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A complete and a truncated U1 snRNA gene of *Drosophila melanogaster* are found as inverted repeats at region 82E of the polytene chromosomes

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ABSTRACT

A phage containing two sequences homologous to U1 snRNA was isolated from a Drosophila melanogaster genomic library, and identified with a previously cloned D. melanogaster U1 snRNA gene. DNA sequence analysis showed that complete and truncated U1 snRNA genes are present, both of which have base substitutions relative to U1 snRNA. These genes show conservation of 5' and 3' flanking regions relative to other U1 and U2 snRNA genes of Drosophila. Intramolecular renaturation experiments and electron microscope mapping demonstrates that the two U1 snRNA sequences are present as inverted repeats about 2.7kb apart, separated by a smaller pair of inverted repeats of an unrelated sequence. These U1 snRNA sequences were located by in situ hybridization at 82E, and related sequences were found at 21D and 95C on the polytene chromosome map.The results are discussed with reference to the origin and function of snRNAs.

INTRODUCTION

Small nuclear RNAs (snRNAs) are abundant, stable, range in size from 90 to 400 nucleotides, and appear to be ubiquitous in eukaryotes. There are six U snRNAs, U1-U6, that have capped 5' ends with the exception of U6, are synthesized by RNA polymerase II, and that have been highly conserved during evolution[1-5]. U snRNAs are associated with proteins in ribonucleoprotein particles[6] (snRNPs) that are themselves highly conserved[7,8]. Examination of the nucleotide sequence of U snRNAs, together with consideration of their nuclear location led to the suggestion that snRNAs are important in RNA processing[6,9,10], since U1 and U2 snRNAs may base pair with the consensus sequences adjacent to intron/exon boundaries. This suggestion is supported by observations that antibodies against snRNPs inhibit HnRNA processing[11-13], that some snRNAs are hydrogen bonded to HnRNA[14-16], and that a U1 snRNA selectively binds to a 5' splice site in

vitro[17]. But so far there has been no direct demonstration of U snRNA function.

snRNA genes are not typical of other genes transcribed by RNA polymerase II. All lack introns, and some lack TATA boxes[18-22]. Those snRNA genes that do have TATA-like boxes have them located in general further 5' to the gene than usual[23-25,36]. Vertebrate snRNA genes share regions of block homology that may be important for control of transcription[18,20,22,24] located 100 to 250 bp 5' to the gene,and share a common 3' sequence that may be a termination signal, but lack a polyadenylation signal[21].

Studies on snRNA genes in Drosophila offer the possibility of combining molecular and genetic analysis. Several groups have isolated snRNAs[26,27] and snRNPs[8,28] from Drosophila. These studies, along with DNA sequence analysis of U1[29] and U2[30] have shown that the coding sequences and associated proteins of snRNA genes in Drosophila are conserved relative to vertebrate genes. Drosophila snRNA genes have a region of block homology between 40 and 70 bp 5' to the gene, but this region is unrelated to vertebrate 5' sequences[25]. The 3' end of Drosophila snRNA genes also differs from vertebrate snRNA genes by having a polyadenylation signal, and by lacking the 3' termination signal[25]. These data suggest that even though Drosophila snRNA genes are conserved structurally, transcriptional regulation may be achieved differently in Drosophila than in vertebrates.

There is no consistent pattern of U1 snRNA gene organization in the genome. The snRNA genes tend to be dispersed in the genome[31] but have also been found as tandem[21,32] or inverted[20] repeats. The number of functional U1 or U1 like genes varies between one in yeast[33] to about thirty in humans[22,34], and there are varying numbers of pseudogenes[20,32,34]. There has been considerable interest in the mechanism by which snRNA pseudogenes have been formed. Some snRNA pseudogenes show conservation of 5' and 3' flanking sequences relative to the functional snRNA gene and arise as a result of DNA duplication[34]. It is thought that U1 snRNA pseudogenes arise from reverse transcribed RNA intermediates, shown by the presence of 3' truncation, adenosine-rich flanking regions, and absence of conservation of flanking sequences[34]. U1 snRNA gene may also arise from association with

repeated elements in the genome, shown by the presence of direct repeats flanking the gene[34,35].

Here we report the sequence of coding and flanking sequences of a complete and a truncated U1 snRNA gene whose flanking sequences are different from Drosophila U1 and U2 genes previously reported, but that retain conservation in the areas of block homology 5' and 3' to the gene. We show that these U1 snRNA sequences are present as inverted repeats in region 82E of the polytene chromosomes. These results are discussed with respect to U1 snRNA structure and function.

