Copies of a *Stellate* Gene Variant Are Located in the *X* Heterochromatin of *Drosophila melanogaster* and Are Probably Expressed

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ABSTRACT

Two variants of *X* chromosome *Stellate* genes responsible for crystal formation in *XO* male primary spermatocytes occupy different genome positions. The majority if not all of the 1250-bp *Stellate* genes are located at the 12E site where the *Ste* locus has been mapped and almost all of the 1150-bp *Stellate* repeats are concentrated in the distal *X* heterochromatin. Sequencing of *Stellate* genes derived from *X* heterochromatin reveals the preservation of their open reading frames and precise matching with some *Stellate* cDNAs reported earlier. At least some heterochromatic *Stellate* genes are suggested to be expressed and, therefore, involved in the interaction with the *Y* chromosome locus *Su(Ste)*, as are the *Stellate* genes from 12E.

MULTIPLE tandemly repeated *Drosophila melanogaster Stellate* genes located on the *X* chromosome determine the appearance of needle- or star-shaped proteinaceous crystals in the primary spermatocytes of *XO* males probably resulting in a disturbance of spermatogenesis (HARDY *et al.* 1984; LOVETT, KAUFMAN and MAHOWALD 1980). It has hypothesized that the *Y*-linked *Su(Ste)* locus contains *Stellate* homologs that suppressed the transcription and/or splicing of *X* chromosome *Stellate* genes and thereby prevented crystal formation (LIVAK 1984).

Two major variants of *X* chromosome *Stellate* genes represented by 1250-bp and 1150-bp *Xba*I repeats were cloned and sequenced (LIVAK 1984, 1990). They differ by a number of nucleotide substitutions and microdeletions and by a 155-bp deletion at the 3′ end that does not disturb the protein coding capacity of the genes. According to genetic and cytological data, the *Stellate* locus has been mapped to the 12E site on the *X* chromosome (HARDY 1980; K. J. LIVAK, personal communication).

In this paper the sequence of two *Stellate* genes from the *X*-linked heterochromatic region cloned in cosmids p171-31 (SHEVELYOV, BALAKIREVA and GVOZDEV 1989) and p171-14 is presented. Sequencing and Southern analysis show that dozens of potentially expressed *Stellate* genes are located in the *X* chromosome heterochromatin and may participate in the complex interaction of *X*- and *Y*-linked *Stellate* sequences.

MATERIALS AND METHODS

Standard procedures were followed for isolation of plasmid DNA, restriction enzyme digestion and electrophoresis of DNA in agarose gels, blotting of DNA onto nitrocellulose, preparation of nick-translated DNA probes, subcloning into the pUC19 vector, library construction in the pJB8 cosmid vector and its screening by the colony-hybridization technique (MANIATIS, FRITSCH and SAMBROOK 1982). Autoradiographs were scanned with an Ultroscan XL laser densitometer (LKB).

Preparation of DNA: To isolate DNA from polytene chromosomes, 250 pairs of Drosophila third instar larvae salivary glands were homogenized in 0.2 ml of 50 mM Tris-HCl (pH 8.0)/25 mM EDTA/0.2% Triton X-100 at 0°. Proteinase K (to 20 μg/ml) and Sarkosyl (to 1%) were added, and after 1 hr of incubation at 37°, the homogenate was sequentially extracted with phenol, with phenol/cloroform/isoamyl alcohol (50:49:1) and with cloroform. DNA in the aqueous phase was precipitated by 2.5 volumes of ethanol and stored at −20°. DNA isolation from eight Drosophila females or from 15 males was performed by the same method.

Sequencing: DNA sequencing reactions were carried out according to MAXAM and GILBERT (1980). The appropriate restriction fragments of the *Stellate* repeat were subcloned in pUC19 and sequenced in both directions.

Drosophila strains: *D. melanogaster* stock 171 is described by PASYUKOVA *et al.* (1986). *X* heterochromatin deficient strains *Df(1)X-1/FM7* and *Df(1)GA-90/B^{s}Y$^+$* (RAHMAN and LINDSLEY (1981) were gratefully obtained from D. L. LINDSLEY. To generate interspecific hybrid females, *D. melanogaster Df(1)X-1/FM7* females were crossed with *Drosophila simulans* males from the Alekseevka strain. Alekseevka flies were collected in a natural Azerbaijan population by E. G. PASYUKOVA, V. G. NIKIFOROV and V. A. GVOZDEV in 1983.

