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Histone H2A.Z Is Widely but Nonrandomly Distributed in Chromosomes of *Drosophila melanogaster**

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Variant histones that differ in amino acid sequence from S-phase histones are widespread in eukaryotes, yet the structural changes they cause to nucleosomes and how those changes affect relevant cellular processes have not been determined. H2A.F/Z is a highly conserved family of H2A variants. H2Av, the H2A.F/Z variant of *Drosophila melanogaster*, was localized in polytene chromosomes by indirect immunofluorescence and in diploid chromosomes by chromatin immunoprecipitation. H2Av was widely distributed in the genome and not limited to sites of active transcription. H2Av was present in thousands of euchromatic bands and the heterochromatic chromocenter of polytene chromosomes, and the H2Av antibody precipitated both transcribed and nontranscribed genes as well as noncoding euchromatic and heterochromatic sequences. The distribution of H2Av was not uniform. The complex banding pattern of H2Av in polytene chromosomes did not parallel the concentration of DNA, as did the pattern of immunofluorescence using H2A antibodies, and the density of H2Av measured by immunoprecipitation varied between different sequences. Of the sequences assayed, H2Av was least abundant on 1.688 satellite sequences and most abundant on the *hsp70* genes. Finally, transcription caused, to an equivalent extent, both H2Av and H2A to be less tightly associated with DNA.

The basic unit of chromatin in eukaryotes is the nucleosome. A nucleosome consists of 146 base pairs of DNA wrapped around an octamer of histone proteins H2A, H2B, H3, and H4 (1). Although chromatin is a highly reiterative structure, cells create heterogeneity in the structure of nucleosomes to facilitate and regulate DNA-mediated processes such as transcription. Heterogeneity is created, in part, by posttranslational modifications of histone proteins, including acetylation, phosphorylation, methylation, ubiquitination, and ADP-ribosylation (2–4). Acetylation status of the amino termini of histones H3 and H4, in particular, plays a important role in transcriptional regulation (2, 5).

Heterogeneity in nucleosome structure also results from incorporation of variant histone proteins into the nucleosome. In contrast to the canonical histones, which are multicopy genes expressed during S-phase of the cell cycle, variant histones are encoded by single copy genes that differ in amino acid sequence from S-phase histones and whose expression is not limited to S-phase (6, 7). Variant histones allow specialization of nucleosome structure for specific purposes. Sperm-specific variant histones, for example, facilitate the dramatic compaction of DNA that occurs during spermatogenesis (4, 8). A specific variant of histone H3 is incorporated specifically at centromeres creating a specialized chromatin structure required for proper function of the kinetochore (9–14), and macro-H2A, a histone H2A variant, localizes preferentially to the inactive X chromosome in mammals and may alter chromatin in a way that helps suppress transcription (15). These and other variants have been identified for histones H2A, H2B, and H3 but not H4. Histone variants are widespread, if not universal, in eukaryotes, yet how variant histones change nucleosome structure and how those changes affect relevant cellular processes have not been determined.

H2A.F/Z is a family of H2A variants that are highly conserved across species and substantially divergent from S-phase H2A in any given species (16, 17). An H2A.F/Z variant has been identified in a wide variety of eukaryotes including *Tetrahymena* (hv1), budding yeast (HTA3 and HTZ1), *Drosophila* (D2, H2A.2, H2AvD, His2AvD, His2Av, and H2Av), chickens (H2A.F), mice (H2A.Z), and humans (H2A.Z) suggesting that incorporation of H2A.F/Z into chromatin is evolutionarily ancient and conserved. H2A.F/Z typically constitutes 5–10% of total H2A proteins in the chromatin of cells (18, 19). H2A.F/Z is essential in *Tetrahymena*, *Drosophila,* and mice, but its essential function is not known (20–22). H2A.F/Z may play a role in transcriptional regulation since in *Tetrahymena* its expression is associated with the transcriptionally active macronucleus, and in *Drosophila* its incorporation into chromatin during development is coincident with the start of zygotic gene expression (23, 24). The chromosome loss phenotype caused by mutation to the H2A.F/Z homolog in fission yeast, *pht1*, suggests that H2A.F/Z could also play a role, directly or indirectly, in chromosome segregation (25).

