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tRNA derived insertion element in histone gene repeating unit of *Drosophila melanogaster*

Yoshinori Matsuo* and Tsuneyuki Yamazaki**

Laboratory of Population Genetics, Department of Biology, Faculty of Science, Kyushu University, Fukuoka 812, Japan

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ABSTRACT

Analysis of 41 histone homologous clones from an isogenic gene library of Drosophila melanogaster showed that non-histone fragments interrupt the histone repetitive clusters at several sites. Long (L) and short (S) forms of the repeating units are distinguished by the insertion of 240 bp into the spacer between H1 and H3 of the L units; Each form appears to be clustered with its own kind. The complete DNA sequence of the histone 5.0 kb repeating unit was determined. Five histone genes(H1, H2A, H2B, H3, H4) were identified in a repeating unit and several sequence blocks common to the five histone genes were found in the 5'- and 3'-regions. The insertion sequence of 240 bp was found to be similar to the Alu family, an element derived from tRNA.

INTRODUCTION

There are two types of histone gene families, a tandem cluster type and a dispersed type. The former is found in Drosophila (1, 2) and the latter, in chicken (3, 4, 5), mouse (6, 7) and human (8-10). In some species, such as Xenopus (11-17) and sea urchin (18-22, 61-62), both types are present together. The order of histone genes in a repeating unit, polarity of transcription and copy number often differ among species (2, 23-25). The orientation of transcription in the sea urchin is unidirectional in all five early histones (H1-H4-H2B-H3-H2A)(20), but not in yeast (H2A-H2B, H3-H4) or Drosophila (H1-H3-H4-H2A-H2B). In the latter species, H2A and H2B, H3 and H4 are transcribed in opposite directions (1, 26, 27). The number of copies in several species has been estimated as: 1-2 in yeast (26, 27), about 10 in chicken (5), 20-60 in Xenopus (12, 13), 110 in Drosophila (1, 28) and several hundred in the sea urchin (20, 22, 23).

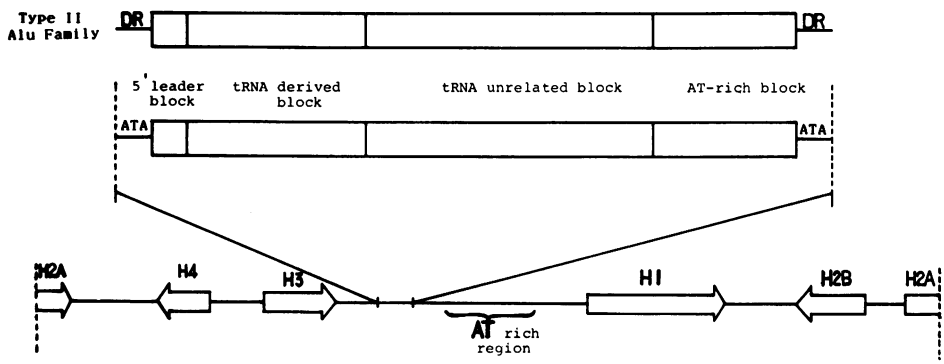


Fig. 1. The structure of the type II Alu family and insertion of histone gene are shown along with the location of insertion sites.

The histone genes of D. melanogaster repeat tandemly in the 39D-E region of chromosome IIR (28), where two kinds of repeating units, L (5.0 kb) and S (4.8 kb) are known to be present (29). The L unit has about 240 bp inserted between H1 and H3 (29)(see Fig. 1). Saigo et al.(30) analyzed the organization of the histone gene family by two dimentional electrophoresis and found that both of these units were clustered. Variant types also exist at low frequency (30). For instance, transposon 297 (a copia-like element) is inserted in the TATA box of H3 (31). As for the structure of the repeating unit, Goldberg (32) determined about 70 % of the DNA sequence of the S repeating unit, but the 3'-coding region and intergenic spacer have yet to be characterized completely.

