Localization of Chromosomal Protein HMG-1 in Polytene Chromosomes of *Chironomus thummi*

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ABSTRACT The distribution of accessible antigenic sites in the chromosomal protein high mobility group one (HMG-1) in *Chironomus thummi* polytene chromosomes is visualized by immunofluorescence. The results indicate that (*a*) HMG-1 is distributed in a distinct banding pattern along the entire length of the chromosomes; (*b*) the banding pattern obtained with fluorescent antibody does not strictly correspond to that observed by phase-contrast microscopy; and (*c*) the amount of HMG-1 increases, and the fluorescent banding pattern changes, during the development of the organism. Our findings suggest that the protein may be involved in the modulation of the structure of selected loci in the chromosome.

The nonhistone chromosomal proteins are an integral part of the eucaryotic genome. Although the function of these proteins is not well understood, there is evidence that some proteins belonging to this group are important in maintaining the structure (1, 36) and regulating the function (9, 35, 41) of chromatin and chromosomes. The difficulties in purifying homogeneous molecular species from this group of proteins are a major obstacle in elucidating their function. Chromosomal protein high mobility group one (HMG-1) is one of the few nonhistone proteins that has been purified to homogeneity (11). This protein is ubiquitous in its distribution, as it is found in several eucaryotic kingdoms (15, 28, 37, 38, 40). Sequence studies revealed that it is unusually rich in charged amino acid residues, and that the negatively and positively charged residues are clustered on the polypeptide chain (39). The proteins bind to histones and DNA and can induce changes in the DNA helical structure (17, 19, 44). Evidence has been presented that this protein is associated with isolated nucleosomes (13).

Antisera, elicited by HMG-1 protein purified from calf thymus, cross react immunologically with HMG-1 derived from several species (33). The antibodies bind to chromatin (4), allowing these antisera to serve as useful cytological tools to study the cellular function of this protein.

In the present study, we investigate the distribution of protein HMG-1 in polytene chromosomes of *Chironomus thummi*. Polytene chromosomes have the same fundamental chromatin fiber structure as that present in all eucaryotic systems (43). Their large size offers the advantage of amplification in studying the location of a particular chromosomal protein and in following structural alterations associated with functional changes in the genome. Antisera to RNA polymerase (18), nonhistone proteins (36), histones (5), and fluorescent concanavalin A (22) have already been successfully used for such studies. The present study is the first investigation on the chromosomal localization of a structurally defined nonhistone protein that has been detected in several eucaryotic kingdoms. Affinity-purified antibodies are used to demonstrate that *Chironomus* contains a protein that is indistinguishable from its homologue purified from calf thymus. Its organization in the chromosomes is compatible with a situation where various effectors can modulate in a specific way the structure of selected loci in the chromosome.

MATERIALS AND METHODS

Staging of Larvae and Identification of Chromosomal Regions

Larvae were grown in aerated boxes containing deionized H_2O, cellulose-absorbent paper, and nettle powder. Each box was initially "seeded" with freshly laid egg-masses to attain the same age larvae in any one chamber. Second instar larvae, that are found after 3.5 d and last 3 d (21), were selected from a box containing 5–6-d-old larva. Third instar larvae were selected from 9–10-d-old larvae (21). Individuals of average size and uniform appearance were selected from each instar. Fourth instar larvae of various stages of development were selected using the criteria of Laufer et al. (25). The characteristics of mid-fourth instar larvae were: separate thoracic segments, smaller body, not yet visible imaginal appendages, and a light red body color. The characteristics of late-fourth instar larvae were: separate thoracic segments, small imaginal appendages, and a darker red color. For early prepupa: fused thoracic segments, transparent and thickened imaginal appendages, visible and developed wings and thoracic legs (but not yet as advanced as in the later stages of development), and larger and stouter larvae, bright red in color. The late prepupa characteristics were: thick fused, thoracic segments, white in color, and a slender, opaque abdomen, pale in color. Chromosomal regions were identifed by the *C. thummi* chromosome maps of Keyl (20) and Hägele (16).