Determining the location of variant histones within chromosomes can provide insight into their function (12, 14, 15). To this end, the location of H2Av, the H2A.F/Z variant histone in *Drosophila melanogaster*, was determined in both polytene and diploid chromosomes in relation to transcriptionally active and inactive genes as well as noncoding sequences, and the results were compared with the localization of S-phase H2A.

EXPERIMENTAL PROCEDURES

Antibody Synthesis—H2Av and H2A polyclonal antibodies were generated by injecting rabbits with synthetic peptides homologous to the carboxyl-terminal 15 amino acids of H2Av or H2A conjugated to keyhole limpet hemocyanin via an added amino-terminal cysteine (Pierce). Se-

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rum was used for all experiments. Affinity purified antibody was generated for H2Av and gave identical results (data not shown).

Immunofluorescence—Immunolocalization of H2Av, H2A, and RNA polymerase II (pol II)[1] to polytene chromosomes was done as described by Shopland and Lis (26). Primary antibodies were H2Av or H2A rabbit antisera used at 1:1000 dilution and a mouse monoclonal antibody, 8WG16 (Babco), was used at a dilution of 1:20. 8WG16 recognizes the heptapeptide repeat of the carboxyl-terminal domain (CTD) of the large subunit of RNA pol II primarily in an unphosphorylated state (27). *In vivo* 8WG16 can still bind phosphorylated pol II that is transcriptionally active if the CTD retains some unphosphorylated repeats but may not bind pol II when the CTD is completely phosphorylated (28, 29). Secondary antibodies were goat anti-rabbit IgG coupled to rhodamine used at a 1:50 dilution (Jackson ImmunoResearch) and goat anti-mouse IgG coupled to Cy2 used at a 1:150 dilution (The Jackson ImmunoResearch). Chromosomes were viewed on a Zeiss Axioscope fluorescence microscope with appropriate filters. Single and double exposure photographs were taken, and the photographic slides were scanned into the computer and prepared using Adobe Photoshop.

Chromatin Immunoprecipitation—Chromatin immunoprecipitations were done essentially as described by Orlando *et al.* (30). S2 cells were maintained in *Drosophila* serum-free medium (Life Technologies, Inc.) at 27 °C. 2–4 × 10^9 cells were cross-linked with formaldehyde for 10 min at 23 °C followed by 50 min on ice. Heat shock samples were heated to 36.5 °C for 1 h before being cross-linked with formaldehyde for 10 min at 36.5 °C followed by 50 min on ice. The cross-linked DNA was sheared by sonication to an average 1 kb in size and was purified by equilibrium sedimentation on a cesium chloride gradient. 30–60 μg of cross-link DNA was used per immunoprecipitation with 5 μl of H2Av antiserum, 5 μl of H2A antiserum, or 10 μl of an affinity purified, rabbit polyclonal antibody directed against the large subunit of pol II expressed in bacteria (31). After reversing the cross-links, the DNA was purified, quantitated using picogreen fluorescence (Molecular Probes), and analyzed by slot-blot hybridization. Hybridization signals were quantitated using stored phosphorimaging screens and ImagQuant software (Molecular Dynamics). Levels of hybridization to immunoprecipitated DNA were measured relative to the level of hybridization observed to the same amount of total, non-precipitated DNA. The *hsp70* probe was a 0.9-kb *Bam*HI-*Sal*I fragment isolated from plasmid 56H8 and is homologous to the 3′-half of all five *hsp70* genes (32). The *hsp26* probe was a 2.2-kb *Bam*I-*Eco*RI fragment isolated from plasmid 202.7 and is homologous to the entire gene and 1.2 kb of 3′-flanking sequences (32). The *hsp83* probe was a 3.2-kb *Bam*HI-*Sal*I fragment isolated from plasmid aDm4.46 and is homologous to the 5′-half of the gene and 0.8 kb of 5′-flanking sequences (32). The *actin* probe was a 0.9-kb *Sal*I fragment isolated from plasmid DmA2 and is homologous to the 3′-half of both the 5C and 42A actin genes (32). The *bicoid* probe was a 3.2-kb *Xba*I fragment isolated from plasmid pUCHSNeo8.7 and is homologous to the entire gene excluding 500 base pairs of the 5′ end (33). The *s19* chorion probe was a 1.5-kb *Eco*RV-*Eco*RI fragment isolated from plasmid pR7.7 and is homologous to the entire gene and 0.7 kb of 3′-flanking sequences (34). The satellite probe was a 424-base pair *Ase*I fragment isolated from plasmid pHD-BPDp and is homologous to 1.688 satellite sequences on *Dp(1;f)1187* (35).