In this study, restriction enzyme mapping and histone homologous fragments were determined for forty-one histone positive clones from the lambda library of a D. melanogaster isogenic strain. The organization of the histone gene family in a genome was deduced. The complete DNA sequence of the histone L unit was determined so as to examine the structure of histone genes.

MATERIALS AND METHODS

D. melanogaster library

To study the organization of the histone gene family in a chromosome, the isogenic strain, AK-194, was used. AK-194 was

previously constructed by extracting both the second and third chromosomes simultaneously from an isofemale line using a balance-lethal system (33). The gene library was constructed by either EcoRI partial digestion using a charon 4 vector (34) or MboI partial digestion using a λEMBL4 vector (35). In order to minimize the production of the artificially ligated DNA of small size (<10 kb) the DNA with the molecular weight of 12-20 kb was purified by sucrose gradient centrifugation. In addition, the Drosophila DNA was treated with alkaline phosphatase prior to ligation to vector DNA. Plaque hybridization was performed to screen the histone genes (36).

Cloning

DNA of a plasmid or phage was prepared by the Alkali-SDS and liquid culture methods, respectively (37). Digested DNA was ligated into the poly-linker site of a pUC9 plasmid (38). Transformation was conducted according to the $CaCl_2$ method (39) using the strain JM83 or TB1.

Labelling of DNA

An EcoRI fragment of pKSL100 (histone 5.0 kb unit of D. melanogaster) was labelled by nick translation (40) using α-^{32}PdCTP.

Southern blotting

Digested DNA was separated by agarose gel electrophoresis and transferred to a nylon membrane filter (Pall) by the method of Southern (41). Hybridization was conducted with a histone gene probe for 24-36 hrs at 68°C (37). The filters were washed with 6xSSC once, 4xSSC twice, and 1xSSC once at 68°C.

DNA sequencing

We determined the DNA sequence by dideoxy chain termination (42, 43) using a denatured plasmid as the template (44). Takara sequencing kit and Amersham's universal primer or oligonucleotide primer (17mer 5'-GCGATGACGCTTGGCG-3') were used for the sequencing reactions.

RESULTS

Organization of the histone gene family in D. melanogaster

The organization of this family in D. melanogaster, that is, the distribution and structure of members of this family in the genome, was deduced by analyzing many independent histone clones

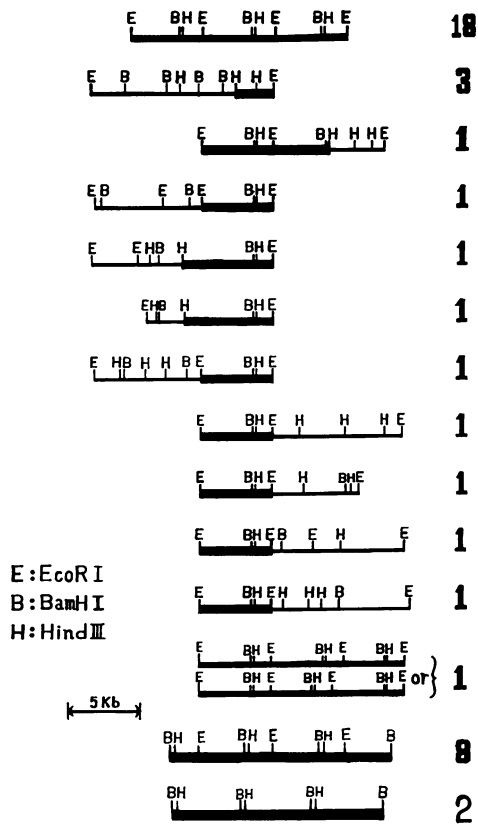


Fig. 2. Restriction mapping of 31 clones from the AK-194 EcoRI library and 10 clones from the BamHI library. In the right column is shown the number of clones with the same mapping patterns. The thick line shows the histone repeating homologous fragments.