RESULTS

The sequencing and molecular analysis of the cloned region underreplicated in polytene nuclei (SHEVELYOV, BALAKIREVA and GVOZDEV 1989) has revealed two 1150-bp *Stellate* gene copies (Figure 1), one of them being interrupted by *mdg1* (TCHURIKOV *et al.* 1981) and *aurora* (Y. Y. SHEVELYOV and D. I. NURMINSKY, in preparation) retrotransposon insertions. The *Stellate* repeat is flanked on one side by a

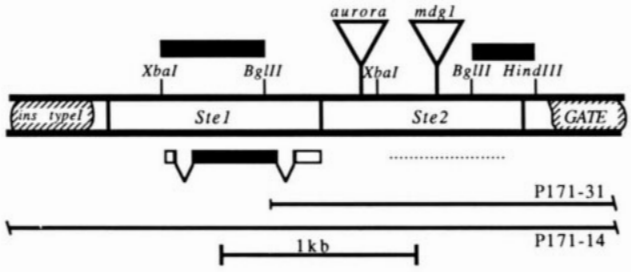

FIGURE 1.—Structural organization of the *Stellate* tandem repeat from the *X* heterochromatin region, cloned in p171-31 and p171-14 cosmids. Triangles mark the sites of *mdg1* and *aurora* insertions; full squares show the hybridization probe to Figure 2; the region sequenced earlier in the opposite direction (SHEVELYOV, BALAKIREVA and GVOZDEV 1989) is dotted; the exon and intron transcript structure shown under the *Ste1* copy is from LIVAK (1990); the *Hin*dIII site appears to be the result of a T to C substitution at the 34th position of a third *Stellate* copy as compared with the *Ste1* and *Ste2* copies.

tandem of ribosomal type I insertions (JAKUBCZAK, XIONG and EICKBUSH 1990) and on the other side by the mobile element *GATE* (DI NOCERA, GRAZIANI and LAVORGNA 1986).

Southern analysis of DNA isolated from salivary gland polytene chromosomes was performed (Figure 2A) to evaluate the distribution of *Stellate* genes between eu- and heterochromatin. According to LIVAK (1984) the 800-bp *Cfo*I hybridizing fragment is derived from the *Y* chromosome, whereas, 1150-bp and 950-bp *Cfo*I fragments represent the 1150-bp and 1250-bp *X*-linked variants of *Stellate* genes. In salivary gland DNA only the 950-bp fragment is well detected (Figure 2A), thus indicating the localization of the 1250-bp *Stellate* genes in euchromatin. The majority if not all of the 1150-bp *Stellate* genes are localized in the *X* heterochromatin, since they are heavily underreplicated in polytene chromosomes. It should be noted that after a long exposure time a weak hybridization signal with the 1150-bp fragment is detected in salivary gland DNA (data not shown).

To corroborate the heterochromatic nature of 1150-bp *Stellate* genes, DNA isolated from *melanogaster/simulans* hybrid females was analyzed by Southern blotting (Figure 2B). The *melanogaster X* chromosome carried in the hybrids contains the heterochromatic deletion *Df(1)X-1* (RAHMAN and LINDSLEY 1981), extending from euchromatic section 20 to at least the *bb* locus; the *simulans X* chromosome and autosomes do not contain any *Ste* homology (LIVAK 1984) (Figure 2B). As is expected, euchromatic *Stellate* sequences (the 950-bp *Cfo*I fragment) are approximately half as intense in *melanogaster/simulans* hybrid DNA as in DNA from *melanogaster* control flies. At the same time, the hybridization of the presumably heterochromatic 1150-bp *Ste* fragment is nearly 10 times less in DNA isolated from *melanogaster/simulans* hybrid females than in *melanogaster* control DNA (Figure 2B). As is shown, *X* heterochromatin also contains other structural variants of *Stellate* sequences.