Preparation of Salivary Gland and Polytene Nuclei Homogenates

Salivary glands from larvae at specific stages were isolated and washed in modified Cannon's medium (31). The glands were then homogenized in 1 mM EDTA and 1 mM phenylmethylsulfonylfluoride (PMSF, Pierce Chemical Co., Rockford, Ill.), using a glass microhomogenizer (Radnoti Glass Technology, Inc., Arcadia, Calif.).

In each preparation, nuclei were isolated from at least 40 salivary glands (32), centrifuged in an Eppendorf centrifuge (Brinkmann Instruments, Inc., Westbury, N. Y.), resuspended, and homogenized in 200 μl of 0.1 mM EDTA and 1 mM PMSF.

Antigens and Antisera

The antigens and antisera used in this study have been previously characterized (4, 33, 34).

Solid-phase Radioimmune Assay

100 μl of the homogenates (nuclear or salivary gland) or the standards (HMG-1 or H4) were serially diluted in phosphate buffered saline (PBS), pH 7.3 and incubated overnight at 4°C in the wells of polyvinyl chloride Microtiter plates (Dynatech Laboratories, Inc., Dynatech Corp., Alexandria, Va.). The plates were washed in PBS and incubated for 4 h at room temperature with 300 μl/well of 1% bovine serum albumin (BSA) in PBS, then washed in PBS and incubated overnight at 4°C with HMG-1 or H4 antisera diluted 1:100 with 1% BSA in PBS. The antibody was removed and the plates washed with PBS. 100 μl/well of ^{125}I-labeled protein A (25,000 cpm) was added, and the plates incubated at 4°C overnight, and then washed extensively with PBS and distilled water. The individual wells were cut out of the plate and the radioactivity was counted in a gamma counter. (For further details on this assay, see Romani et al. [34].

Salivary Gland Squash Preparation of Chromosomes

Salivary glands were isolated and washed in *Chironomus* Ringer's (32), which contained 0.2% Nonidet P-40 (Bethesda Research Laboratories, Rockville, Md.) and 0.1% Triton X-100 (Research Products International Corp., Elk Grove Village, Ill.). They were fixed for 5 min in 87 mM NaCl, 5 mM phosphate buffer, pH 7.3, 3.2 mM KCl, 2 mM $MgCl_2$, 3% formaldehyde , 0.2% Nonidet P-40, and 0.1% Triton X-100, then washed and squashed in 45% acetic acid. The coverslips were floated off in Tris-buffered saline (TBS: 0.9 mM NaCl, 10 mM Tris, pH 7.3), and the preparations washed in the same solution for at least 1 h.

Immunofluorescence Procedure

Indirect immunofluorescence was performed with affinity-purified antibodies and rhodamine- or fluorescein-labeled goat anti-rabbit IgG (Cappel Laboratories, Cochranville, Pa.). After extensive washing in TBS, 100 μl of affinity-purified, rhodamine-conjugated HMG-1 antisera, diluted 1:100, was applied to each preparation, and these were incubated at 37°C for 1 h in a moist chamber. The preparations were washed overnight at room temperature, mounted in Aquamount (Lerner Laboratories, Stamford, Ct.), and photographed using a Zeiss Photomicroscope III.

Diazobenzyloxymethyl Paper Radioimmune Assay

100 salivary glands were boiled in 50 μl of 250 mM sucrose, 10 mM phosphate buffer, pH 7.2, 3% 2-mercaptoethanol, and 0.1% SDS. The preparation was then centrifuged, and the supernatant fluid run with other protein standards on an 18% polyacrylamide-SDS gel, according to LeStourgeon and Rusch (26). The separated proteins were transferred and covalently bound to diazobenzyloxymethyl (DBM) paper (available as aminobenzyloxymethyl paper from Schleicher and Schuell, Inc., Keene, N. H.) essentially using the method of Renart et al. (30). The preparation was reacted with HMG-1 antisera diluted 1:50 in 150 mM NaCl, 50 mM Tris-HCl, pH 7.4, 5 mM EDTA, 0.25% gelatin wt/vol and 0.05% Nonidet P-40 wt/vol, washed, and reached with ^{125}I-labeled protein A (150 μCi, 5–10 μCi/μg) 2 h at 37°C. The preparation was washed overnight with 1 M NaCl, 50 mM EDTA, 0.4% Sarkosyl wt/vol (Ciba-Geigy Corp., Ardsley, N. Y.), and autoradiographed with Kodak x-ray film (30).