from an isogenic strain (AK-194). To avoid biased selection of a cloning enzyme, thirty-one and ten clones were obtained from EcoRI and BamHI libraries, respectively. In Fig. 2, the histone homologous repetitive fragments (thick line) with and without the EcoRI site are considered to be the histone L (5.0 kb) and S (4.8 kb) units, respectively. As expected from the tandemly clustered structure, most of the clones (28 out of 41: 18 clones of 3L units from the EcoRI library, 8 clones of 3L units from the BamHI library and 2 clones of 3S units from the BamHI library) each had 3 units of the same length in tandem, indicating that each unit was grouped with its own kind. This is consistent with the

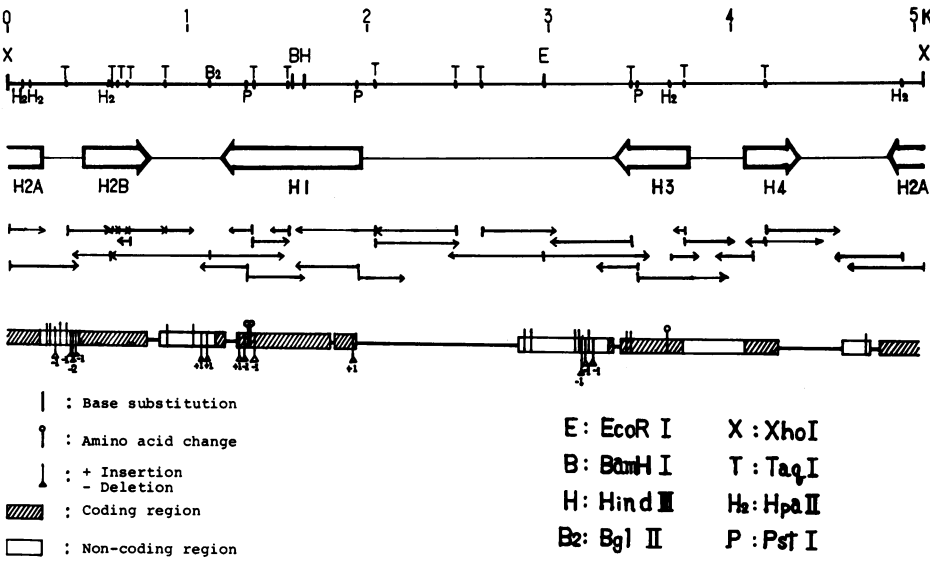


Fig. 3. Sequencing strategy of the histone 5 kb unit. DNA sequences were determined mainly from PstI, TaqI, and HpaII sites. Boxed regions at the bottom of the figure indicate those sequenced previously by Goldberg (32).