RESULTS

Distribution of H2Av in Polytene Chromosomes by Immunofluorescence—A polyclonal antibody was generated against *Drosophila* H2Av to determine where this H2A.F/Z histone variant is located in chromosomes. The antibody recognized a 14.6-kDa protein present in nuclei of embryos and S2 tissue culture cells as well as in larval salivary glands (Fig. 1). The protein was absent from larval imaginal disc cells homozygous for the *His2Av*^{810} null allele of the *His2Av* gene (20) demonstrating that the 14.6-kDa protein is H2Av (Fig. 1).

The antibody was used to localize H2Av in polytene chromosomes of third instar larval salivary glands by indirect immunofluorescence (Fig. 2). H2Av was widely distributed in polytene chromosomes, being present in thousands of bands throughout the euchromatin, and was also present in the heterochromatic chromocenter (Fig. 2*a*). Some level of fluorescence was detectable along virtually the entire length of each chromosome arm. Low levels of fluorescence were unlikely to be due to nonspecific background, since control antibodies, such as those to acetylated isoforms of histone H4, gave no detectable signal between strong bands of fluorescence.[2] Therefore, even low levels of immunofluorescence probably reflected the presence of H2Av. Earlier studies had also reported the banded appearance of H2Av localization in polytene chromosomes (20, 23).

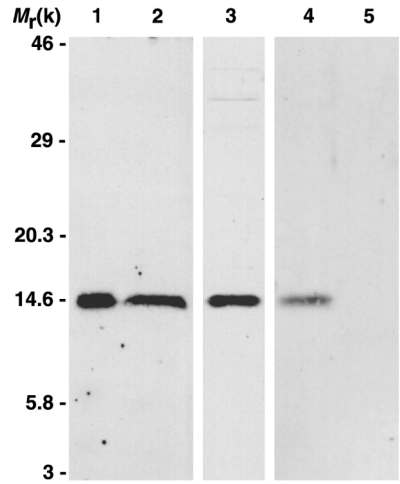

FIG. 1. **An antibody to *Drosophila* H2Av.** Equal amounts of protein from embryo nuclei (*lane 1*), S2 cell nuclei (*lane 2*), and from larval salivary glands (*lane 3*) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and assayed by Western blot hybridization. The H2Av antiserum detected a 14.6-kDa protein in all samples. The 14.6-kDa protein was detected in wing discs from wild type larvae (*lane 4*) but not from larvae homozygous for the *His2Av*^{810} null allele of *His2Av* (*lane 5*). No signal was observed with preimmune sera (data not shown).

Immunocytological studies of *Drosophila* histone H2A.2, which was likely to have been the same protein we now know to be H2Av, suggested that H2Av localization was limited to interband regions of the polytene chromosome, and therefore, H2Av might play a general role in determining the band-interband structure of polytene chromosomes (36). This hypothesis was directly tested by double labeling chromosomes with the H2Av antibody and the DNA stain 4′,6-diamidine-2′-phenylindole dihydrochloride (DAPI), which reveals the band-interband structure of the chromosome. H2Av localization was not limited to interband regions and showed a complex pattern of staining that did not correlate with the banded structure of the polytene chromosome (Fig. 2*b*). Localization of H2Av, therefore, does not simply parallel the concentration of DNA along the polytene chromosome.

