IN SITU HYBRIDIZATION MAPPING OF HISTONE GENES IN *ANOPHELES ALBIMANUS*

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ABSTRACT. The histone genes of *Anopheles albimanus* were mapped by *in situ* hybridization to 6 bands in Region 34A on the right arm of chromosome 3. A genomic library was made by cloning fragments of 15 to 23 kb (derived from partial *Eco*RI digestion) into the phage vector, EMBL4, and probed with the histone gene repeat of *Drosophila melanogaster*. Thirty-two phages containing histone gene sequences were isolated from about 10^5 plaque-forming units (pfu). Complete *Eco*RI digestion of DNA from 5 of the 32 recombinant phages and the genomic DNA of *An. albimanus* yielded a single 3.84-kb fragment that contained sequences homologous to the 5 histone genes of *D. melanogaster*. This 3.84-kb unit of mosquito histone genes was subcloned into puc19 plasmid, and the resulting clone (palbi34A) was used for *in situ* hybridization to salivary gland chromosomes.

INTRODUCTION

As a part of our research on the genetics of anopheline mosquitoes, we are mapping genes on the polytene chromosomes through *in situ* hybridization. Recently, we used heterologous clones of the histone genes from *Drosophila melanogaster* Meigen to clone the histone genes from *Anopheles albimanus* Wied. and subsequently determined through *in situ* hybridization the location of the histone loci on the polytene chromosomes from salivary glands of *An. albimanus*.

Histone genes are a class of middle repetitive DNA sequences that occur as highly organized tandemly repeated quintets in *Drosophila* and other insects. In other organisms, the histone genes are randomly arranged dispersed clusters, e.g., in several species of sea urchins. These genes are usually turned on during DNA synthesis, consist of 5 classes of basic proteins, and are associated with DNA in organizing chromatin architecture by compacting DNA to form nucleosomes, the basic units of chromatin and thus the chromosomes. Histone genes are typically clustered in the eukaryote genome, and for that reason they can serve as a good marker in the genome for locating other closely linked genes (Maxson et al. 1983).

MATERIALS AND METHODS

Enzymes and molecular weight marker DNA were purchased from Life Technologies, Inc. (Gaithersburg, MD). The phage lambda replacement vector (EMBL4), DNA and the packaging extract (Packagene) were purchased from Promega Biotech (Milwaukee, WI). Growth media, buffer formulation, and maintenance of bacterial strains and phages are the same as described in Maniatis et al. (1982). A clone of the histone gene repeat, 1-aDm 3000-1, of *D. melanogaster* and clones of individual Hae II fragments of the histone gene repeat (source of individual histone genes) were obtained from D. S. Hogness, Stanford University, CA.

A genomic DNA library, consisting of large (15- to 23-kb) random, *Eco*RI genomic DNA fragments from *An. albimanus* was established by existing techniques (Bingham et al. (1981) for isolation of DNA; Maniatis et al. (1982) for digestion of DNA with *Eco*RI; Frischauf et al. (1983) for cloning into the EMBL4 vector). A clone of *Drosophila* histone gene repeat was used to screen the genomic library for phages containing sequences of the histone genes of *An. albimanus* by using a standard hybridization protocol (Maniatis et al. 1982). Duplicate positive phages were used for 2nd and 3rd screening to purify individual phages. A 3.84-kb *Eco*RI insert was isolated from positive phages and subcloned into puc19 plasmid. The recombinant plasmid was designated as palbi34A.

Single and double digests of the histone clone, palbi34A, with restriction endonuleases were probed with individual histone genes (H1, H2A, H2B, H3 and H4) of *D. melanogaster* to determine the relative positions of individual genes of mosquito in the 3.84-kb insert.

In situ hybridization of the mosquito histone gene clone (palbi34A) to the polytene chromosomes of *Anopheles albimanus* was done by the procedure of Pardue and Gall (1975). For each slide, 20 μl of hybridization solution containing 4×10^5 to 1×10^6 CPM probe (plasmid palbi34A) was used. The slides were washed in buffers of varying stringencies ($2 \times$ to $6 \times$ SSC) to detect possible regions of weaker hybridization. Sixteen slides, each with at least 5 well spread polytene chromosome complements, were used for *in situ* hybridization. A group of 4 slides each was used for hybridization using $6 \times$ TNS, $4 \times$ TNS, $3 \times$ TNS and $2 \times$ TNS buffer concentration, respectively. Within each group, one slide each was washed in $6 \times$ SSC, $4 \times$ SSC, $3 \times$ SSC

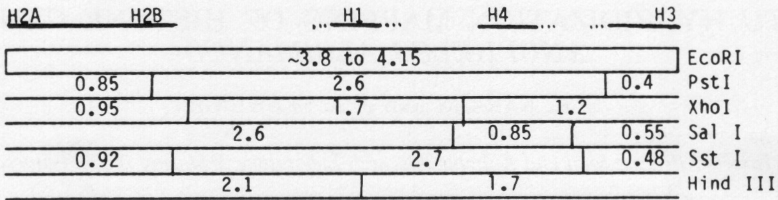

Fig. 1. Schematic representation of the gene order in the histone gene repeat, palbi34A, in *Anopheles albimanus*.