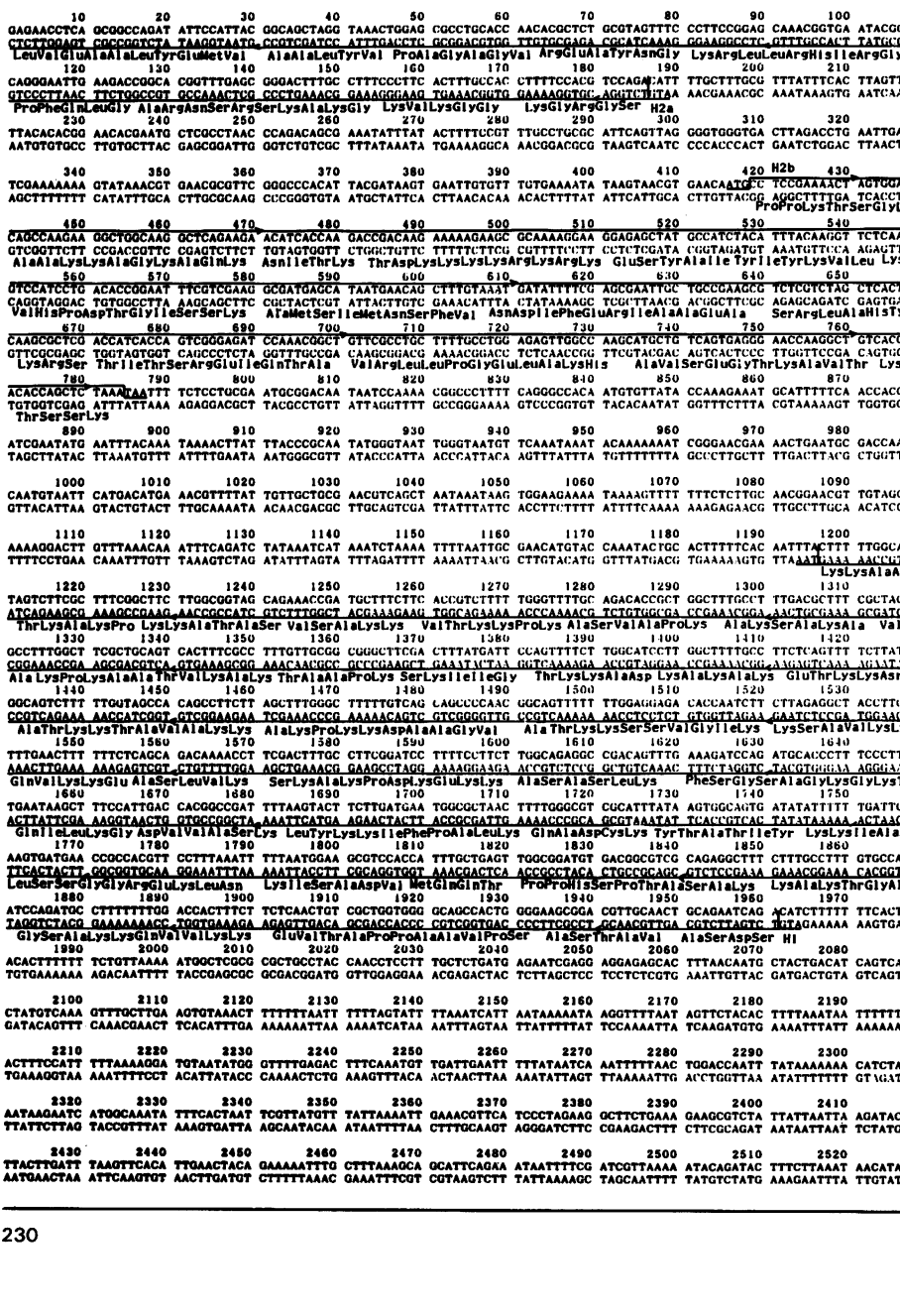
conclusion arrived at by a different method (30). Twelve of the remaining clones, however, had histone non-homologous fragments as well as one or two histone units. Although at most two different clones of these fragments may be at the ends of a histone cluster, some may be situated in the middle of histone loci. This is because the histone genes were found to be located in the 39D-E region of chromosome IIR by in situ hybridization (28). Thus, some parts of the histone gene cluster may possibly be interrupted by non-histone DNA. Southern blotting experiments of genomic DNA digested with BamHI showed the presence of histone homologous fragments with various sizes when blotted against the histone probes, confirming the above interpretation about the structure of histone gene families (data not shown).

Complete DNA sequence of the 5.0 kb histone gene repeating unit

One L unit was cloned (AK-194-19) and its DNA sequence was determined. The sequencing strategy and 5041 bp sequence determined are shown in Figs. 3 and 4, respectively. Five histone coding regions were identified within a repeating unit by

computer analysis and amino acid sequence (45-48).

