A Histone Variant, H2AvD, is Essential in *Drosophila melanogaster*

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H2AvD, a *Drosophila melanogaster* histone variant of the H2A.Z class, is encoded by a single copy gene in the 97CD region of the polytene chromosomes. Northern analysis shows that the transcript is expressed in adult females and is abundant throughout the first 12 h of embryogenesis but then decreases. The H2AvD protein is present at essentially constant levels in all developmental stages. Using *D. melanogaster* stocks with deletions in the 97CD region, we have localized the H2AvD gene to the 97D1-9 interval. A lethal mutation in this interval, *l(3)810*, exhibits a 311-base pair deletion in the H2AvD gene, which removes the second exon. P-element mediated transformation using a 4.1-kilobase fragment containing the H2AvD gene rescues the lethal phenotype. H2AvD is therefore both essential and continuously present, suggesting a requirement for its utilization, either to provide an alternative capability for nucleosome assembly or to generate an alternative nucleosome structure.

INTRODUCTION

The core histones are among the most highly conserved proteins known, as might be anticipated from their central role in generating the compact octamer structure used in DNA packaging (Kornberg and Thomas, 1974; Argents *et al.*, 1991). Minor variations that do occur in the primary structure of the histones fall into two categories (reviewed by Wu *et al.*, 1986). In the first category are the evolutionary changes that have occurred, generally amino acid substitutions or the addition or deletion of a few amino acids. These changes frequently occur in the charged N- and C-terminal "tails." Relatively little change is seen in the two-thirds of the protein involved in histone-histone interactions; there is virtually no change in the free energy of binding between histones derived from the same or different eukaryotic organisms (with the exception of the ciliates), reflecting conservation in the interactions that generate the histone octamer core of the nucleosome (Spiker and Isenberg, 1977). In the second category are those histone sequences that have varied within the same species, are represented by different genes, and are expressed under different regulatory systems. These include clusters of different histone genes expressed at different times in

development, the best studied cases being in sea urchin embryogenesis (Newrock *et al.*, 1978); histone variants expressed in specific terminally differentiated cells, such as the histone H5 specific to nucleated red blood cells (Neelin *et al.*, 1964; Ruiz-Carrillo *et al.*, 1974); and variants expressed in the majority of the nuclei but uncoupled from the cell-cycle regulation that dominates bulk histone synthesis (Franklin and Zweidler, 1977; Zweidler, 1984). Among the latter are several with distinctly different amino acid sequences, which might be capable of generating a nucleosome structure that differs significantly in its shape, stability, or other properties when compared with a nucleosome assembled from the cell-cycle-regulated core histones.

The H2A.Z variant is of particular interest because of its abundance in the nucleus, its conserved structure, and its inferred association with euchromatin. The variant H2A.Z was originally characterized in vertebrates, where it represents 5–10% of the H2A in all cell types and species examined (West and Bonner, 1980). Subsequently, the H2A.Z protein and/or its gene have been characterized in creatures as diverse as chick (Harvey *et al.*, 1983), sea urchin (Ernst *et al.*, 1987), *Drosophila* (van Daal *et al.*, 1988), and *Tetrahymena* (White *et al.*, 1988). H2A.Z has several characteristics that distinguish it from the major cell-cycle-regulated H2A, designated H2A.1. In general, the H2A.Z variant is only ~60% identical in amino acid sequence with the corresponding

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H2A.1 in a given species. H2A.Z does have the characteristic sequence AGLQFPVGR at amino acids 23–31; this characteristic region, known as the H2A box, clearly defines H2A.Z as an H2A histone (Wu *et al.*, 1986; Hatch and Bonner, 1988). However, there are significant differences. For example the *D. melanogaster* H2A.Z protein, designated H2AvD, differs from its H2A.1 cognate in having an N-terminal alanine rather than serine; in having an N-terminal peptide (amino acids 1–22) with a net charge of +6 rather than +8; in having an internal peptide (amino acids 38–43) of RTTSHG, changed from KGNVYAE; and in having a longer C-terminal peptide (starting at amino acid 122), of 19 amino acids instead of 3 (van Daal *et al.*, 1988). That these changes are significant is implied by the observation that, aside from the C-terminal tail, the sequences of the H2A.Z proteins of *Drosophila* and mammals differ in only 3 of 121 amino acids. Comparison of the protein sequences of the Z variant and cell-cycle-regulated histone H2As from six species suggests that the Z variant and the cell-cycle-regulated H2A genes diverged before the separation of *Schizosaccharomyces pombe* from the rest of the eukaryotic lineage (van Daal *et al.*, 1990). No Z variant has been reported for *Saccharomyces cerevisiae*. Although *S. cerevisiae* has two genes encoding H2A (each paired with a gene for H2B), the H2A protein products differ only by a change of Ala-Thr to Thr-Ala at amino acid positions 124 and 125 (Choe *et al.*, 1982). Although loss of one of the H2A-H2B gene pairs (*TRT2*) has virtually no phenotype, loss of the other (*TRT1*) alters the mitotic and meiotic cycles. This appears to be due to the fact that only the *TRT1* gene pair can compensate by changing the levels of gene expression (Norris and Osley, 1987).

