Chromosomal Mapping of the Human Histone Gene H2AZ to 4q24 by Fluorescence *in Situ* Hybridization

Nicholas Popescu,* Drazen Zimonjic,*
Christopher Hatch,†[1] and William Bonner†[2]

*Laboratory of Biology, CPCP, DCE, and †Laboratory of Molecular Pharmacology, DTP, DCT, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

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H2A.Z is a replication-independent histone protein species of the H2A family (1, 2) that has maintained its identity throughout evolution as a separate H2A species (6). A comparison of H2A amino acid sequences shows that the H2A.Z protein contains regions of low homology with other H2A protein species alternating with regions of high homology (1). In *Drosophila,* the homologous protein has been shown to be essential from early development by a knockout and rescue experiment (7). These findings indicate that H2A.Z performs an essential cellular function and may form a different type of nucleosome structure in chromatin. The H2A.Z gene, at locus H2AZ, con-

[1] Present address: Journal of the National Cancer Institute, 9030 Old Georgetown Road, Bethesda, MD 20814.
[2] To whom correspondence should be addressed at NCI-NIH, Building 37, Room 5D17, Bethesda, MD 20892. Telephone: (301) 496-5942. Fax: (301) 402-0752.

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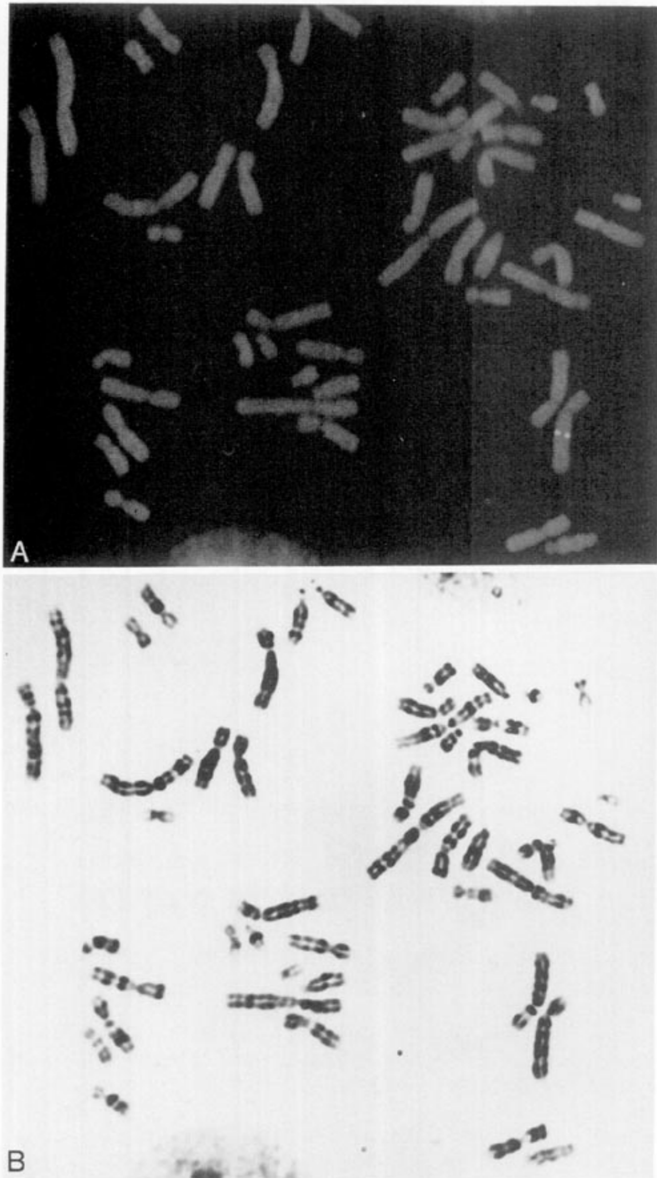

FIG. 1. Localization by fluorescence *in situ* hybridization (FISH) of the human gene H2AZ to human chromosome 4q24. **(A)** A metaphase chromosome spread after FISH with a biotinylated genomic DNA probe shows fluorescent signals on both chromatids of the long arm of chromosome 4. **(B)** The same spread after G-banding with Wright's stain permits the localization of the signal to region 4q24.

tains introns and encodes a polyadenylated mRNA species in contrast to the replication-linked histone genes that lack introns and encode short mRNA species that terminate in a stem–loop structure (2).