To determine if H2Av localization was correlated with transcriptional activity, the pattern of H2Av staining was compared with sites of transcription by co-localizing pol II and H2Av (Fig. 2*c*). Loci of elevated pol II immunofluorescence were found throughout the euchromatin with a limited number of loci having conspicuously higher pol II immunofluorescence identifying sites where pol II density and transcriptional activity were particularly high. It is likely that not all sites of transcriptional activity were detected because the antibody used, 8WG16, preferentially binds hypophosphorylated pol II (see "Experimental Procedures"). The distribution of H2Av immunofluorescence was generally more widespread than pol II

[1] The abbreviations used are: pol II, RNA polymerase II; CTD, carboxyl-terminal domain; ChIP, chromatin immunoprecipitation; DAPI, 4′,6-diamidine-2′-phenylindole dihydrochloride; kb, kilobase pair.

[2] T. J. Leach, M. Mazzeo, H. L. Chotkowski, J. P. Madigan, M. G. Wotring, and R. L. Glaser, unpublished observations.

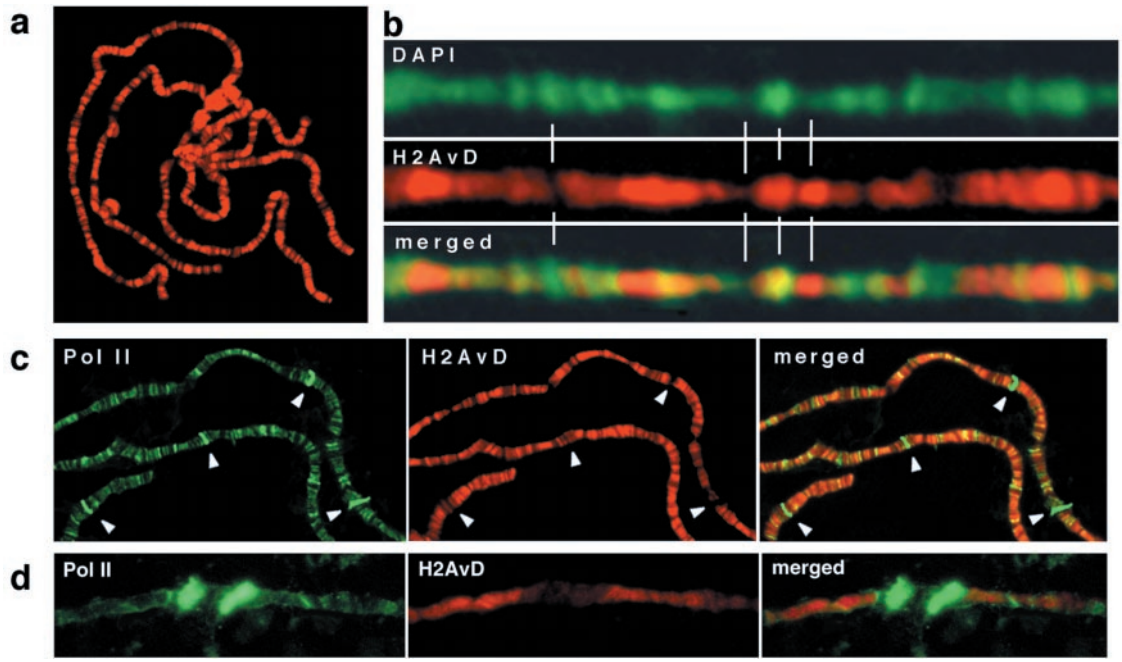

FIG. 2. **Immunolocalization of H2Av in polytene chromosomes.** *a*, H2Av antibodies were bound to salivary gland polytene chromosomes and detected with a secondary antibody conjugated to rhodamine. No fluorescence was observed with preimmune sera (data not shown). *b*, after localizing H2Av (*red*), the chromosomes were counterstained with DAPI (*green*). The merged image demonstrates the incongruity of the H2Av and DAPI patterns. Four areas that differ in their relative intensities of DAPI and H2Av fluorescence are highlighted by *vertical lines*. *c*, chromosomes were double labeled with antibody to RNA pol II (*PolII*) (*green*) and to H2Av (*red*). Regions of highest pol II density were regions of low H2Av density (*arrowheads*). *d*, larvae were heat-shocked at 36.5 °C for 1 h and their chromosomes double-labeled with RNA pol II (*green*) and H2Av (*red*). The high RNA pol II density and the low H2Av density at the 87A and 87C loci, which contain the *hsp70* genes, are evident.