In higher eukaryotes, histone variant genes also differ significantly from the cell-cycle-regulated genes in their mode of regulation and in their organizational characteristics. In all species studied to date, H2A.Z is synthesized throughout the cell cycle, whereas H2A.1 is synthesized predominantly in S-phase (Wu and Bonner, 1981). The mRNA for H2A.1, and other cell-cycle-regulated histones, is not polyadenylated, whereas the mRNA for H2A.Z, and other variants expressed throughout the cell cycle, is polyadenylated (Old and Woodland, 1984). In addition, the H2A.Z variant genes from several different species contain introns, the positions of which have been conserved (van Daal *et al.*, 1990), whereas *Drosophila* H2A.1, and cell-cycle-regulated histone genes in general, have no introns.

In *D. melanogaster*, the gene for the H2AvD variant is single copy and is not linked to the histone gene cluster at 39D2-3 through 39E1-2 (Pardue *et al.*, 1977), which contains 110 copies of the major (presumably cell-cycle-regulated) *D. melanogaster* histone genes (Lifton *et al.*, 1978). The unique nature of this variant gene, along with its antiquity and its highly conserved structure, suggests that the H2A.Z protein plays an important role.

The homologous *Tetrahymena* H2A variant, hvl, is found in the transcriptionally active macronucleus but is absent from the transcriptionally inert micronucleus, suggesting that this variant is associated with "active" chromatin (Allis *et al.*, 1980). However, the nature of its function, or even its necessity, has not been demonstrated previously for any H2A.Z-type variant. In this report we show that while the H2AvD gene is primarily expressed early in *D. melanogaster* embryogenesis, the protein is present at all developmental stages. We demonstrate that this H2A variant is essential in *D. melanogaster* by rescuing a lethal mutation using P-element-mediated transformation with the H2AvD gene.

MATERIALS AND METHODS

Isolation and Analysis of RNA

RNA was isolated by a hot phenol/chloroform extraction procedure (Jowett, 1986). RNA was electrophoresed on a 1.5% agarose, 2.1% formaldehyde gel in MOPS buffer (0.04 MOPS, pH 7.0, 10 mM sodium acetate, 1 mM EDTA) and transferred to nylon membrane, prehybridized at 65°C for 2 h in 2× Denhardt's solution (0.04% Ficoll, 0.04% polyvinyl pyrrolidone 360, 0.04% bovine serum albumin [BSA]), 5× SSC (SSC, 0.15 M NaCl, 0.015 M sodium citrate), 6% polyethylene glycol (PEG), 0.2% sodium dodecyl sulfate (SDS), and hybridized at 65°C for 20 h in prehybridization mixture plus 50 µg/ml denatured salmon sperm DNA and 2 × 10⁶ cpm/ml of probe. Probes were labeled with ³²P-dNTPs by nick translation. Filters were washed twice for 30 min each at room temperature and twice at 65°C in 0.2× SSC, 0.2% SDS. Filters were exposed to preflashed XAR-5 (X-omat AR; Kodak, Rochester, NY) film. Autoradiographs were scanned using a computing densitometer (model 300A; Molecular Dynamics, Sunnyvale, CA).

Isolation and Analysis of Histone Proteins

Histone proteins were extracted from *D. melanogaster* nuclei prepared according to Elgin and Hood (1973). The nuclei were washed once in 0.35 M NaCl, 10 mM tris(hydroxymethyl)aminomethane (Tris), pH 7.4, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 1 mM N-ethylmaleimide and once in 7% perchloric acid. Core histones were extracted by homogenization of the nuclei in 0.4 N sulfuric acid. Nuclear debris were removed by centrifugation at 400 × g, and the histones were precipitated in 6 volumes of acetone. The histones were collected by centrifugation at 4400 × g and resuspended in phosphate-buffered saline. Histones were electrophoresed on a 15% SDS polyacrylamide gel in Tris-glycine buffer (0.25 M Tris, pH 8.8, 1.9 M glycine) and electrotransferred to nitrocellulose at 6 V/cm in Tris-glycine buffer plus 0.01% SDS. The nitrocellulose filter was incubated for 30 min in TBST (10 mM Tris, pH 8.0, 150 mM NaCl, 0.05% Tween-20), 1% BSA. H2AvD antiserum diluted to 1:100 was added and the antibodies allowed to bind for 30 min. Excess antibodies were removed by three washes in TBST, 5–10 min each. Goat anti-rabbit IgG coupled to peroxidase was added (1:7500) and the filter incubated for 30 min. Excess secondary antibody was removed by washing as above. The reaction was visualized by incubating the filter with 0.0032% nitroblue tetrazolium, 0.0016% 5-bromo-4-chloro-3-indolyl phosphate in 100 mM Tris 9.5, 100 mM NaCl, 5 mM MgCl₂. The colorimetric reaction was stopped by transferring the filter to water.