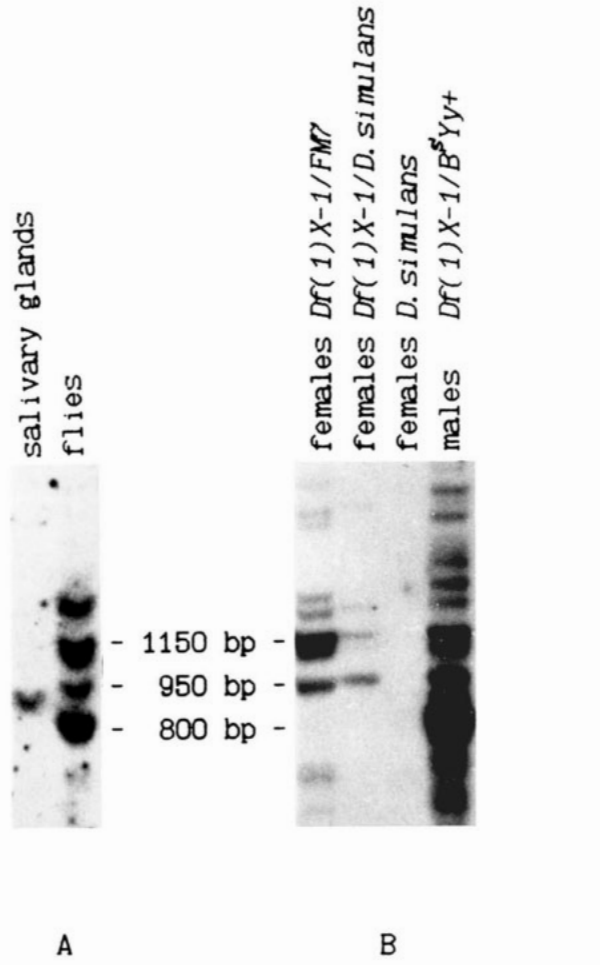

FIGURE 2.—Southern analysis experiments indicating the heterochromatic origin of almost all of the 1150-bp *Stellate* genes. DNA from 125 pairs of salivary glands and an equal amount (approximately 2 μg) of total DNA from *D. melanogaster* stock 171 (panel A), DNAs from eight females with the following genotypes: *Df(1)X-1/FM7*; *Df(1)X-1/D. simulans*; *D. simulans/D. simulans*; and from 15 *Df(1)X-1/B^{s}Yy^{+}* males (panel B) were digested with *Hha*I (*Cfo*I isoschizomer), electrophoresed in a 1% agarose gel, blotted and hybridized with a *Ste* probe, comprising a mixture of 0.55-bp *Xba*I-*Bgl*II *Ste1* and 0.3-bp *Bgl*II-*Hin*dIII *Ste2/Ste3* fragments (see Figure 1). The fragments sizes are from LIVAK (1984).

Figure 3 represents nucleotide sequences of the two *Stellate* genes (*Ste1* and *Ste2*) from *X* heterochromatin cloned in the p171-31 and p171-14 cosmids compared with the *Stellate* sequences determined by LIVAK (1990) in the pSX83.4 plasmid and in a cDNA clone (cDNA4). As was inferred from the Southern analysis data, the sequence of the 1150-bp *Stellate* gene from pSX83.4 is most similar to the heterochromatic *Stellate* copies. The 1150-bp variant differs from the 1250-bp *Stellate* genes mainly by deletions in the 3′- and 5′-noncoding regions. Comparison with cDNA4 shows that the *Ste1* sequence differs from it by a single nucleotide substitution in the 5′-noncoding region (Figure 3), while pSX83.4 has seven mismatches and