with many loci containing H2Av but little or no pol II, and some loci contained equivalent levels of pol II and H2Av immunofluorescence (Fig. 2*c*). H2Av immunofluorescence was reduced at those loci containing the most pol II and presumably the highest levels of transcription (*arrowheads* in Fig. 2*c*). Reductions in H2Av immunofluorescence were also observed at the *hsp70* heat shock loci after a 1-h heat shock of larvae, which produces high densities of pol II concomitant with transcriptional induction of the *hsp70* genes (Fig. 2*d*). No obvious reduction in H2Av immunofluorescence was observed at heat shock loci under nonheat shock conditions (data not shown), and the reduction in H2Av immunofluorescence observed after heat shock is likely to be transient, since no persistent change in H2Av immunofluorescence was observed at heat shock loci in larvae subject to daily heat shocks during earlier development (data not shown).

Distribution of H2Av in Diploid Chromosomes by Chromatin Immunoprecipitation—Levels of H2Av immunofluorescence observed in polytene chromosomes might reflect not only the density of H2Av but also the influence of chromatin structure, such as the state of DNA condensation or decondensation associated with chromosome puffing. To avoid such complications and provide an alternative methodology for determining the distribution of H2Av in *Drosophila* chromosomes, chromatin immunoprecipitations (ChIP) were used to measure the association of H2Av with different types of DNA sequences in the diploid chromosomes of tissue culture cells.

DNA:protein cross-links were generated by formaldehyde treatment of cells, and complexes were immunoprecipitated using H2Av antibodies. The immunoprecipitated DNA was analyzed by slot-blot hybridization, and the hybridization signal was compared with the hybridization signal for a comparable mass of total, nonprecipitated DNA. A ChIP/total value of 1 means the sequence was neither enriched nor depleted relative to the abundance of the same sequence in totals. Negligible DNA was precipitated with preimmune sera or in no-antibody controls (Fig. 3*a*).

Immunoprecipitations were initially done using an antibody to pol II and probing for the coding sequences of the *hsp70*, *hsp26*, and *hsp83* heat shock genes to establish the validity of the protocol. Association of pol II with *hsp70*, *hsp26,* and *hsp83* increased 44-, 22-, and 6-fold, respectively, after a 1-h heat shock (Fig. 3, *a* and *b*), consistent with earlier observations (32). mRNA levels for these genes also increased 43-, 23-, and 4-fold, respectively, after a 1-h heat shock (data not shown).

Immunoprecipitations were then done using the H2Av antibodies, and the association of H2Av with a variety of gene sequences was measured (Fig. 3*c*). H2Av was associated with the constitutively expressed *hsp83* and cytoplasmic *actin* genes, the uninduced and induced *hsp70* and *hsp26* genes, and the nonexpressed developmental *bicoid* and chorion *s19* genes (Fig. 3*c*). H2Av was also associated with noncoding sequences 5′ and 3′ of both *bicoid* and *hsp70* (data not shown). Levels of H2Av on all sequences tested were similar with ChIP/total ratios around 1, suggesting that H2Av is fairly uniformly distributed in chromatin irrespective of whether a sequence is transcribed, potentially transcribed, or noncoding. H2Av was even associated with heterochromatic satellite sequences, although at lower levels (Fig. 3*c*), suggesting that some amount of H2Av is present even in heterochromatin, a conclusion consistent with localization of H2Av to the heterochromatic chromocenter of polytene chromosomes (Fig. 2*a*).