Antibody Production

A synthetic peptide containing 15 amino acids from the C-terminal region (amino acids 126–140) of a conceptual translation of the H2AvD cDNA was produced, starting from an N-terminal cysteine residue. An antibody was made using the procedure of Green *et al.* (1982).

Briefly, this peptide was linked to keyhole limpet hemocyanin (KLH) via the cysteine residue. Twelve milligrams KLH in 75 ml 10 mM sodium phosphate, pH 7.3, was incubated with 2.1 mg maleimido benzoyl-N-hydroxy succinimide ester (MBS) in dimethylformamide for 30 min at room temperature. The KLH-MBS complex was isolated using a 40 × 0.9-cm Sephadex G-25 column in 0.5 M sodium phosphate, pH 6.0. This was incubated with 15 mg peptide for 16 h at room temperature. The complexed peptide was isolated from a 40 × 0.9-cm Sephadex G-25 column in 0.5 M sodium phosphate, pH 6.0. One hundred micrograms antigen in Freund's complete adjuvant (1:1) was injected interdermally into a rabbit. This was followed by five booster injections of 100 µg antigen in Freund's incomplete adjuvant (1:1). Specificity of the antiserum was established using a Western blot in the analysis of total *Drosophila* histones.

Polytene Chromosome Staining

D. melanogaster third instar larval polytene chromosomes were prepared and stained as described in Silver and Elgin (1977). H2AvD antiserum was used at a 1:2500 dilution. Fluorescein-conjugated goat anti-rabbit IgG was used at a 1:100 dilution.

Southern Analysis

DNA was isolated by homogenization of *D. melanogaster* embryos or adults in 0.1 M Tris, pH 8, 50 mM NaCl, 1% SDS, 0.15 mM spermine, 0.5 mM spermidine. Proteinase K was added to a final concentration of 100 µg/ml and the mixture was incubated at 50°C for 2–16 h with occasional gentle agitation. Three extractions with an equal volume of phenol:chloroform were performed. The aqueous phase was dialysed against three changes of TE (10 mM Tris, 1 mM EDTA, pH 8.0). RNAase was added to a final concentration of 100 µg/ml, and the solution was incubated for 1–4 h at 37°C. Phenol:chloroform extractions and dialysis were repeated as described. Samples were run on a 0.8% agarose gel and analyzed by Southern blot, using the same hybridization and wash conditions as described for RNA analysis.

Sequence Amplification

DNA sequences were amplified using the polymerase chain reaction. 0.1 µg *Drosophila* DNA was incubated with 20 µM primers, 200 µM each dATP, dCTP, dGTP, dTTP, and 2.5 units Taq polymerase in 5 mM KCl, 1 mM Tris-HCl, pH 7.4, 0.15 mM MgCl₂, 10 µg/ml gelatin. The primers used were P1 = 5'AAACCGAATTCCGTAGAA and P2 = 5'CACGGCTGCAGTGGCTC. Thirty-five rounds of amplification were performed (one round = 1 min 94°C, 1 min 46°C, 2 min 72°C) using a DNA thermal cycler (Perkin Elmer-Cetus, Norwalk, CT).

RNA sequences were copied into a DNA template and then amplified as above. 0.1 µg total RNA and 200 µM primer were heated to 85°C for 3 min and allowed to anneal by cooling to 42°C over a 10-min period. Six hundred micromoles each dATP, dCTP, dGTP, dTTP, 10 units RNasin, and 10 units reverse transcriptase in 50 mM Tris-HCl, pH 7.6, 60 mM KCl, 10 mM MgCl₂, 1 mM dithiothreitol was added and DNA synthesis allowed by incubation at 42°C for 1 h.

Sequencing

Polymerase chain reaction (PCR) products were separated on polyacrylamide gels and recovered by elution in 10 mM Tris, 1 mM EDTA, 100 mM NaCl at 37°C for 16 h. The fragments were cloned into M13mp18 and M13mp19 and sequenced using Sequenase (United States Biochemicals, Cleveland, OH).