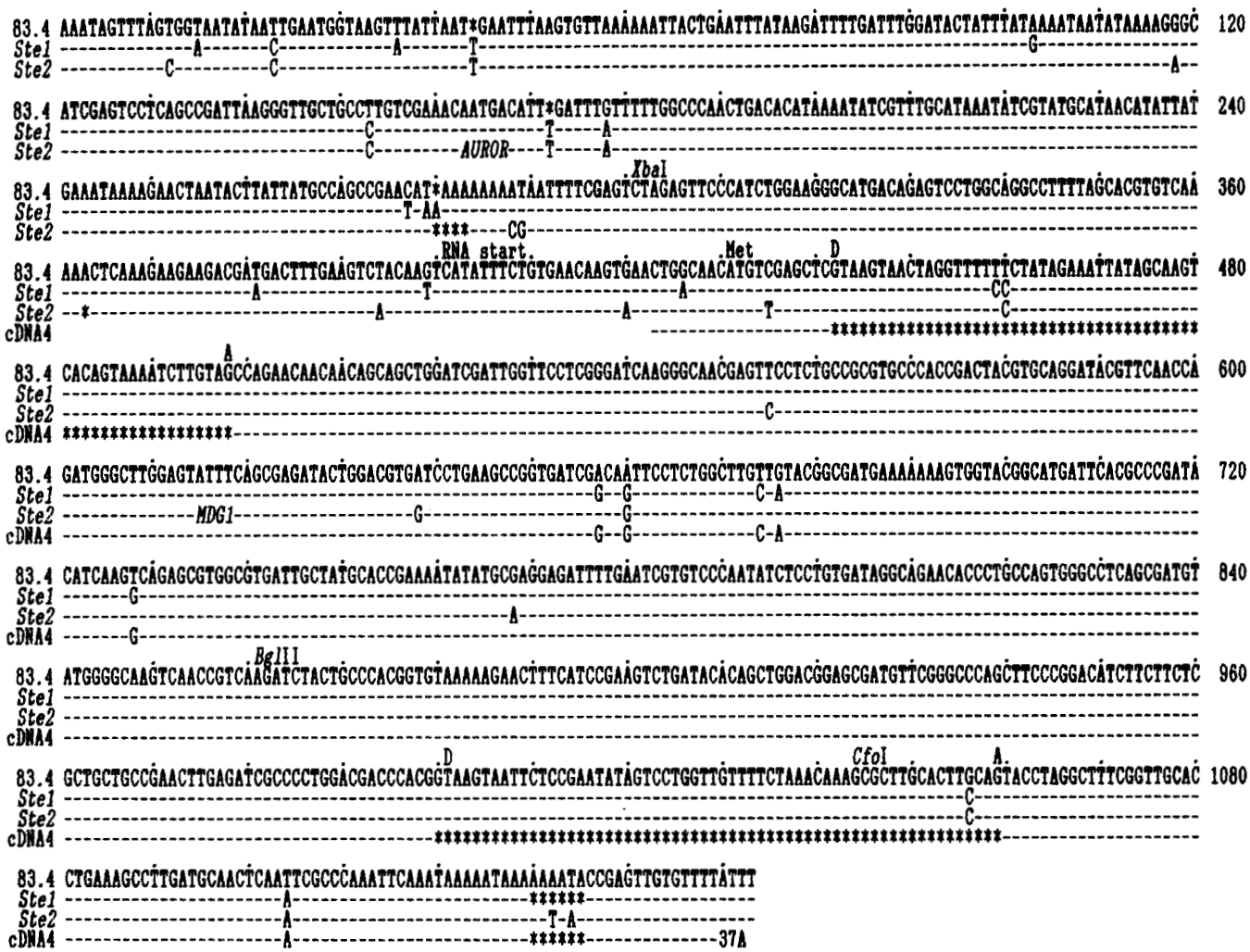

FIGURE 3.—Sequence comparison of the heterochromatic *Ste1* and *Ste2* copies with the *Stellate* gene from the pSX83.4 clone and *Stellate* cDNA4 (LIVAK 1990). The *Ste1* sequence from the beginning to the *Bgl*II site at position 861 is derived from the p171-14 clone, the *Ste1* remnant and the *Ste2* sequence are from the p171-31 clone. A hyphen means the same base as in pSX83.4 and an asterisk means the absence of the base. The characters *MDG1* and *AURORA* mark four and five bases, correspondingly, duplicated after *mdg1* and *aurora* insertions. RNA start, first Met codon, splice donor (D) and acceptor (A) sites are from LIVAK (1990).

the *Ste2* copy, damaged by the *mdg1* and *aurora* insertions, has nine mismatches. Nevertheless, none of the substitutions destroys the *Stellate* open reading frame (ORF). This indicates either the recent transfer of functioning genes to heterochromatin or, more likely, the expression of *Stellate* genes in the *X* heterochromatin.

DISCUSSION

At least two copies of *Stellate* genes were found in the X heterochromatin: The following data demonstrate the *X* heterochromatic origin of cloned *Stellate* genes: the *Eco*RI fragment from the cloned region is heavily underreplicated in polytene chromosome DNA (SHEVELYOV, BALAKIREVA and GVOZDEV 1989; their Figure 4A); this fragment is not *Y*-linked as it is represented in Drosophila female DNA (SHEVELYOV, BALAKIREVA and GVOZDEV 1989, their Figure 6A), and is not autosome-derived since LIVAK (1984) has demonstrated the absence of *Ste* homologous sequences in *D. melanogaster* autosomes; according to *in situ* hybridization data of PASYUKOVA *et al.* (1986) there are no *mdg1* copies in the 12E site of stock 171, used to prepare the cosmid library.