Comparison of H2Av immunoprecipitations from normal *versus* heat shock cells suggested that transcription causes H2Av to cross-link less frequently to DNA (Fig. 3*c*). In uninduced cells, H2Av was associated with all three heat shock genes (Fig. 3*c*). After transcription was induced by heat shock, immunoprecipitation levels for all three genes decreased as follows: 1.7-fold for *hsp70*, 1.5-fold for *hsp26*, and 1.4-fold for *hsp83*

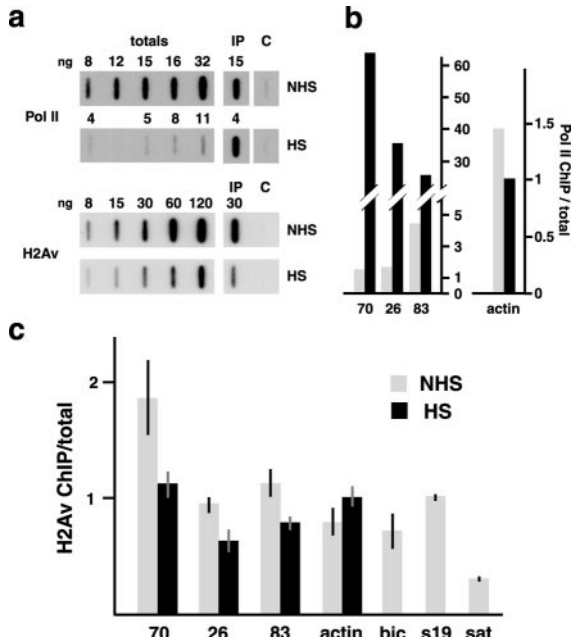

FIG. 3. **H2Av localization to diploid chromosomes by ChIP.** *a*, slot-blot hybridization results are shown for *hsp70* sequences precipitated with RNA pol II (*PolII*) or H2Av antibodies from non-heat shock (*NHS*) or heat shock (*HS*) cells. The immunoprecipitation (*IP*), no-antibody control (*c*), and non-precipitated totals are shown for each sample with the amount of DNA blotted indicated in nanograms (*ng*). Relative levels of enrichment or depletion can be seen by comparing the amount of hybridization signal in the immunoprecipitation slot to the amount of hybridization in the slot containing the same mass of total DNA. *b*, quantitative slot-blot hybridization results are shown for *hsp70* (70), *hsp26* (26), *hsp83* (83), and *actin* gene sequences immunoprecipitated with RNA pol II antibodies from cells that were not heat-shocked (*light*) or were heat-shocked (*dark*) prior to cross-linking. ChIP/total values above 1 indicated enrichment of the sequence in the precipitate relative to totals, and values below 1 indicate depletion. *c*, quantitative slot-blot hybridization results are shown for *hsp70* (70), *hsp26* (26), *hsp83* (83), *actin*, *bicoid* (bic), and chorion *s19* (s19) genes and 1.688 satellite sequences (*sat*) immunoprecipitated with H2Av antiserum from cells that were not heat-shocked (*light*) or heat-shocked (*dark*) prior to cross-linking. ChIP/total values are presented as described for *b*. Mean and S.D. were calculated from results of at least three independent immunoprecipitations.