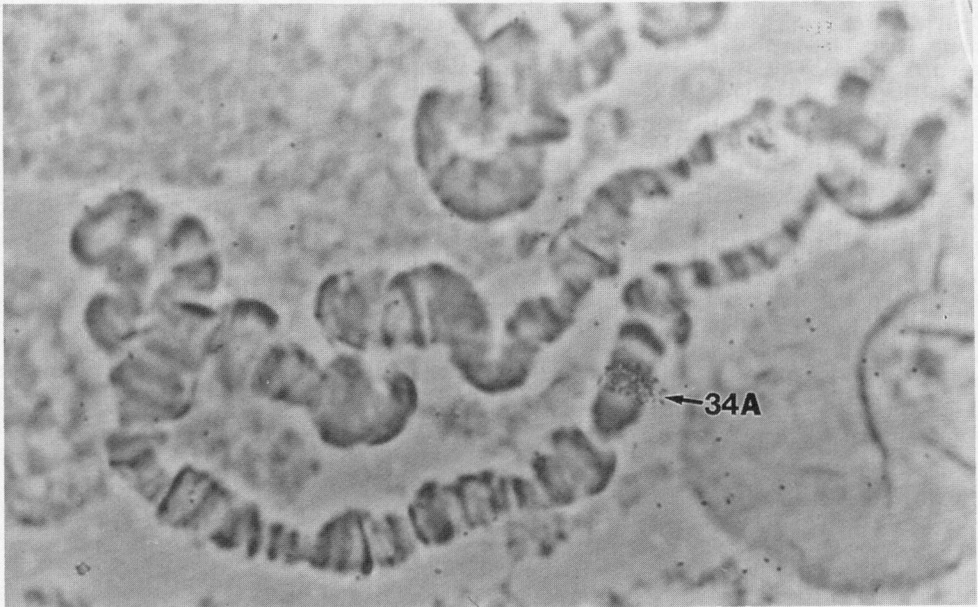

Fig. 2. The histone gene repeat, palbi34A, hybridized to region 34A on the right arm of chromosome 3 of *Anopheles albimanus* (shown by arrow).

and 2 × SSC, respectively (refer to Pardue and Gall (1975) for buffers and standard procedure of *in situ* hybridization). These variables in buffer concentrations were used with the aim of identifying the location of chromosome regions, containing either histone genes or cluster of genes with variable degrees of homologies to the cloned histone sequences in a sample of 16 mosquito larval genomes (approximately 80 chromosome complements).

RESULTS AND DISCUSSION

Thirty-two phages containing histone gene sequences were isolated from about 10^5 plaque-forming units (pfu). Complete *Eco*RI digestion of DNA from 5 of the 32 recombinant phages and the genomic DNA of *An. albimanus* yielded a single 3.84-kb fragment that contained sequences homologous to the 5 histone genes of *D. melanogaster* as determined by Southern hybridization tests. One of the 3.84-kb *Eco*RI fragment (designated as palbi34A) containing the histone gene repeat was selected for further characterization and the *in situ* hybridization experiment. The palbi34A clone also showed hybridization with each of the 5 histone genes of *Drosophila*, indicating that the *Eco*RI fragment represents a repeat unit of the histone genes of *An. albimanus* (Fig. 1). The results of *in situ* hybridization experiments (Fig. 2) showed that the binding of radiolabeled probe of palbi34A was very specific and limited to 6 neighboring bands in region 34A on the right arm of chromosome 3. Therefore, our evidence indicates that the histone loci of *An. albimanus* were shown to occur in clusters in region 34A of 3R. The results of the Southern hybridizations conducted with the 5 histone genes of *D. melanogaster* also indicated that the genes are likely arranged in tandem repeats.

Histone genes also occur in tandem repeats in *D. melanogaster* (Lifton et al. 1977). This tan-

dem arrangement is in contrast to the irregular arrangement of histone genes of mouse, chicken and human (Maxson et al. 1983). Polymorphic organization of histone genes has also been reported in yeast (Hereford et al. 1979); toad, *Xenopus laevis* (Van Dongen et al. 1981); and in newt, *Notophthalamus viridiscens* (Stephenson et al. 1981).

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REFERENCES CITED

Bingham, P. M., R. Levis and G. M. Rubin. 1981. Cloning of DNA sequences from the white locus of *Drosophila melanogaster* by a novel and general method. Cell 25:693–704.

Frischauf, A. M., H. Lehrach and M. Murray. 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. 170:827–842.

Hereford, L., K. Fahrner, J. Woolford, M. Rosbash and D. B. Kaback. 1979. Isolation of yeast histone genes H2A and H2B. Cell 18:1261–1271.

Lifton, R. P., M. L. Goldberg, R. W. Karp and D. S. Hogness. 1977. The organization of the histone genes in *Drosophila melanogaster*. Functional and evolutionary implications. Cold Spring Harbor Symp. Quant. Biol. 42:1047–1051.

Maniatis, T., E. F. Fritsch and J. Sambrook. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbor Labs., Cold Spring Harbor, NY.

Maxson, R. R., R. Cohn and L. Kedes. 1983. Expression and organization of histone genes. Annu. Rev. Genet. 17:239–277.

Pardue, M. L. and G. Gall. 1975. Nucleic acid hybridization to the DNA of cytological preparations, *In:* pp. 1-16. *In:* D. M. Prescott (ed.), Methods of cell biology Vol. 10.

Stephenson, E. C., H. P. Erba and J. G. Gall. 1981. Histone gene clusters of the newt *Nutophthalmus* are separated by long tracts of satellite DNA. Cell 24:639–647.

Van Dongen, W., L. De Laaf, R. Zaal, A. Moorman and O. Destree. 1981. The organization of the histone genes in the genome of *Xenopus laevis*. Nucleic Acids Res. 9:2297–2311.