All or almost all of the 1150-bp *Stellate* genes are located in the *X* heterochromatin: The Southern analysis of DNA isolated from salivary gland polytene chromosomes and from *melanogaster/simulans* hybrid females, carrying the *Df(1)X-1* deletion in *D. melanogaster X* heterochromatin, localize the 1250-bp *Stellate* genes in the euchromatin, probably at the 12E site on the *X* chromosome. At the same time, the strong underreplication of 1150-bp *Stellate* genes in polytene chromosome DNA and weakening of hybridization to the corresponding fragment in heterochromatin deficient DNA from *melanogaster/simulans* hybrids sug-

gest the *X* heterochromatic nature of all or almost all copies of this structure. The hybridization with the 1150-bp *Stellate* fragment seen in the DNA isolated from *Df(1)X-1/B^{s}Yy$^+$* males (Figure 2B, lane 4) does not controvert the above suggestion since the *B^{s}Yy$^+$* chromosome contains some *X* heterochromatin material (WILLIAMSON 1976; GATTI and PIMPINELLI 1983).

The hybridization with the 1150-bp repeat detected in polytene chromosome DNA after long exposure time may be the result of either the presence of a few such copies in euchromatin or the incomplete underreplication of heterochromatic genes, typical for β-heterochromatin (reviewed by MIKLOS and COTSELL 1990).

The nucleotide sequence of two *Stellate* genes from the *X* heterochromatin region: To answer the question whether the heterochromatic *Stellate* sequences are real genes or pseudogenes, two copies from the cloned heterochromatic region were sequenced and compared with *X*-linked *Stellate* genes sequenced earlier (LIVAK 1990). The heterochromatic *Stellate* copies from p171-31 and p171-14 correspond to the 1150-bp variant from the pSX83.4 clone. Both heterochromatic *Ste1* and *Ste2* copies do not contain nucleotide insertions/deletions and stop codons in the ORF region with the exception of *mdg1* and *aurora* insertions in the *Ste2* copy.

The nearly full identity of the *Ste1* sequence to three of six randomly selected *Stellate* cDNAs sequenced by LIVAK (1990) is even more important. Assuming that this cDNA frequency reflects the *Stellate* transcript representation in their common pool, it can be concluded that in the Drosophila strain from which cDNAs were cloned a substantial part of all the transcripts from *X*-linked *Stellate* genes is from the 1150-bp variant. It should be mentioned that many copies of *Stellate* genes are located in the *X* heterochromatin. This is indicated by Figure 2 of this paper, Figure 4A of SHEVELYOV, BALAKIREVA and GVOZDEV (1989) and by the available heterochromatic YAC clone containing about 10 tandemly repeated 1150-bp *Stellate* units (G. L. KOGAN, personal communication). It seems unlikely that a few euchromatic copies of the 1150-bp variant, if they exist, can yield a similar amount of transcripts as many dozens of 1250-bp *Stellate* genes at the 12E site. It is more probable that the 1150-bp heterochromatic *Stellate* genes are transcribed in the primary spermatocytes of *D. melanogaster* males and participate in spermatogenesis disturbance in males lacking the *Y*-linked *Su(Ste)* locus, as are the *Ste* genes from the 12E site.

The transcription of Drosophila heterochromatic loci has been shown for several genes, such as rRNA genes located in the *X* and *Y* chromosome heterochromatin (RITOSSA and SPIEGELMAN 1965), male fertility factors forming giant loops on the *X* chromosome of *Drosophila hydei* (see for instance HAREVEN, ZUCKERMAN and LIFSCHYTZ 1986) and *D. melanogaster* (BONACCORSI *et al.* 1990), and the *light* locus from the *D. melanogaster* chromosome *2* heterochromatin (DEVLIN, BINGHAM and WAKIMOTO 1990). The analysis of sequences determining the transcriptional activity of heterochromatic genes is of interest. It should be mentioned in this connection, that eu- and heterochromatic *X*-linked *Stellate* genes differ in the 5′-region by four microdeletions and many nucleotide substitutions (LIVAK 1990; this paper). It would be advisable to perform *P* element-mediated transformation of Drosophila embryos with the heterochromatic *Stellate* gene, as has been done with the euchromatic copy (LIVAK 1990), in order to test whether it undergoes "the reverse position effect" in euchromatin like the *light* gene (WAKIMOTO and HEARN 1990).

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