Units of the histone repeats have 200-1400 bp of nontranscribed spacer sequence. The largest spacer between H1 and H3 is 1400 bp and contains the AT-rich region (about 77 % of



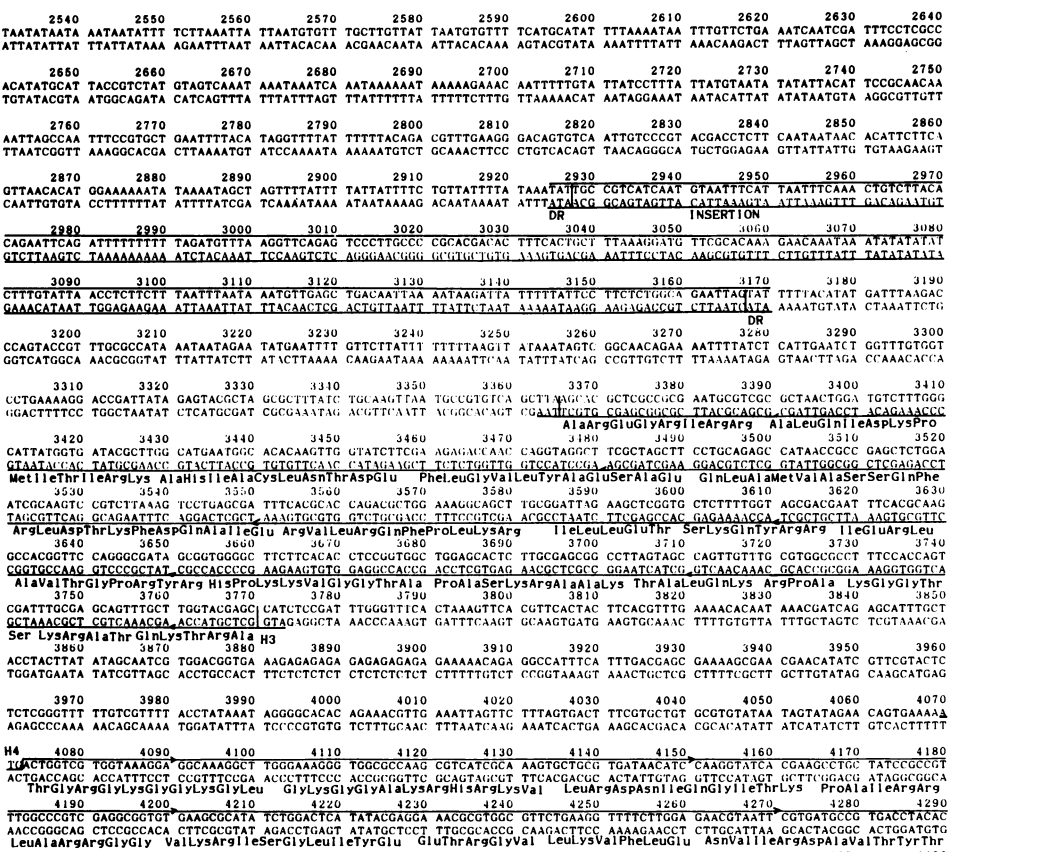


Fig. 4. Complete DNA sequence of the histone 5 kb unit. Position No. 1 is the XhoI site. Five histone structural genes are indicated by underlines. The boxed sequences show tRNA derived insertions (Alu-like family).