Genetic Stocks

Developmental analysis was carried out using *D. melanogaster* Oregon R. Deficiency stocks *Df(3R)ro^{80b}* (Lewis, unpublished data; Anderson *et al.*, 1985), *Df(3R)ro^{xB3}* (Lewis, unpublished data; Tomlinson *et al.*,

1988), *Df(3R)TI^{9QRX}* (Anderson *et al.*, 1985), and *Df(3R)Ser^{+R2f}* (Lewis, unpublished data; Tomlinson *et al.*, 1988) were obtained from Kathryn Anderson, University of California, Berkeley. Strains carrying lethal mutations within the 97D1-9 interval were provided by Rob Saint, University of Adelaide, Australia [*l(3)15* and *l(3)113*], and by Kathryn Anderson [*l(3)155*, *l(3)810*, *l(3)835*].

RESULTS

Developmental Expression of the H2AvD Transcript

RNA from sequential developmental stages of *D. melanogaster* was probed with the H2AvD cDNA clone 10₁ (van Daal *et al.*, 1988) (Figure 1) and with the genomic clone 4_{1C}, which contains the transcribed region and ca. 4.0 kilobase (kb) upstream and 9.5 kb downstream of surrounding DNA (identical results; unpublished data). Two transcripts are observed: an abundant transcript of 0.9 kb and a minor transcript of 1.2 kb. Note that the former is the same size as the cDNA previously sequenced (van Daal *et al.*, 1988). The identity of the larger transcript is not known, but its expression parallels that of the smaller more abundant transcript. Densitometer scanning of the autoradiograph shows that the 1.2-kb transcript is present at approximately one-fiftieth the level of the 0.9-kb species. These observations sug-

Figure 1. Expression of H2AvD. Ten micrograms of RNA isolated from 0- to 2-, 2- to 4-, 4- to 8-, 8- to 12-, and 12- to 18-h embryos, larvae, pupae, and female and male adults were electrophoresed on a 1.5% denaturing agarose gel. After transfer to nylon membrane, the filter was probed with the full-length H2AvD cDNA clone 10₁ (van Daal *et al.*, 1988).

Figure 2. Developmental profile of H2AvD protein. Fifteen micrograms of histone from 0- to 6-, 6- to 12-, and 12- to 18-h embryos, first instar larvae, and pupae and 1 μ g of histones from adults were electrophoresed on a 15% SDS polyacrylamide gel and electrotransferred to nitrocellulose. The filter was incubated with H2AvD antiserum and stained as described in MATERIALS AND METHODS. The level of H2AvD is relatively constant.

gest that the larger transcript is an aggregated or incompletely denatured form of the 0.9-kb transcript, although other possibilities, such as incomplete RNA processing, have not been ruled out. Northern analysis using a probe that detects transcripts from the H2A.1 and H2B.1 genes (*Hae* II fragment) (Gilmour, personal communication) shows the same developmental profile as is seen for the H2AvD gene (unpublished data), confirming earlier results of Anderson and Lengyel (1984). The presence of the H2AvD transcripts in adult females, but not adult males, and in 0-2 h embryos suggests that the gene is maternally transcribed and the RNA loaded into the developing oocyte.

Developmental Profile of the H2AvD Protein

Histones were isolated from nuclei prepared from embryos, larvae, pupae, and adult flies and electrophoresed on a 15% SDS polyacrylamide gel. An antibody specific for H2AvD (directed against the unique C-terminal peptide, as described in MATERIALS AND METHODS) was used to determine the developmental profile of the H2AvD protein. The result, illustrated in Figure 2, shows that the H2AvD protein is present throughout all stages of development; one major protein is observed. The H2AvD protein appears to be present at an essentially constant level relative to the major core histones, which are themselves quite stable (Wu *et al.*, 1986). Immu-

nofluorescent staining of polytene chromosome preparations from third instar larvae shows a widespread, but not general, distribution of H2AvD (Figure 3).

Localization of the H2AvD Gene

The H2AvD gene was previously mapped to the 97CD region on the right arm of chromosome 3 by in situ hybridization to third instar larval polytene chromosomes (van Daal *et al.*, 1988). Higher resolution mapping was accomplished using four strains of *D. melanogaster* carrying heterozygous deletions in the 97CD region, indicated on the map in Figure 4. Genomic DNA from these strains was digested with *Eco*RI, size separated by gel electrophoresis, and transferred to a nitrocellulose filter. To determine the number of copies of the H2AvD gene present in each strain, the filter was probed with a genomic clone encompassing the H2AvD gene (4_{1G})

Figure 3. Distribution of H2AvD in the polytene chromosomes. Polytene chromosomes from third instar larvae stained using an antiserum specific for H2AvD. Top, phase contrast; bottom, immunofluorescence.