RESULTS

Presence of HMG-1 Protein in Chironomus

A prerequisite to the study of the organization of HMG-1 chromosomal protein in the polytene chromosomes of *Chironomus* is an unequivocal demonstration that this protein, or molecules that are antigenically cross reacting, are indeed present in this organism. Homogenates of salivary glands, and of nuclei purified from the salivary glands, were tested for the presence of HMG-1 chromosomal protein by a solid-phase radioimmunoassay (34). Antisera to histone H4, which is highly conserved during evolution (8), served as a positive control for the reaction. Fig. 1 presents the dependence of ^{125}I-protein A binding on the concentration of the homogenates of either nuclei (*a*) or salivary glands (*b*). The homogenates bound antisera both to HMG-1 protein and to histone H4 specifically, because addition of nonimmune serum to the homogenate did not result in significant binding of ^{125}I-protein A. Examination of the data reveals that anti-HMG-1 sera bound more extensively to homogenates from salivary glands than to homogenates of nuclei, whereas anti-H4 bound more efficiently to homogenates of nuclei. At the highest concentration of antigen used, the ratio, bound from the nucleus, of HMG-1 to H4 was 0.75, whereas, using the unfractionated salivary gland, it was 1.51. These ratios, of course, represent the amount of antibodies bound to the two proteins and do not reflect the absolute amount of HMG-1 and H4 in the tissue. Because HMG-1 is bound to the internucleosomal region (27), although most of the antigenic determinants in chromatin-bound H4 are steri-

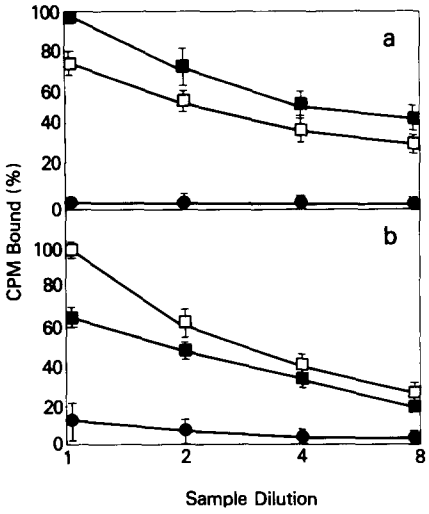

FIGURE 1 Presence of HMG-1 protein in salivary glands and nuclei. Solid-phase radioimmune assay analysis of anti-HMG-1, anti-H4, and normal rabbit serum binding to *C. thummi* fourth instar polytene nuclei and salivary gland homogenates. (*a*) Nuclear homogenate reacted with HMG-1 antisera (□), H4 antisera (■), and normal rabbit serum (●). Maximum radioactivity of ^{125}I-labeled protein A bound to H4 antibodies is 1,700 cpm. (*b*) Salivary gland homogenate reacted with HMG-1 antisera (□), H4 antisera (■), and normal rabbit serum (●). Maximum radioactivity of ^{125}I-labeled protein A bound to HMG-1 antibodies is 2,800 cpm. Data points represent the arithmetic mean of three experiments. The brackets indicate the standard error of the mean. All antisera were diluted 1:100. The concentrate sample dilution was the homogenate of 10–20 salivary glands, or 40–60 nuclei from salivary glands suspended in 750 μl of 0.1 mM EDTA, 1 mM PMSF, pH 7.5.

cally hindered (10), it can be expected that antibodies to HMG-1 will bind to chromatin more readily than antibodies to H4. These results suggest that protein HMG-1 or proteins that immunologically cross react with HMG-1 are present both in the cytoplasm and the nuclei of the salivary glands. This fact has been reported in several types of cells (6).