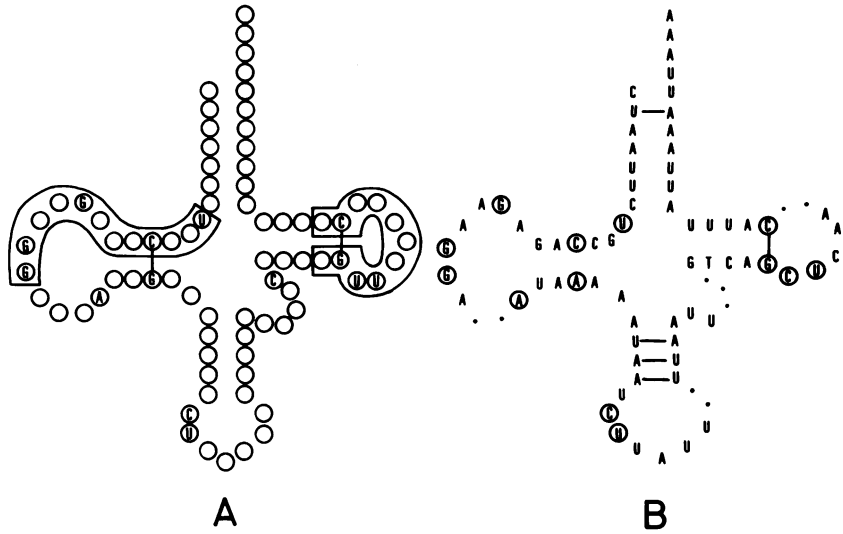
the sequence is A or T in Nos. 2115-2927 of Fig. 4) and 240 bp insertion (Nos. 2927-3167 of Fig. 4). The AT-rich region may have a specific structure and perform certain functions (49). The repeat of a simple sequence (GA)10 was found in the spacer between H3 and H4 (Nos. 3882-3902 of Fig. 4). A similar repeating structure was found in the histone repeating unit of sea urchin (50) and in the gammer-globin genes of human (51).

t-RNA derived insertion sequences (240 bp) in the L unit

The structure and sequence of the insertion in the spacer between H1 and H3 were examined in detail. The insertion site was inferred from the sequences of the S (4.8 kb) and L units (5.0 kb)(32, 52 and Fig. 4). These sequences have several features in common with elements derived from t-RNA. First, the length of the insertion of each of these sequences (240 bp) is comparable to that of the Alu-like family (approximately 70-300 bp). Secondly, the structure of the sequence is quite similar to that of the Alu-like sequences (53) as described below. Thirdly, direct repeat (DR) and insertion sites are very AT-rich as suggested by Daniels and Deininger in Alu-like sequences (54). A typical structure of Alu-like sequences is shown in Fig. 1. There is a DR block at either end and inbetween, 57-leader, t-RNA derived, t-RNA unrelated and AT-rich blocks. Insertion sequences found in histone L repeating unit have similar structures and putative DR (ATA)(Fig. 1). The DNA sequence of the insertion corresponding to the tRNA derived block is shown in Fig. 5B and the conserved sequence in the tRNA family, in Fig. 5A. Twelve out of 14 positions in the latter were found in the former (55). t-RNA is transcribed by RNA polIII and contains its promoter within the molecule (boxed in Fig. 5A)(56). The general promoter for polIII proposed by Sharp et al.(56) is indicated at the bottom of Fig. 5 as well as the insertion sequences corresponding to this region. Conserved sequences of the polIII promoter were found in the insertion sequences at the same position (15 out of 16 were matching). From these findings the structure of these insertion sequences appears similar to that of the Alu-like family and the insertion sequences may possibly be derived from t-RNA.

Common sequences among five histone genes in D. melanogaster

The 5'-upstream positions of five histone genes in D.



General promoter*

^{6}NNT$^{G}_{A}$N$^{T}_{C}$NNA$^{G}_{A}$N.GGN..AN..N$^{G}_{A}$NNN^{27}-------^{49}NNNNG$^{A}_{T}$TC$^{T}_{A}$ANNCN^{62}

^{6}TCTGCCAGAGAAGGA..AT..AAAAA^{27}-------^{44}GTCAGCTCAA..CA^{55}

Fig. 5. A: Conserved sequences in the all t-RNA derived family and all t-RNA are shown in the position of the t-RNA structure. Promoters of RNA polIII are boxed. B: Insertion sequences of histone genes are shown in the form of the t-RNA structure. Conserved positions are shown by circled letters. Bottom: General promoters of RNA polIII (the upper sequence) and insertion sequences of the histone thought to correspond to the promoter of RNA polIII are shown as lower sequences. ":" indicates the conserved sites in both sequences. *: General promoter shown in this figure is slightly modified from Sharp et al (56).