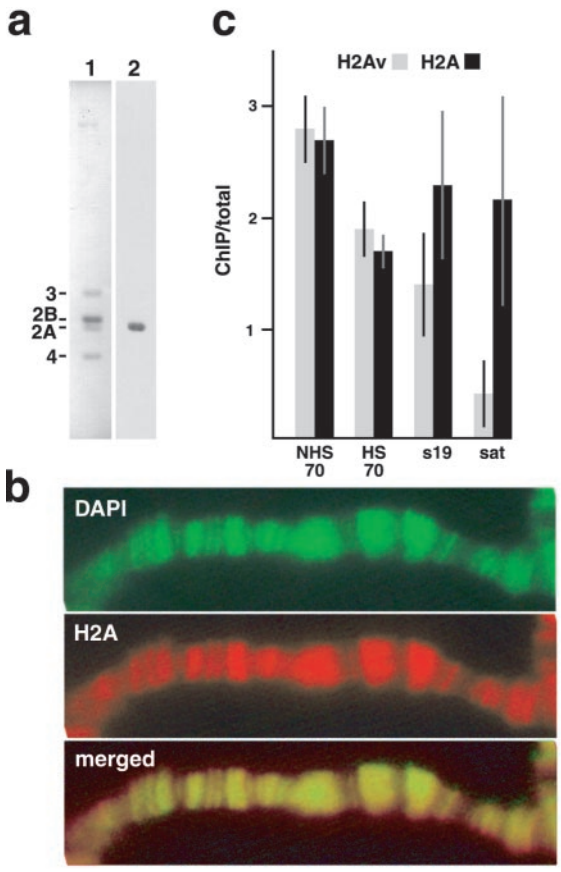

FIG. 4. **Localization of *Drosophila* H2A in polytene and diploid chromosomes.** *a*, equal amounts of protein from embryo nuclei were fractionated by SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membrane. One lane was stained with Coomassie Brilliant Blue to reveal histones H2A, H2B, H3, and H4, which are the predominant proteins in nuclei preparations (*lane 1*). The other lane was assayed by Western blot hybridization using the H2A antiserum. A single protein was identified that co-migrated with H2A (*lane 2*). No signal was observed with preimmune sera (data not shown). *b*, H2A antibodies were bound to salivary gland polytene chromosomes and detected with a secondary antibody conjugated to rhodamine (*red*). The chromosome was then counterstained with DAPI (*green*). The merged image demonstrates the congruity of the H2A and DAPI patterns. *c*, chromatin immunoprecipitations were done as described for Fig. 3 using H2Av (*light*) and H2A (*dark*) antibodies done in parallel on the same preparations of cross-linked DNA. Quantitative results for the *hsp70* (70), chorion *s19,* and 1.688 satellite sequences (*sat*) are shown. *Hsp70* sequences were precipitated from both nonheat-shocked (*NHS*) and heat-shocked (*HS*) cells. Chorion *s19* and 1.688 satellite sequences were precipitated from nonheat-shocked cells. Mean and S.D. were calculated from results of three independent immunoprecipitations.

(Fig. 3*c*). Immunoprecipitation levels for the actin genes appeared to increase after heat shock, which would be consistent with the decrease in transcription that occurs to these genes (32). These results suggest that transcription causes H2Av to be less frequently or less tightly associated with DNA, a conclusion consistent with the reduced immunofluorescence observed at actively transcribed loci in polytene chromosomes (Fig. 2*c*).

The Distribution of S-phase H2A in Polytene and Diploid Chromosomes—S-phase H2A is likely to be uniformly distributed in chromosomes and therefore provides a good control for the pattern of H2Av localization. A polyclonal antibody was generated against the carboxyl-terminal 15 amino acids of H2A. The antibody recognized a single nuclear protein on Western blots that migrated at the position of bulk H2A protein (Fig. 4*a*). The antibody was used to localize H2A in polytene chromosomes of third instar larval salivary glands by indirect immunofluorescence. H2A immunofluorescence paralleled precisely the banded structure of the chromosome revealed by DAPI staining (Fig. 4*b*). This result agrees with earlier studies (37) and is consistent with a uniform distribution of H2A-containing nucleosomes along the chromosome paralleling the density of DNA. The H2A result contrasts markedly with the complex immunofluorescence pattern observed for H2Av, which did not correlate with the banded structure of the chromosome (compare Figs. 2*b* and 4*b*).

The distribution of H2A was also determined in diploid chromosomes by chromatin immunoprecipitation. H2A and H2Av immunoprecipitations were done in parallel on the same preparations of cross-linked DNA (Fig. 4*c*). Nearly identical amounts *hsp70* sequences were precipitated by both antibodies, and the same transcription-induced reduction in DNA association was observed between uninduced and heat shock-induced samples. In contrast, H2A and H2Av antibodies precipitated different amounts of *s19* and satellite sequences (Fig. 4*c*). The H2A antibody precipitated about the same amount of DNA from both the unexpressed *s19* gene and 1.688 satellite sequences, whereas the H2Av antibody precipitated 1.6-fold less *s19* and 5-fold less satellite DNA. These differences suggest that H2Av is less abundant on these sequences.