To find out the number and types of protein present in the salivary glands that can react with anti-HMG-1 sera, we electrophoresed homogenates of salivary glands in 18% polyacrylamide gels in the presence of SDS. The proteins were transferred from the gel to DBM paper, which they attach to covalently (30). Then, the paper was treated with anti-HMG-1 and ^{125}I-protein A, and the location of antigenic bands that bound HMG-1 antibodies was visualized by autoradiography. The results are presented in Fig. 2. Among the many protein bands present in the salivary gland, there is a band with a mobility identical to that of protein HMG-1 purified from calf thymus (Fig. 2*a*).

A gel of these proteins, after transfer to the DBM paper, is presented in Fig. 2*b*, together with the corresponding autoradiograph, Fig. 2*c*. The autoradiograph indicates that the antibodies bind to the HMG-1 standard and to a band present in the salivary gland homogenate whose mobility is indistinguishable from that of protein HMG-1 purified from calf thymus. We conclude, therefore, that *Chironomus* salivary glands contain principally one type of protein that can bind anti-HMG-1 sera. Because the mobility of this protein is indistinguishable from that of purified HMG-1, and because it is known that the primary sequence of HMG-1 has been conserved during evolution (12, 33, 38), we assume that the *Chironomus* protein that bound the antibodies is either similar or identical to protein HMG-1 purified from calf thymus.

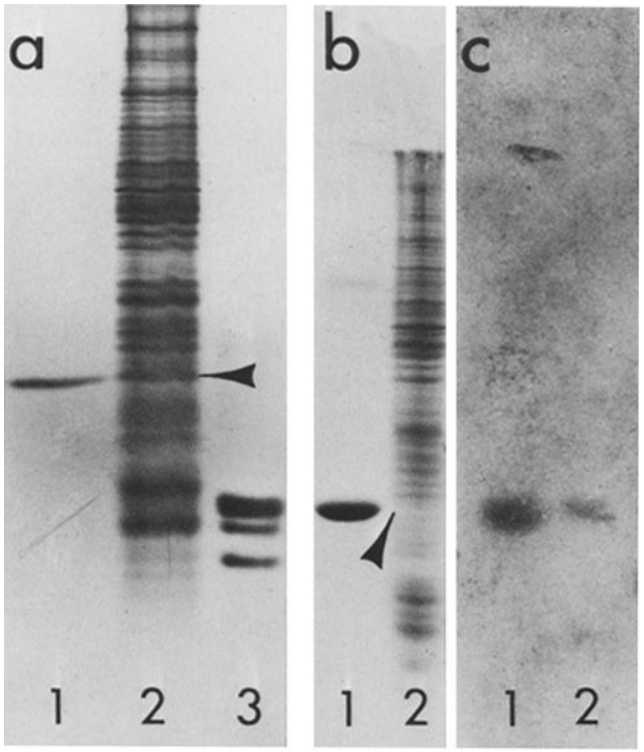

FIGURE 2 *C. thummi* salivary glands contain a single molecular species of HMG-1. (*a*) 18% SDS slab gel electropherogram of calf thymus HMG-1 (lane *1*), salivary gland extract prepared from 40 fourth instar salivary glands (lane *2*), and chick erythrocyte nucleosome histones (lane *3*). Arrow indicates band in salivary gland homogenate that has an electrophoretic mobility corresponding to the HMG-1 sample in lane *1*. (*b*) Photograph of the 18% SDS slab gel electrophoregram after the proteins were transferred and covalently bound to DBM paper (see Materials and Methods): calf thymus HMG-1 (lane *1*) and salivary gland homogenate (lane *2*). Arrow indicates location of transferred *Chironomus* HMG-1 band. (*c*) Corresponding autoradiograph of the transferred proteins bound to DMB paper and reacted with HMG-1 antisera diluted 1:100 followed by ^{125}I-labeled protein A. Lanes *1* and *2* as in *b*.