melanogaster (500 bp examined) were compared by computer. Two common sequence blocks, "TATA box" and "AGTGAAA" were found in the 5'-region (boxed in Fig. 6A). AGTGAAA was present at -8 bp to -25 bp from the initiation codon and nearly identical sequences were also noted further upstream (-31 to -46 bp, underlined in Fig. 6A). A few similar sequences were found in the histone genes of other species (see 47). The 3'-regions (500 bp downstream from stop codon examined) were also compared. Two common sequence blocks, a " hair pin loop" structure block and " AATAAA" were observed in the 3'-region (shown in Fig. 6B). It is well known that Drosophila histone m-RNA is not polyadenylated, in contrast

Fig. 6. 5' regions and 3' regions are compared in 5 histone genes in Drosophila melanogaster. Only 200 bp upstreams from the start codon and 250 bp downstreams from the stop codon are shown in the figure. Common sequences are boxed.

to Xenopus (23) and yeast (24). The hair pin loop structured block (AATCGGTCCTTTTCAGGACCACAA) found at about 40-100 bp downstream from the stop codon appears important for the 3'-end formation of the m-RNA (57, 58). This sequence block was conserved in most of the histone 3' region (23). The AATAAA sequence thought to be a polyadenylation signal, is rarely present in published DNA sequences of histone genes (47).

DISCUSSION

Several common sequence blocks were found in the 5'- and 3'-regions of five histone genes of D. melanogaster. The AGTGAAA sequence block was found in the 5' region at almost the same position, but a homologous sequence could not be detected in other species such as sea urchin (18-22), yeast (26, 27), chicken (3-5), or human (8-10). Thus, this sequence block may be important for the specific expression of Drosophila histone genes. In addition to the " hair pin loop " structure block, the AATAAA se-

quence block was found in the 3'-region. From this, it is conceivable that Drosophila histone genes produce two kinds of m-RNA, one carrying poly(A) and one that does not.

Recently, the Alu-like family, rodent type 2 Alu family, rat ID sequences, rabbit C family, and the bovine or goat 73-base-pair repeat were found to be similar to tRNA (53, 54, 59). The insertion sequence in the AT-rich spacer region of histone repeat unit is also similar to t-RNA. This insertion could be transcribed by RNA polIII, since the conservative sequence of the RNA polIII promoter is in this insertion. The insertion site is very close to the region reported as the binding sites for protease sensitive components (49). Thus, the element may perform some role in gene expression or constructing the chromatin structure. The functions of these sequences (AGTGAAA, AATAAA, Alu-like insertion) remain to be determined by future research.

In regard to the organization of the histone gene family in D. melanogaster, the L and S units are polymorphic and clustered, respectively. Variant types having non-histone fragments are sometimes present in the histone cluster (31, 60). In some Drosophila species such as D. mauritiana, D. teissieri, D. erecta, and D. orena, the length type is constant (61, 62). It appears that variant types accidentally increase or become fixed in the histone clusters of certain Drosophila species and several subclusters segregate in the polymorphic state in D. melanogaster.

The interruption of a histone cluster by non-histone fragments may at times reduce the rate of genetic exchange among the members of a family. Disruption of homogenization would cause the histone clusters to differ from those of the main cluster. If these "orphan" genes (63) come to have different function(s) as in the case of the late histone genes in sea urchin (18, 19), the processes responsible for hindering the exchange of genetic information among the members of a family, will also be important for the progressive evolution of organisms.

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* Present address: Department of Ecology, Ethology and Evolution, University of Illinois, Shelford Vivarium, 606 E. Heaky St., Champaign, Illinois 61820, USA

** To whom correspondence should be addressed.

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