MATERIALS AND METHODS

Library Screening, Cloning Procedures

A Drosophila genomic library[37] was screened with a 4.3kb Eco R1 fragment containing the LSP-1β gene[38] and the U1 snRNA gene (see below) using the technique of Benton and Davis[39]. Filters were hybridized overnight at 42°C in 50% formamide, 5xSSC (1xSSC is 0.15M NaCl, 0.015M NaCitrate, pH 7.0), 1xDenhardt's solution[40], 250 μg/ml calf thymus DNA, and 10% dextran sulphate, and then washed in 0.5xSSC at 42°C and exposed to X-ray film with intensifying screens. Positive phages were picked, plated at lower density and rescreened with the same probe to establish pure cultures. Afterwards, all phages hybridizing to a probe containing only LSP-1 sequences were discarded and the putative snRNA containing phages retained for further analysis. Restriction mapping, filter hybridization, and subcloning conditions have been previously described[41]. Appropriate restriction fragments were subcloned into pUC8 (Figure 1).

DNA Sequencing

DNA sequencing was carried out according to the procedure of Maxam and Gilbert[42], and in every case both strands of the DNA were sequenced.

Electron Microscopy and In Situ Hybridization

Intramolecular renaturation mapping was performed as described[43].

In situ hybridization was carried out as described by Brock and Roberts[44] using probes labelled by nick-translation[41] with (^{125}I-dCTP).

RESULTS

Isolation and Mapping

A Drosophila genomic library was screened at low stringency with a probe containing the LSP-1β gene, and an unidentified small gene located 3' to the LSP-1β gene to obtain sequences homologous to the LSP-1β gene[45]. In this screen, two phages were recovered that presumably contained sequences homologous to the small gene 3' to the LSP-1β gene since they did not have restriction maps corresponding to any known LSP gene, did not hybridize to poly(A^+) RNA isolated from fat body where LSPs are synthesized, and unlike LSP-1β DNA, did not hybridize to phages containing LSP-1α and γ sequences. These two phages were restriction mapped and found to be overlapping, so only Dm525 was studied further.

At this time, Mount and Steitz[29] cloned a U1 snRNA gene found on a 4.3kb EcoR1 fragment located at region 21D of the polytene chromosomes. The LSP-1β gene is also located at 21D on a 4.3kb Eco R1 fragment. This coincidence, together with the observations by Mount and Pardue (personal communication) that U1 snRNA genes are found at regions 21D, 82E, and 95C, and by us (see below) that phage Dm525 also hybridizes to these regions, led us to hybridize plasmid pDmU1.4d containing 90bp of the U1 snRNA gene (generously donated by S. Mount) to phage Dm525. As shown in Figure 1, the pDmU1.4d plasmid hybridizes to a Pst I fragment containing the 3' end of the LSP-1β gene plus some 3' flanking sequences, and in addition hybridizes to two fragments after digestion of Dm525 by Kpn I and BamH1.

To further investigate this homology, a plasmid containing the two regions homologous to the U1 snRNA in Dm525 plus some flanking sequence was constructed (pDm6B). Using the pDmU1.4d plasmid as a probe, a detailed restriction map was prepared and the putative U1 snRNA sequences were precisely localized (Figure 1). This map shows that the two putative U1 snRNA genes are separated by over 2.5kb of DNA, and that no restriction site homology is observed in the flanking DNA.

DNA Sequence Analysis

Each U1 snRNA gene and flanking regions was sequenced on both strands using the strategy shown in Figure 2. Figure 2

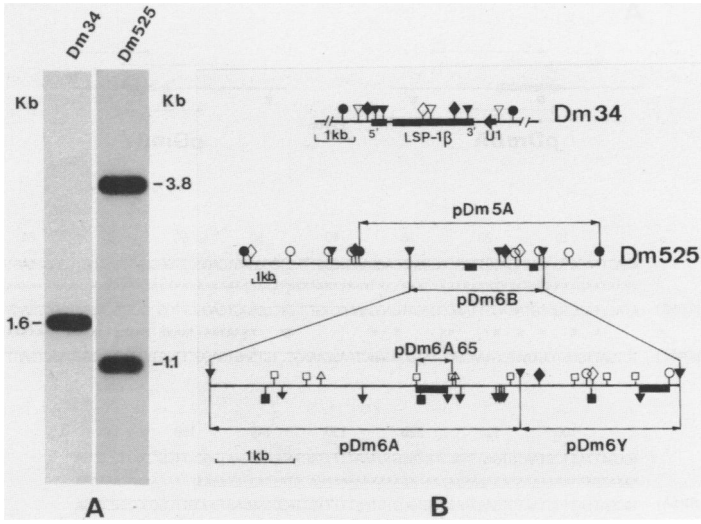
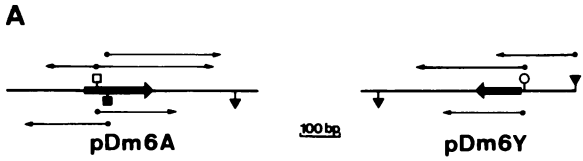