Localization of Protein HMG-1 in Polytene Chromosomes

The presence of HMG-1 protein in polytene chromosomes was revealed by using rhodamine-labeled affinity-purified anti-HMG-1 antibodies, or by the indirect immunofluorescence technique, using affinity-purified anti-HMG-1 and rhodamine-labeled gamma globulins from goat anti-rabbit gamma globulin. The phase (a) and fluorescence (b) micrographs presented in Fig. 3 reveal that the antibodies bound to the chromosomes, producing an intense and distinct banding pattern. In the absence of specific antibodies, the chromosomes did not fluoresce. The glandular material surrounding the chromosomes was significantly more fluorescent when reacted with anti-HMG-1 than when reacted with a control sera. Possibly, this reflects the presence of HMG-1 not intimately associated with the chromosomes.

Relation between Phase and Fluorescence Banding Patterns

Examination of chromosomes in several squash preparations revealed that the banding pattern observed under phase-contrast optics does not strictly correspond to the banding patterns seen under fluorescence optics after the chromosomes were reacted with anti-HMG-1. Some dense chromomeres fluoresce intensely whereas others show minimal fluorescence. An example of chromosome regions displaying the various patterns

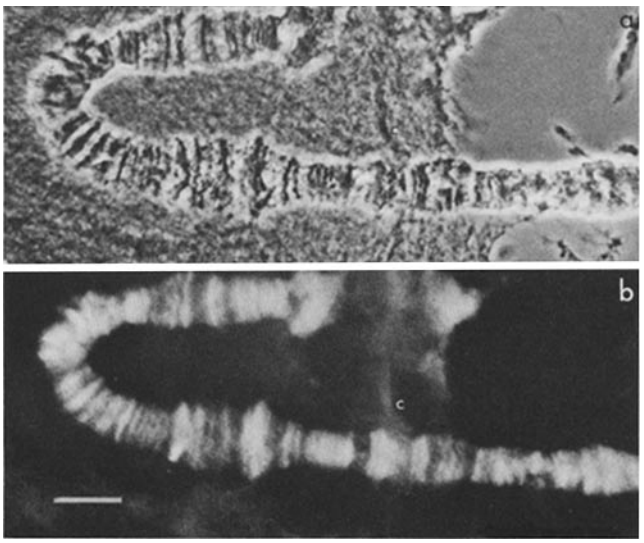

FIGURE 3 Banding pattern of chromosome III reacted with anti-HMG-1. Fourth instar chromosome III reacted with affinity-purified, rhodamine-labeled HMG-1 antisera diluted 1:100. (*a*) Phase-contrast micrograph. Glandular material including nucleoplasm and cytoplasm is indicated by the letter *c* (*b*) Corresponding fluorescence micrograph. Bar, 10 μm.

observed is presented in Fig. 4. An extended region from polytene chromosome I, photographed with both phase-contrast and fluorescence optics, has lines drawn between corresponding chromosomal areas (identification according to Keyl [20] and Hägele [16]). The regions have been identified by measuring relative distances from an identifiable reference point. Generally, there appears to be some correlation between chromomere density and fluorescence-staining intensity. There are, however, some interesting exceptions: bands designated IB3j, IB4d, IB4f, IB4h, IC3c, and ID1d appear dark (dense) with phase-contrast optics (*a*); however, these chromomeres show minimal fluorescence in the corresponding fluorescence micrograph (*b*). Bands numbered IC1c, IC3d, and ID1f in *a* exhibit a higher level of fluorescence in *b*.

The fluorescence intensity is an indication of the number of antibodies bound at a particular locus. The number of antibodies bound is dependent on the accessibility of the antigenic determinants in the chromosome to antibodies, and should not be taken as an indication of the absolute amount of HMG-1 in a particular region. It is concluded, therefore, that the various regions of the chromosome differ in the exposure of antigenic determinants of protein HMG-1.