Figure 4. Southern analysis of *Drosophila melanogaster* stocks carrying deletions in the 97CD region. DNA was isolated from four strains carrying deletions in the 97CD region, *Df(3R)ro^{80b}* (Lewis, unpublished data; Anderson *et al.*, 1985), *Df(3R)ro^{XB3}* (Lewis, unpublished data; Tomlinson *et al.*, 1988), *Df(3R)TI^{9QRX}* (Anderson *et al.*, 1985), and *Df(3R)Ser^{+R82f}* (Lewis, unpublished data; Tomlinson *et al.*, 1988). The map below indicates the relative positions of the break points; the regions deleted are indicated by the bracketed lines. Ten micrograms of DNA from each strain was digested with *EcoRI*, electrophoresed on a 0.8% agarose gel, transferred to nylon membrane, and probed with the H2AvD genomic clone 4_{1G} and clone 88.3 (Corces *et al.*, 1980) covering the heat shock *hsp26* gene. The first lane contains 4_{1G} DNA digested with *EcoRI*. The bands corresponding to the H2AvD and *hsp26* genes are indicated.

and with a genomic clone encompassing heat shock gene *hsp26* (88.3) (Corces *et al.*, 1980), a single copy gene used for quantitative reference. Figure 4 illustrates that the bands corresponding to the H2AvD gene are present at one-half the intensity of the band corresponding to the *hsp26* gene in the *Df(3R)ro^{80b}* and *Df(3R)ro^{XB3}* deletion strains, whereas the bands corresponding to the two genes are present at equal intensity

in the *Df(3R)TI^{9QRX}* and *Df(3R)Ser^{+R82f}* deletion strains. This indicates that the H2AvD gene falls within the region 97D1-9.

This finding was confirmed by the observation that some genomic clones containing all or part of the *rough* gene, previously mapped to region 97D5-7 (Tomlinson *et al.*, 1988) overlap (by hybridization) with the H2AvD-containing genomic clone 4_{1G} (unpublished data).

Identification of a Lethal Mutation in 97D1-9 that Alters the H2AvD Gene

Lethal complementation groups in the 97D1-9 region have been defined using ethylmethanesulphonate (EMS) mutagenesis by the laboratories of Dr. K. Anderson, University of California, Berkeley, and Dr. R. Saint, University of Adelaide. DNA samples from five heterozygous lines carrying lethal mutations in the 97D1-9 region were digested with *Hinc* II or *Dde* I, size separated by electrophoresis on an agarose gel, transferred to nitrocellulose by Southern blot, and probed with an H2AvD genomic clone (5_{1G}). Figure 5A shows that the *l(3)810* mutation is associated with a single additional band in these digests, which could be due to a deletion of ~300 base pair (bp). This putative deletion was further localized by digesting DNA from heterozygous flies carrying the *l(3)810* mutation with additional restriction enzymes (see Figure 5B). The locations of the restriction sites in the wild-type H2AvD gene are shown in the map at the bottom of the figure. Figure 5B shows that the 3.4-kb *EcoRI* fragment is less intense in *l(3)810* DNA compared with *l(3)835* DNA, and that there is a 3.1-kb band present in *l(3)810* DNA digest not seen in the *l(3)835* DNA digest. The 3.8-kb fragment in the *Pst* I digest of *l(3)810* DNA (resulting from the *Pst* I site within the gene and one to the left on the map) is reduced in intensity and a 3.5-kb band is seen as well. This places the deletion between the *EcoRI* and *Pst* I sites as indicated on the map. Oligonucleotide primers P1 and P2, shown on the map, were used in a PCR with *l(3)810* DNA. The resulting fragments of ~800 and 490 bp were cloned into M13 and sequenced. The larger fragment gave exactly the sequence of the wild-type H2AvD gene, whereas the shorter fragment showed a deletion of 311 bp as indicated in Figure 6. The precise region of the deletion is from nucleotide 287 to nucleotide 597 (this is nucleotide 555 to nucleotide 865 in Figure 2 of van Daal *et al.*, 1990), which removes the region surrounding and including the second exon. This deletion does not alter the reading frame of the H2AvD protein but would result in a protein product 26 amino acids shorter than the wild-type product, lacking the N-terminal tail and part of the H2A box. Western analysis, using antibodies specific for the C-terminal tail of H2AvD, of histone isolated from flies heterozygous for the *l(3)810* mutation showed no evidence of such a product (unpublished data). RNA from *l(3)810* flies was

Figure 5. Southern analysis of lethal complementation groups in the 97D1-9 region. (A), *l(3)810* is heterozygous in the H2AvD gene region. Ten-microgram aliquots of DNA from each of five stocks with EMS-induced lethal mutations in the 97D1-9 interval, *l(3)15*, *l(3)113*, *l(3)155*, *l(3)810*, and *l(3)835*, were digested with *Hinc* II and *Dde* I, electrophoresed on a 0.8% agarose gel, transferred to nylon membrane, and probed with the H2AvD genomic clone 5_{1C}. (B), *l(3)810* carries a deletion between the *Eco*RI and *Pst* I sites. Ten-microgram aliquots of DNA from flies carrying the *l(3)810* and *l(3)835* mutations were digested with *Bam*HI, *Bgl* II, *Dra* I, *Eco*RI, and *Pst* I, electrophoresed on a 0.8% agarose gel, transferred to nylon membrane, and probed with the H2AvD genomic clone 5_{1C}. Map of the H2AvD gene region (shown below). The hatched regions represent transcribed but not translated sequences; the filled boxes represent translated sequences of the H2AvD gene. Restriction enzymes are indicated as follows: A, *Dra* I; B, *Bgl* II; D, *Dde* I; E, *Eco*RI; H, *Hinc* II; M, *Bam*HI, and P, *Pst* I. P1 and P2 are the oligonucleotide primers used for PCR analysis (see Figure 6).