Figure 1 : A. Hybridization of the ^{32}P labelled pDmU1.4d plasmid to Dm34 phage DNA digested by PstI and to Dm525 phage DNA digested by Bam HI and KpnI. B. Restriction maps. Phage Dm34 : only a detailed map of the 4.3 Kb EcoRI fragment used as a screening probe and containing the LSP-1β and U1 snRNA genes is shown. The complete map of phage Dm34 was published previously[45]. Phage Dm525 and plasmid pDm6B : Black boxes indicate the location of the putative U1 snRNA sequences as detected by hybridization with pDm U1.4d. The pDm5A, pDm6A, pDm6B, pDm6Y and pDm6A65 plasmids were constructed by subcloning restriction fragments into the pUC8 plasmid. Restriction sites are: ● EcoRI, ▼ Bam HI, ◇ KpnI, ○ Hind III, ♦ sstI, ▽ PstI, □ Sau3A, △ BglII, ■ BalI, ▼ HinfI.

shows a comparison of each sequence with the U1 snRNA sequence by Mount and Steitz[29]. The U1 snRNA gene found in pDm6A is complete and has one base change relative to the U1 snRNA, a G>T transition at position 134. The U1 snRNA gene found in plasmid pDm6Y is truncated at its 5' end and is missing the first 50 bases. In addition this U1 sequence has three base changes relative to the remainder of the U1 snRNA: C>T, G>A, and T>C transitions at position 64, 76, and 131 respectively.

Also shown in Figure 2 is a comparison of the 5' and 3' flanking sequences of the complete U1 snRNA gene, and the 3' flanking sequence of the incomplete U1 snRNA gene with the flanking sequences of other Drosophila U1 and U2 genes published previously[25]. The 5' region of the complete U1 snRNA gene shows



B

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                    10        20        30        40        50        60        70        80        90
U1 21D              ATACTTACCTGGCGTAGAGGTTAACCGTGATCACGAAGGCGGTTCCTCCGGAGTGAGGCTTGGCCATTGCACCTCGGCTGAGTTGACCTC
                    xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
U1 82E (pDm6A)     ATACTTACCTGGCGTAGAGGTTAACCGTGATCACGAAGGCGGTTCCTCCGGAGTGAGGCTTGGCCATTGCACCTCGGCTGAGTTGACCTC
                      x  x  x  x  x     xx     x  x          xx  xxxxxxxxxxxxx xxxxxxxxxxx xxxxxxxxxxxxx
U1 82E (pDm6Y)     TCTGATTGCGTCCTAATCGAAACCCCTATGCCGAGCTAAGCAAAGCTTGTGAGTGAGGCTTGGTCATTGCACCTCAGCTGAGTTGACCTC

                    100       110       120       130       140       150       160
U1 21D              TGCGATTATTCCTAATGTGAATAACTCGTGCGTGTAATTTTTGGTAGCCGGGAATGGCGTTCGCGCCGTCCCGA
                    xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
U1 82E (pDm6A)     TGCGATTATTCCTAATGTGAATAACTCGTGCGTGTAATTTTTGTTAGCCGGGAATGGCGTTCGCGCCGTCCCGA
                    xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
U1 82E (pDm6Y)     TGCGATTATTCCTAATGTGAATAACTCGTGCGTGTAATTTCTGGTAGCCGGGAATGGCGTTCGCGCCGTCCCGA
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C

5' flanking sequences:

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                    -70       -60       -50       -40       -30       -20       -10
U1 21D              ACTGCAATAATTCCCAACTGCTTCTGGCCATCAGCTCATGGAAA-CCC-ATTCCCTGAGCTGAGGAAAGC

U1 82E (pDm6A)      ACCACAATAATTCCCAACTAGTTCTAGTTGCGCCCTCATGGAAATTCC-TATGCC-GAGCTAAGCAAAGC

U2 131A              CACTTATAATTCCCAACTGCTTCTGGCCGTTTGGTCATGGAGAGACCTGTTCGTTTCCGTTTCAAGTT

U2 131B             TGTGCTACAATTCCCAACTGCTTCTGGCCGTTTGGTCATGGAGAGCCC-GTTCGTTTCCGTTTCAAGTT

U2 141B             AGTAAAGTAATTCCCAACTGATTTTAGCTGCAGTCGCATGAAG--TCCTTTGTGCTGGGAAGGAGTAGTT
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3' flanking sequences:

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                        10        20
U1 21D              CATCTGC-AATAAATTTTAAAAGTATCAT

U1 82E (pDM6A)      CATTCGTAAATAAATAT------GTA-CAT

U1 82E (pDM6Y)      CATTTATAAATAACTAT------GTAAAAA

U2 131A             -CTTAAT-AATAAATATAAAACCAATTTG

U2 131B             -CTGAAT-AATAAATATTTAA-CTATTGT

U2 141B             -CTGAAT-AATAAATATTTAA-TTATAAA
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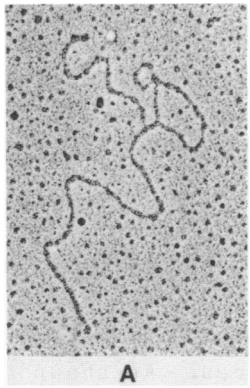
extensive homology with the previously reported sequence but gaps must be introduced into the sequences to maximize alignment. The 3' flanking sequences of both the complete and incomplete U1 snRNA gene conserve the polyadenylation signal ATAAAT but the homology is less than at the 5' end. No inverted repeats were found 3' to the gene.

Genomic Organization

Human U1 snRNA pseudogenes are often surrounded by direct repeats. Because one of the U1 snRNA sequences reported here is clearly a pseudogene, it was of interest to determine if these genes were surrounded by repeated sequences. As shown above, direct sequence analysis of the DNA immediately surrounding these U1 snRNA genes revealed no direct repeats. To examine the longer range organization of these genes, intramolecular renaturation analysis followed by electron microscopy was performed on the phage Dm525, and on the plasmids pDm5A and pDm6B. As shown in Figure 3, a stem loop structure that itself contains a stem loop within it was clearly visible. Measurement of DNA length permitted localization of the outer pair of inverted repeats within the plasmid pDm5A. The outer pair of inverted repeats corresponds to the two U1 snRNA genes (Figure 3). Renaturation experiments with pDm6Y allowed localization of the inner pair of inverted repeats, which are 70bp long and separated by 600bp of DNA. A plasmid containing foldback sequences[43] did not hybridize to pDm6Y. It was observed that pDm6Y contains highly repeated sequences that were shown by in situ hybridization to be largely present in the chromocentre (unpublished data).

In situ hybridization studies were performed initially with phage Dm525. This phage hybridizes strongly to region 82E of the polytene chromosomes. Because the ratio of U1 coding DNA to

Figure 2 : A. Sequencing strategy of the U1 snRNA genes contained in plasmids pDm6A and pDm6Y. B. Comparison of the sequences of the U1 snRNA genes located at 21D[29] with the sequence of the U1 snRNA genes in pDm6A and pDm6Y. C. Comparison of the 5' and 3' flanking sequences of the U1 snRNA gene in pDm6A and the 3' flanking sequences of the U1 snRNA gene in pDm6Y with the flanking sequences of other Drosophila U1 and U2 snRNA genes published previously[25]. Positions are indicated relative to the first and last nucleotides of the mature snRNAs. Gaps were introduced to maximize alignment. Identical nucleotides in at least 3 of the sequences have been underlined.



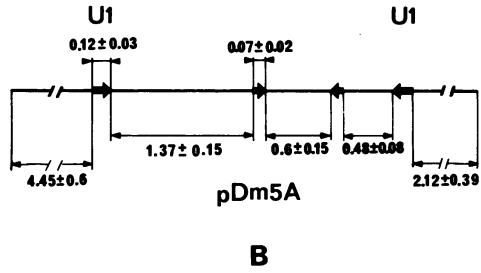


Figure 3 : Localization of the U1 snRNA genes as inverted repeats within the plasmid pDm5A. A. The 10 Kb EcoRI fragment from pDm5A after 30 mn of intramolecular renaturation at room temperature. B. Length measurements of renatured EcoRI fragments from pDm5A. Lengths are in Kb ± standard deviation.

flanking DNA is low, detection of other areas in the genome that might also contain U1 snRNA sequences was difficult. To increase the likelihood of finding such dispersed sequences, the plasmid pDm6A65 containing 136bp of U1 coding sequence plus about 350bp of 3' flanking sequence was hybridized *in situ* to polytene chromosomes. As shown in Figure 4, hybridization signals were observed in regions 21D, 82E, and 95C.