DISCUSSION

The distribution of H2Av appears to be widespread in the *Drosophila* genome. Some level of H2Av immunofluorescence was detectable along virtually the entire length of each polytene chromosome (Fig. 2). In addition, every DNA sequence tested was immunoprecipitated by the H2Av antibody at levels significantly above background irrespective of the transcriptional status or coding capacity of the sequence (Fig. 3).[2] Thus, the immunofluorescence and immunoprecipitation results suggest that H2Av-containing nucleosomes are widespread, if not ubiquitous, in the genome. The distribution of H2Av along the length of the chromosome, however, also appears to vary. The banded pattern of H2Av immunofluorescence was complex and did not simply parallel the concentration of DNA, as was the case for H2A (Figs. 2*b* and 4*b*), and significantly different amounts of *s19* and satellite sequences were precipitated by the H2Av and H2A antibodies (Fig. 4*c*). Thus, the immunofluorescence and immunoprecipitation results also suggest that the density of H2Av-containing nucleosomes is different on different sequences.

H2A.Z variant histones are likely to be involved in transcriptional regulation. H2A.Z is associated with transcriptionally active nuclei in both *Tetrahymena* and *Drosophila* (23, 24), and recent genetic evidence suggests that H2A.Z regulates transcription in budding yeast.[3] The distribution of H2Av in *Drosophila* chromosomes appeared to correlate with transcriptional potential, in general, but not necessarily with specific sites of active transcription. For example, by comparison to H2A, noncoding satellite sequences had the least H2Av, the coding but nonexpressed *s19* gene had more H2Av, and the inducible *hsp70* gene had the most H2Av (Fig. 4*c*). In contrast, there was little difference in H2Av immunoprecipitation levels between the constitutively expressed *hsp83* and *actin* genes and the nonexpressed *bicoid* and *s19* genes (Fig. 3*c*). In addition, sites of active transcription in polytene chromosomes were not sites of elevated H2Av immunofluorescence (Fig. 2*c*). So even if H2Av is involved in transcriptional regulation, its incorporation into chromatin does not appear to be transcription-dependent. H2Av is therefore unlikely to act as a replacement histone that replaces H2A lost from actively transcribed genes (38). Similarly, mammalian H2A.Z also fails to accumulate in chromatin of nondividing rat neurons as would be expected of a replacement histone (39).

If transcription is not responsible for the incorporation of H2Av into chromatin, then what is the origin of H2Av's widespread but nonrandom pattern of localization? H2Av could be localized throughout the genome, including heterochromatic sequences, if it were incorporated into nucleosomes during DNA replication. If this were the case, however, H2Av-containing nucleosomes would need to be preferentially assembled onto some sequences, like euchromatic genes, *versus* other sequences, like satellite repeats, to create the observed variations in H2Av density. Alternatively, H2Av incorporation during S-phase could be stochastic if mechanisms exist that could subsequently change the level of H2Av on specific sequences in the context of pre-existing chromatin.

The function of H2Av is likely to involve conformational changes to the structure of the nucleosome. The αC helix region of H2Av is required for H2Av function and is the only region of H2Av for which the amino acid sequence of H2A cannot be substituted without loss of function (40). The αC helix of H2Av along with the α3 helix and carboxyl-terminal tail forms a "docking" domain that interacts with the carboxyl-terminal tail of H4 within the core of the nucleosome and might be expected to influence the conformational stability of the nucleosome (1). Transcription-induced changes in nucleosome structure are likely to be the cause of reductions in *hsp70* immunoprecipitation levels observed after heat shock (Figs 3*c* and 4*c*). An earlier cross-linking analysis of histone associations with the *hsp70* genes suggested that transcription causes a conformation change in nucleosome structure that results in reduced cross-linking of histone globular domains to DNA, whereas cross-linking of tail domains is unaffected (41). This particular transcription-induced alteration in nucleosome structure appeared to be the same for H2Av-containing and H2A-containing nucleosomes since equivalent reductions in immunoprecipitation levels were observed using both antibodies (Fig. 4*c*). Although this result did not reveal a unique aspect of H2Av function, it is still possible that quantitative differences between the H2Av and H2A immunoprecipitations were obscured by the very high levels of *hsp70* transcription induced by heat shock and that important differences in the behavior of H2Av-containing and H2A-containing nucleosomes might be revealed at lower levels of transcription or on different, developmentally regulated genes.

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[3] M. Smith, personal communication.

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