used to generate cDNA for a PCR reaction with primers P1 and P2. Two products of 312 and 234 bp were sequenced and shown to represent the wild-type H2AvD coding sequence and a product where the first exon is spliced to the third exon, as anticipated. This smaller product was seen in approximately the same amount as the wild-type species, suggesting that transcription of both the wild-type and mutant genes occurs at the same level.

Rescue of Lethality

The 4.1-kb *Bgl* II fragment encompassing the gene (Figure 5) was cloned into the *Bam*HI site of the P-element vector Casper, which carries a miniwhite marker gene (Pirota, 1988). This DNA was injected into *white* embryos; two transformed lines were established. A line

in which the inserted DNA was shown to be on chromosome 2 (by its segregation away from a second chromosome balancer *SM6a*, *Cy*) was used to show rescue of lethality of the *l(3)810* mutation on chromosome 3. This was demonstrated by crossing *l(3)810* flies carrying the inserted copy of the H2AvD gene to flies heterozygous for the deficiency *Df(3R)ro^{XB3}* as illustrated in Figure 7. Flies carrying the *l(3)810* mutation over this deficiency of the 97D1-9 region were seen from the final cross at a frequency of ~30% of emerging flies, indicating rescue due to the presence of the inserted copy of H2AvD gene. *l(3)155* and *l(3)835* flies carrying the inserted H2AvD gene were also crossed to flies heterozygous for the deficiency *Df(3R)ro^{XB3}*. No flies of the *l(3)155/Df(3R)ro^{XB3}* or *l(3)835/Df(3R)ro^{XB3}* genotype were seen, confirming that rescue was specific for the *l(3)810* mutation.

		P1							
wt:	1	<u>AAACCGAATT</u>	<u>CCGTAGAAAC</u>	AAC TCGCAGC	CTCCGGTTTC	GTGTTGCAAC	AAAATAGGCA		
l(3) 810:		<u>AAACCGAATT</u>	<u>CCGTAGAAAC</u>	AAC TCGCAGC	CTCCGGTTTC	GTGTTGCAAC	AAAATAGGCA		
wt:	61	TTCCCATCGC	GGCAGTTAGA	ATCACCGAGT	GCCCAGAGTC	ACGTTTCGTAA	GCAGGCGCAG		
l(3) 810:		TTCCCATCGC	GGCAGTTAGA	ATCACCGAGT	GCCCAGAGTC	ACGTTTCGTAA	GCAGGCGCAG		
wt:	121	TTTACAGGCA	GCAGAAAAAT	CGATTGAAGA	GAAATGgtaa	tattgcggtg	aatttttgag		
l(3) 810:		TTTACAGGCA	GCAGAAAAAT	CGATTGAAGA	GAAATGgtaa	tattgcggtg	aatttttgag		1
		Met							
wt:	181	cggcagcgca	tctcgctttt	cccactagcg	ctgcccccg	gaattaaata	ttgaagtgcg		
l(3) 810:		cggcagcgca	tctcgctttt	cccactagcg	ctgcccccg	gaattaaata	ttgaagtgcg		
wt:	241	gggattacgg	agacgcggac	aacaaatgca	cgcaaatgga	caccacgtag	gccgcaacaa		
l(3) 810:		gggattacgg	agacgcggac	aacaaatgca	cgcaaatgga	caccac----	-----		
wt:	301	caaggagcag	cgaaatcggc	gggcattgtt	attgtgctgc	tagcgggcaa	tcggccgtaa		
l(3) 810:		-----	-----	-----	-----	-----	-----		
wt:	361	cctcactttg	ggcagcggtg	cctccatttt	gtgttttctt	ctgcgactcg	tgccacattc		
l(3) 810:		-----	-----	-----	-----	-----	-----		
wt:	421	gctttaattc	gttattttta	gaaatgcatt	gtgctgtgtc	tattcgagG	CTGGCGGTAA		
l(3) 810:		-----	-----	-----	-----	-----	-----	A	laGlyGlyLy 5
wt:	481	AGCAGGCAAG	GATTCGGGCA	AGGCCAAGGC	GAAGGCGGTA	TCGCGTTCCG	CGCGCGCGGG		
l(3) 810:		-----	-----	-----	-----	-----	-----		
		sAlaGlyLys	AapSerGlyL	ysAlaLysAl	aLysAlaVal	SerArgSerA	laArgAlaGl	25	
wt:	541	TCTTCAGgtg	agtttttaca	tctgtacctc	ggttttggcg	ccttttcggt	acccactctg		
l(3) 810:		-----	-----	-----	-----	-----	-----	ctg	27
		yLeuGln							
wt:	601	ccaacgagca	tattccacac	ataacagggt	ggcccgccct	cgttggttgg	ttgggggtga		
l(3) 810:		ccaacgagca	tattccacac	ataacagggt	ggcccgccct	cgttggttgg	ttgggggtga		
wt:	661	caaaaacata	acaagccagc	cggtcaagcc	aattaaccag	ttacctgcct	cttatagtcc		
l(3) 810:		caaaaacata	acaagccagc	cggtcaagcc	aattaaccag	ttacctgcct	cttatagtcc		
wt:	721	cggacagTTC	CCCGTGGGTC	GCATCCATCG	TCATCTCAAG	AGCCGCACTA	CGTCACATGG		
l(3) 810:		cggacagTTC	CCCGTGGGTC	GCATCCATCG	TCATCTCAAG	AGCCGCACTA	CGTCACATGG		
		Phe	ProValGlyA	rgIleHisAr	gHisLeuLys	SerArgThrT	hrSerHisGl	45	
		P2							
wt:	781	<u>ACGCGTCGGA</u>	<u>GCCACTGCAG</u>	<u>CCGTG</u>					
l(3) 810:		<u>ACGCGTCGGA</u>	<u>GCCACTGCAG</u>	<u>CCGTG</u>					
		vArgValGlv	AlaThrAlaA	laVal					53