The human gene locus H2AZ was assigned to chromosome 4 by challenging a panel of 27 human–hamster hybrid cell lines with oligonucleotide probes specific to two regions of the human gene. The human gene H2AZ locus has three *Eco*RI sites, yielding 2.9-kb upstream and 4.7-kb downstream fragments after digestion (see Fig. 1 of Ref. 2). Commercial Southern blots were obtained with *Eco*RI-digested DNA preparations from the 27 lines (Bios Corp.). An oligonucleotide probe, taagagaacgctagagggagctggtgttca, to intron 3 of the gene gave one human-specific band on these blots consistent in size with the expected 4.7-kb downstream *Eco*RI fragment; this band was mapped to chromosome 4 (2 hybrid lines with chromosome 4, 25 without it; 27 concordances, no discordances). A 5′ utr oligonucleotide probe, tgccttgcttgcttgagcttcagcggaatt, to the upstream 2.9-kb fragment yielded two bands on these Southern blots. The smaller band, consistent in size with the expected 2.9-kb *Eco*RI gene fragment, was also mapped to chromosome 4 (27 concordances, no discordances). The larger, approximately 6-kb band was mapped to chromosome 21 (7 hybrid lines with chromosome 21, 20 without it; 26 concordances, 1 discordance) and may result from a possible pseudogene (2). From these results, the human gene H2AZ is assigned to chromosome 4; a possible pseudogene is assigned to chromosome 21. Thus, the human gene H2AZ is not part of the clusters of human replication-linked histone genes that have been assigned to chromosomes 1, 6, and 12 (5).

The H2AZ gene locus was determined by fluorescence *in situ* hybridization (FISH) as described by Pinkel *et al.* (3) using H2AZ genomic fragments, including 3.2 kb of upstream, 0.9 kb of cDNA, 1.3 kb of intron, and about 2.6 kb of downstream sequences. The 7.2-kb *Cla*I–*Cla*I fragment and the partially overlapping downstream 4.8-kb *Eco*RI–*Eco*RI fragment shown in Fig. 1 of Hatch and Bonner (2), were labeled by nick-translation with biotin-11-dUTP. Chromosome spreads were prepared from methotrexate-synchronized normal peripheral leukocyte cultures. After pretreatments to digest RNA and denature the DNA, the spreads were hybridized with the biotin-labeled DNA probe (200 ng) for 18 h at 37°C in a solution consisting of 2× SSC, 50% (v/v) formamide, 10% (w/v) dextran sulfate, 2× Denhart's solution, 1% Tween 20 (v/v), and 50 μg human C_0t-1 DNA (GIBCO BRL). After hybridization, the spreads were washed in 50% formamide, 2× SSC at 42°C and in 0.1× SSC at 60°C. The biotin-labeled DNA was detected by fluorescein isothiocyanate (FITC)-conjugated avidin DCS and antiavidin antibodies (Vector Laboratories). Chromosomes were counterstained with propidium iodide and examined with a Olympus BH2 epifluorescence microscope.

For each chromosomal spread, two consecutive 8-bit grayscale images (FITC and propidium iodide) were recorded, precisely overlaid (GeneJoin Layers), merged (GeneJoin MaxPix), and digitally printed on a Phaser IISDX printer. For chromosome banding, the slides with recorded labeled metaphases were stained with DAPI (0.2 μg/ml). If the DAPI staining gave unsatisfactory banding patterns, the slides were destained, dehydrated, dried, digested with trypsin (GIBCO BRL) diluted in Hanks' balanced salt solution (1:50) for 30 s, and stained with Wright's stain (4). The chromosome spreads were relocated and the images compared.

As shown in Fig. 1, the H2AZ gene was mapped to chromosome 4q24 using this methodology. Only spreads with chromosomes exhibiting symmetrical fluorescent signals on both chromatids were recorded and included for analysis. Fluorescent doublets on chromosome 4 were observed in 71 of 100 metaphases or prometaphases examined on two independent hybridizations with the probe. Fluorescent doublets were not observed on any other chromosome. Double symmetrical signals on both chromosome 4 homologues were initially found on only four of the examined metaphases, but after an additional amplification with fluorescein avidin/antiavidin antibodies, the percentage increased to 60%. However, the signal to background ratio was unacceptable for further analysis.

The location of the signal on the long arm of chromosome 4 was determined on trypsin- or DAPI-banded chromosomes using on-screen measurements and comparison of enlarged digital images of both labeled and banded chromosomes. Of a total

of 44 individual chromosomes from 38 metaphase spreads with symmetrical doublets, the signal was localized on a [illegible]-band idiogram to chromosome region 4q24, to which we assign the locus of the human gene H2AZ.

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