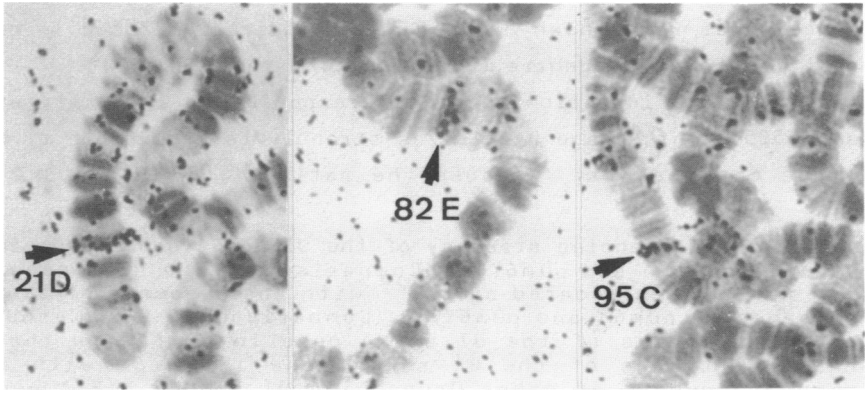


Figure 4 : *In situ* hybridization of the pDm6A65 to salivary gland chromosomes of the *Drosophila melanogaster* Canton S strain. Sites of hybridization are indicated by an arrow.

DISCUSSION

It is not possible to decide if the complete U1 snRNA at 82E is functional. The sequence differs by one base from the sequence of the U1 snRNA reported previously[29], and this change falls in a region conserved between fly, human, mouse, and chicken U1 snRNAs. This suggests that the base change in the complete U1 snRNA gene may be important for gene function, and supports the idea that this sequence could be a pseudogene. The U1 snRNA purified from a Drosophila Kc cell line is unique[27,29], and therefore finding a DNA sequence that differs from the RNA sequence also supports the hypothesis that the complete 82E snRNA gene is a pseudogene. However, there are different snRNAs genes expressed in different chicken tissues[46], and multiple snRNAs differentially expressed during oogenesis and embryogenesis of Xenopus[47], which suggests the need for caution when interpreting results obtained for a single cell type. It may be that different Drosophila tissues, have different U1 snRNA genes expressed. The observation that extensive conservation in 5' and 3' flanking sequences of the complete U1 gene relative to other expressed snRNA genes supports the idea that this gene has been functional in the past, and suggests that it may still be transcribed. Only *in vivo* transcription studies will permit definition of the status of the complete snRNA sequence at 82E. These studies together with *in vitro* mutagenesis studies will also throw light on the regulation of U1 snRNAs.

The truncated U1 snRNA sequence reported here is clearly a pseudogene since it completely lacks one third of the coding sequence at the 5' end. So far as we are aware, it represents the first example of an snRNA pseudogene with a 5' truncation. The 3' flanking sequence of the truncated U1 snRNA sequence shows good conservation relative to the complete U1 snRNA gene close by, suggesting that these two genes arose as a result of a DNA duplication event. This hypothesis is supported by the observations that the truncated pseudogene is not flanked by direct repeats, and does not have adenosine-rich flanking sequences, making it highly unlikely that this pseudogene arose as a result of an RNA intermediate. There is no evidence to link the inverted repeats found between the two U1 snRNA sequences at

82E with the duplication of these genes. It may be that the duplication of the U1 snRNA sequences preceeded the truncation of one of these genes, and that the truncation occurred later by a different, unknown, mechanism.

It will be of interest to examine the U1 snRNAs from the 95C region in order to better understand the regulation and evolution of the U1 snRNA genes in Drosophila.

In this report we show that the U1 snRNA genes of Drosophila are present as a small family of genes in three genomic locations: 21D, 82E, and 95C, and show that two of this family are present at 82E. The in situ locations of the U1 snRNAs genes correspond to those found by Saluz et al.[26] for an snRNA purified from Drosophila and given by them the neutral designation of $snRNA_2$ since clear evidence of homology between their purified snRNAs and previously characterized vertebrate snRNAs was not available. We suggest that this snRNA found by Saluz et al.[26] corresponds to U1 snRNA. This group estimated that 7 $snRNA_2$ (U1 snRNA) genes exist in Drosophila. Since Mount and Steitz[29] have found one U1 snRNA gene at 21D, and we report a further two U1 snRNA sequences at 82E, it is likely that the remainder of the U1 snRNA genes will be found at 95C. Drosophila U1 snRNA sequences can be closely linked in the genome, as has been previously reported for other snRNA genes in rat, Xenopus and sea urchin[20,21,32], and are the second group of snRNA genes to be found as inverted repeats.

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