Developmental Stage Differences in the Location of HMG-1

A stringent examination of the reproducibility of the fluorescent pattern, observable in polytene squashes prepared from different individuals, revealed that absolute reproducibility could be observed only in corresponding chromosomes from sister salivary glands. This is demonstrated in Fig. 5, where lines have been drawn to connect corresponding areas of phase-contrast and fluorescence micrographs of chromosome III from sister salivary glands squashed and prepared on different slides. The pattern of fluorescence is also the same for chromosomes of sister salivary glands at different levels of polyteny. In *F*, a lower, polytenic-level chromosome from one of the cells at the base of the saliva duct shows a fluorecence pattern comparable to that found in the larger chromosomes from the same pair of salivary glands (*B* and *D*) when stained with HMG-1 antisera. In contrast, polytene chromosomes from various individuals show variations in the fluorescence distribution produced by HMG-1 antibodies. In Fig. 6, phase-contrast and fluorescence micrographs have been aligned, and corresponding chromosomal regions connected by lines. Bands IIIB3o, IIIB4b, IIIB4f, IIIC4h, IIID1d, and IIID2b correspond in *d* and *f*, but not in *b*, whereas bands IIIB4d, IIIC1a, IIIC2b, and IIIC3d correspond in *b* and *d*, but not with *f*. Region IIIC4c fluoresces in *b* and *f*, but not in *d*. Only regions IIIC1e and IIIC2f show corresponding levels of fluorescence in *b*, *d*, and *f*. Although it is possible that some of the differences in fluorescence distribution may reflect variations in the preparatory or staining procedure, this type of artifact was not observed in sister salivary glands that were treated on separate