Figure 6. Comparative sequence of wild-type and *l(3)810* mutant DNAs encoding H2AvD. The DNA sequence between P1 and P2, the two oligonucleotide primers used to amplify the region containing the deletion. P1 and P2 are underlined. Nucleotides are numbered on the left, whereas amino acids are numbered on the right with the translation start as +1. Only the 5'→3' strand is shown for the larger (wild-type [wt]) and smaller [*l(3)810*] PCR products obtained. DNA sequence from exons is given in upper case letters, whereas that from introns is given in lower case letters. Dashes are used where there is no corresponding sequence in the *l(3)810* PCR product.

DISCUSSION

While the existence of histone variant proteins has been documented for some time and their incorporation into nucleosomes established (reviewed by Wu *et al.*, 1986), their specific functions have remained unknown. The histone H2A variants of the H2A.Z type were first characterized in mammals and have since been shown to be evolutionarily conserved. The H2A.Z protein differs from H2A.1 in several features that could effect histone-DNA and histone-histone interactions. We have previously isolated and characterized cDNA and genomic clones for the *D. melanogaster* H2A.Z gene, H2AvD (van Daal *et al.*, 1988, 1990). The highly conserved nature

of the protein and the conservation of gene structure observed for *Tetrahymena* (van Daal *et al.*, 1990), *Drosophila* (van Daal *et al.*, 1990), chicken (Dalton *et al.*, 1989), and humans (Hatch and Bonner, 1990) suggests that this protein has an important function in the cell.

Developmental Expression of H2AvD

Transcripts from the H2AvD gene are present in adult females and are abundant during the first 12 h of embryogenesis, peaking at ca. 6 h, the time of maximal DNA synthesis. The H2AvD transcripts thus show the same developmental profile over time as do the bulk of the histone transcripts, those from the histone genes

encoded at region 39D2-3 to 39E1-2, including the H2A.1 transcript (Anderson and Lengyel, 1984). Because the H2A.1 mRNA and protein are generally synthesized predominantly during the S-phase of the cell cycle, it is not surprising that the *Drosophila* H2A.1 transcript is not abundant after 12 h, because mitotic divisions largely cease at about this time in *D. melanogaster* embryogenesis (Madhavan and Schneiderman, 1977). Although one would not expect expression of the variant to be regulated within the cell cycle, the similarity of the developmental profiles of the H2A.1 and H2AvD transcripts suggests that synthesis of H2AvD, while constant throughout the cell cycle, may be related to the relative rate of cell division. The findings suggest a requirement for H2AvD as the genome is replicated.

observed in other organisms (e.g., West and Bonner, 1980).

The H2AvD gene is single copy; its location at 97CD was confirmed and refined by Southern analysis of overlapping deletions in this region. The gene lies in the 97D1-9 interval, within 10 kb of the *rough* locus. EMS mutagenesis of this region has defined five lethal complementation groups (Anderson and Saint, personal communication). One of these, *l(3)810*, contains a 311-bp deletion that begins in the middle of the first intron and extends to the middle of the second intron of the H2AvD gene. PCR analysis of cDNA from RNA of flies carrying the *l(3)810* mutation indeed indicates the presence of transcripts lacking the second exon.

