt's reagent, 0.1% SDS, and 5 μg/ml of yeast tRNA, at 42 °C for 16 h with uniformly labeled 4-8 S RNAs of Novikoff hepatoma cells. The filters were washed at 42 °C with 3 × SSC and 1.5 × SSC and the hybridized RNAs eluted and fractionated on a 10% polyacrylamide, 7 M urea gel. Lane 1, Novikoff hepatoma 4-8 S RNA used for hybridization; lanes 2, 3, 4, and 5, λDU6 DNA, pDU6-2 DNA, pU6-3 DNA and pDU6-1 DNA, respectively. Two minor RNAs, labeled A and B, were consistently observed and their relation to U6 RNA is not known.

![Image]

Fig. 4. Transcription of Drosophila U6 RNA genes in vitro. The in vitro transcriptions were carried out using [α-32P]GTP and 1 pmol of DNA template as described under "Materials and Methods." The RNAs labeled after in vitro transcription were purified by phenol extraction and ethanol precipitation. The RNAs were fractionated on a 10% polyacrylamide, 7 M urea gel and subjected to autoradiography. Lane 1, Novikoff hepatoma 4-8 S RNA used as marker RNAs. Lanes 2, 3, and 4, Drosophila pDU6-1 clone, containing also a tRNA gene, transcribed using Drosophila nuclear extract, Novikoff hepatoma cell extract, and HeLa cell extract, respectively. Lane 5, Drosophila pDU6-3 DNA transcribed with Drosophila nuclear extract. Lanes 6, 7, and 8, Drosophila pDU6-2 DNA transcribed with Drosophila nuclear extract, Novikoff hepatoma cell extract, and HeLa cell extract, respectively. Lanes 9, 10, and 11, mouse U6 gene transcribed with Drosophila nuclear extract, Novikoff hepatoma cell extract, and HeLa cell extract, respectively. Lane 12 and 13, no DNA added and pSP65 DNA, respectively, transcribed with Novikoff hepatoma cell extract. Lane 14, the transcripts from lane 2 hybrid selected with pDU6-3. Lane 15, the transcripts from lane 3 hybrid selected with pDU6-3. Lane 16, Novikoff hepatoma 4-8 S RNA used as markers.

![Image]

Fig. 5. T1-RNase fingerprint of Drosophila U6 RNA synthesized in vitro. The U6 RNA band obtained by transcription of DU6-2 DNA in vitro was purified on an acrylamide gel (see Fig. 4, lane 6) and the RNA recovered from the gel. The labeled U6 RNA was digested with T1-RNase and subjected to fingerprinting and autoradiography. The RNA was labeled with [α-32P]GTP as precursor. The T1-RNase oligonucleotides were numbered according to Epstein et al. (1981) used for rat U6 RNA. The primed numbers indicate oligonucleotides with different mobilities due to sequence differences between Drosophila and rat U6 RNAs.
Fig. 7. A possible secondary structure of Drosophila U4/U6 RNA complex. The sequence of Drosophila U4 RNA was from Myslinski et al. (1985), and Drosophila U6 RNA sequence was derived from DNA sequence (Fig. 3). The secondary structure is essentially the same as proposed by Hashimoto and Steitz (1984) for vertebrate U4/U6 RNA complex. The base pairing between U4 and U6 RNAs found by Rinke et al. (1985) is also incorporated into this model. The dots represent base pairing between complementary nucleotides. The large arrows indicate part of U6 RNA (corresponding to nucleotides 49–56) base pairing with U4 RNA.

### Table I
**Conservation of U-snRNAs and 5 S RNA through evolution**

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RNA genes. This sequence from -43 to -71 is over 60% homologous among the Drosophila U6 RNA genes but is absent in the mouse U6 RNA gene (Fig. 3) and it is a possible candidate responsible for species-specific transcription.

**Transcription of U6 Genes Appears to Be Species-specific—**

All the three Drosophila U6 RNA genes were transcribed in vitro to yield U6 RNA when Drosophila nuclear extract was used (Fig. 4); however, these were not transcribed with rat or HeLa cell extracts (Fig. 4) or when injected into frog oocytes (results not shown). A tRNA was transcribed and processed in all the three extracts (Fig. 4). These results show that unlike tRNA genes and 5 S RNA genes which can be transcribed across species (Sharp et al., 1984), U6 RNA genes require species-specific factors. The Drosophila tRNA was more efficiently transcribed than any of the Drosophila U6 snRNA genes (Fig. 4). Whether this is due to the method used in preparing the extract used for transcription or whether this represents the in vivo situation is not known. It is worth noting that U-snRNAs have a low turnover (Weinberg and Penman, 1968) and the relative efficiencies observed (see Fig. 4) may reflect the in vivo situation.

**Secondary Structure of Drosophila U4/U6 RNA Complex—**

Hashimoto and Steitz (1984) proposed a hypothetical secondary structure, showing maximum base pairing between rat U4 RNA and U6 RNAs. Recently a base-paired interaction between U4 and U6 RNAs in intact ribonucleoprotein particles from HeLa cells was analyzed by psoralen cross-linking which indicated that nucleotides UGGCUAGUU (corresponding to nucleotides 56–63) of U4 RNA hydrogen bonded to GAUUAGCA (corresponding to nucleotides 49–56) of U6 RNA (Rinke et al., 1985).

An attempt was made to deduce a secondary structure for Drosophila U4/U6 RNA complex, incorporating the features proposed by Hashimoto and Steitz (1984) and by Rinke et al. (1985). Although Drosophila U4 RNA is only 75% homologous to rat U4 RNA (Myslinski et al., 1984; Saba et al., 1986) and Drosophila U6 RNA deduced from the DNA sequence differs from rat U6 RNA in five positions (this study), the hydrogen bonding proposed by Hashimoto and Steitz (1984) and Rinke et al. (1985) was conserved in Drosophila U4/U6 complex (Fig. 7). In this secondary structure, nucleotides 49–56 of U6 RNA are shown to be hydrogen bonded to nucleotides 56–63 of U4 RNA (Fig. 7). These two regions present in distinct loops, may come together in space for base pairing. These data provide supportive phylogenetic evidence for the secondary structure of U4/U6 RNA complex.

The information on structural organization and transcription of U6 RNA genes obtained in our study could form the basis for elucidating the sequences and factors involved in the expression of these genes. It is significant to note that the in vitro transcription systems, where U6 RNA genes are transcribed in a species-specific manner, has the potential to be developed into a "complementation" assay to purify species-specific transcription factors. Such an approach would be analogous to that used to isolate Xenopus TFIIA (Engelke et al., 1980). Also, the availability of an in vitro system where the U6 RNA is accurately capped would make the studies on this novel cap structure feasible.

**Acknowledgments—**

We wish to thank Dr. Vincent Pirrotta for providing the Drosophila genomic library and genomic DNA and Dr. Carl Parker for generously providing Drosophila nuclear extracts used for in vitro transcription studies. We would also like to thank Dr. David Wright for injection of cloned DNA containing Drosophila U6 genes and mouse U6 genes into frog oocyte nuclei and Dr. Subramanyam Chirala for useful suggestions and help in computer analysis.

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