Deletion of the second exon results in an in-phase deletion of the first 26 amino acids of the protein. The predicted smaller protein was not detected on gels or Western blots in histone samples from flies carrying the *l(3)810* mutation, suggesting that the smaller protein product is either not made, is unstable, is not incorporated into nuclear structures, or is not isolated from nuclei with the acid treatment used to extract histones. P-element-mediated transformation with a 4.1-kb *Bgl* II fragment of the H2AvD gene, which includes about 600 bp of sequence upstream of the transcription start site and 2 kb of sequence downstream of the poly A addition site, was able to rescue lethality of the *l(3)810* mutation, indicating that the lethality is indeed caused by mutation of the H2AvD gene.

The H2A.Z protein is only 60% similar to the major cell-cycle-regulated H2A protein. The high level of conservation of the variant (97% identical between mammals and *Drosophila*, excluding the C-terminal tail beyond residue 121) strengthens arguments that it has a function in nucleosomes distinct from that of H2A.1. The large differences seen in the H2A.Z protein sequence compared with that of H2A.1 suggest that these two proteins could interact differently with neighboring histone molecules and/or with DNA. H2A is known to be in a critical position in that it is located in the region where DNA enters and exists from the nucleosome, presumably a region that would influence folding and unfolding of the DNA (Mirzabekov *et al.*, 1978; Richmond *et al.*, 1984). The observation that the H2A variant in *Tetrahymena* is found exclusively in the transcriptionally active macronucleus and is absent from the transcriptionally inert micronucleus has led to the suggestion that the nucleosome containing the H2A variant might be important for differential packaging related to the transcription process (Allis *et al.*, 1980).

It should be noted that the nucleosome structure as studied to date is an average of nucleosomes containing the cell-cycle-regulated histones only and nucleosomes containing the cell-cycle-regulated histones plus the basally expressed variants. Analysis of the histones from *Xenopus* oocytes has shown that the histones of this system, often used for nucleosome reconstitution, contain a high proportion of the H2A.Z variant (Shimamura and Worcel, 1989). It should be possible to use purified histones and histone variant proteins to selectively reassemble nucleosomes containing either the cell-cycle-regulated histones or the variant histones for comparative analysis. Inclusion of a variant H2A molecule could potentially result in significant alterations of the octamer structure and in its interaction with DNA. Significant differences within the portion of the histone responsible for histone-histone contacts were pointed out in the INTRODUCTION. The N-terminal tail of H2AvD lacks a Ser at the N-terminus (a site of modification for H2A.1) and has a different net charge from H2A.1 (+6 instead of +8). The H2AvD protein has a C-terminal tail that is 16 amino acids longer than H2A.1 of *D. melanogaster*. It is known that ubiquitin can covalently modify H2A at lysine 119, indicating that this region is not sterically hindered (reviewed by McGhee and Felsenfeld, 1980). The C-terminal tail should therefore be available for interaction with nonhistone proteins and/or DNA. These structural differences observed between H2A.1 and H2AvD may have important functional consequences.

In addition to the structural differences implied by the large protein sequence divergence between H2A.1 and H2A.Z-type variants, the regulation of the H2A.1 and H2A.Z genes in mammals is also markedly different. Unlike the major histones, which are synthesized primarily during S-phase, H2A.Z is synthesized throughout the cell cycle (Wu and Bonner, 1981). H2A.Z is therefore available to replace any H2A molecules that might be displaced from the DNA in the nonreplicative phases of the cell cycle, for example, during the transcription process. However, the very different protein sequence of H2A.Z suggests that this is not the only functional reason for maintaining a separate gene. Two features of the H2AvD gene differ markedly from those of the H2A.1 genes: the H2A.Z gene has introns and the mRNA is polyadenylated. In contrast, the cell-cycle-regulated histone genes lack introns and do not produce a polyadenylated mRNA, but have a stem-loop structure in the 3'-untranslated tails that has been shown to be involved in determining mRNA processing and stability (Schümperli, 1988). Both features seem designed to maximize histone H2A.1 synthesis in S-phase. The stem-loop structure is not seen in the H2AvD transcripts. The lack of a potential stem-loop, combined with the presence of a polyA tail, may contribute to the presence of the H2A.Z transcript throughout the cell cycle. These questions can now be addressed by utilizing *in vitro*

manipulation of the gene, followed by P-element-mediated transformation and analysis in *D. melanogaster*.

The work described in this paper is the first unequivocal demonstration of the necessity and importance of the H2A.Z class of histone variant proteins. With the H2AvD gene in *D. melanogaster* clearly defined, we now expect to be able to analyze the function of this histone variant and to answer many questions as to the role of this protein in chromatin structure and gene expression.

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