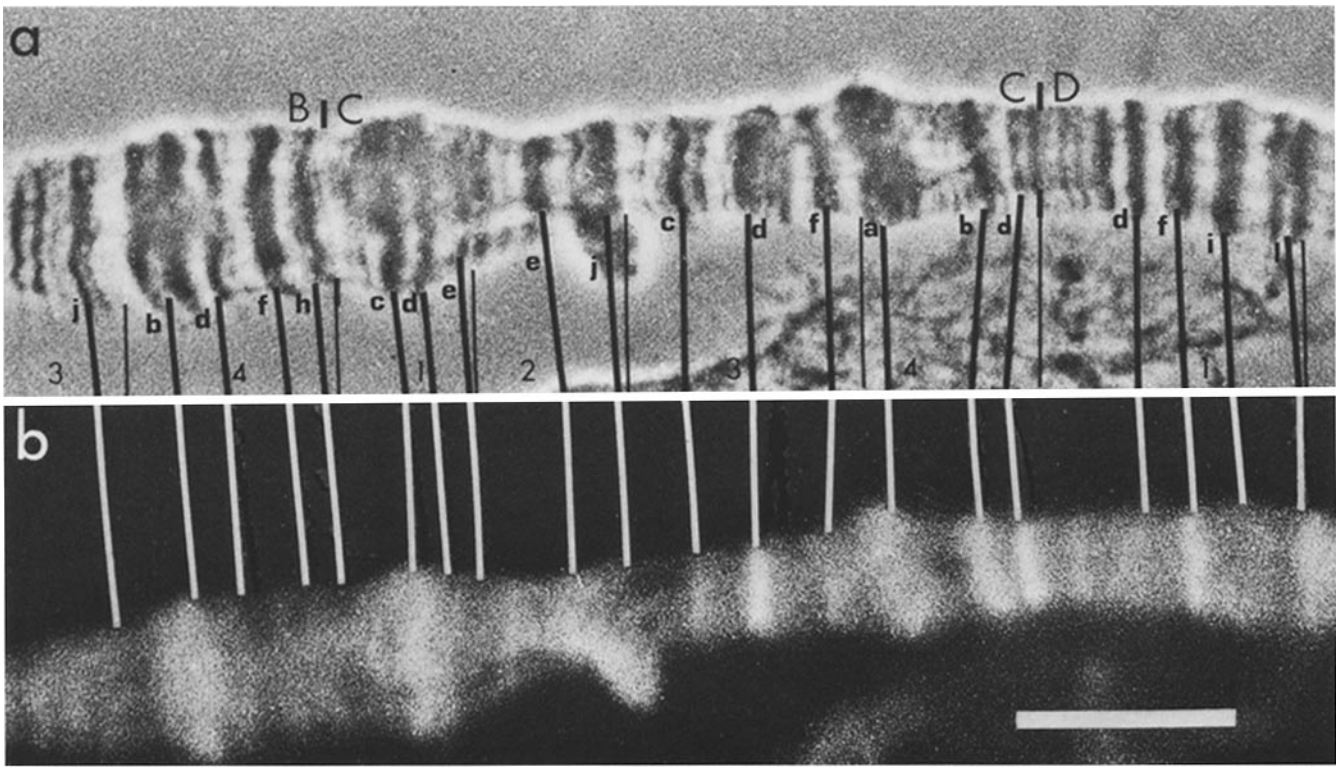

FIGURE 4 Localization of rhodamine-labeled HMG-1 antisera in various chromosomal regions. (*a*) Phase-contrast micrograph of a selected region from chromosome I. (*b*) Corresponding fluorescence micrograph. Points between corresponding chromosomal areas were determined by selecting a landmark on the phase-contrast micrograph and measuring the distance between the landmark and a selected chromosome region with a transparent measuring template. The template was then placed on the same landmark in the corresponding fluorescence micrograph and the chromosome region at the specified distance from the landmark noted. A minimum of three determinations were made for each pair of points connected by the black and white lines. The lines are not always parallel because they connect the bottom of the chromosomal regions (note the variations in the skew) in the phase contrast micrograph to the top of the corresponding regions in the fluorescence micrograph. Chromosome areas were identified by use of the *C. thummi* chromosome maps of Keyl (20) and Hägele (16). Bar, 16 μm.

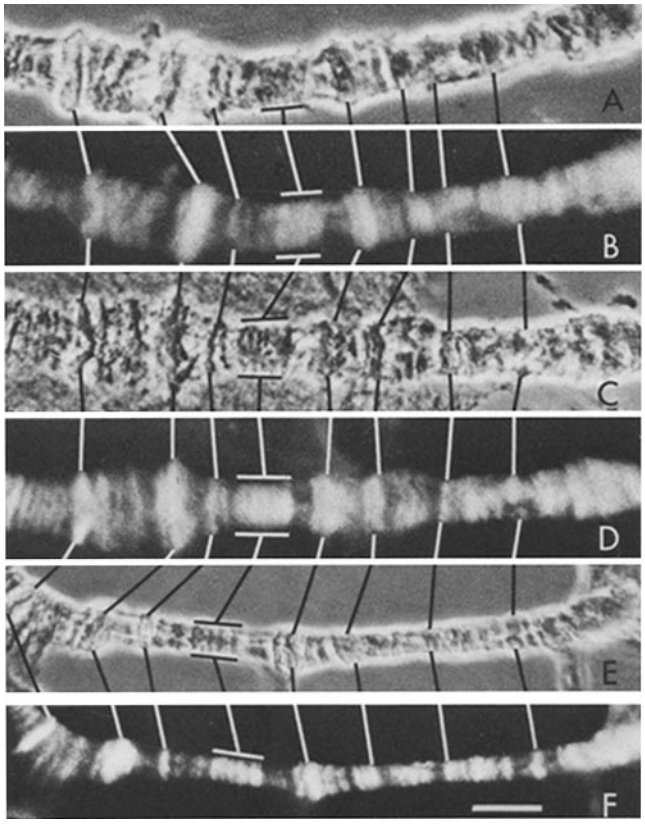

FIGURE 5 Reproducibility of fluorescent banding patterns in corresponding chromosomes from sister salivary glands. Chromosome III from a pair of sister salivary glands (from one larvae) reacted with affinity-purified, rhodamine-labeled HMG-1 antibodies diluted 1:100. (*A*) Phase-contrast micrograph of chromosome III from the left sister salivary gland. (*B*) Corresponding fluorescence micrograph. (*C*) Phase-contrast micrograph of chromosome III from the right sister salivary gland. (*D*) Corresponding fluorescence micrograph. (*E*) Phase-contrast micrograph of chromosome III from the right salivary gland at a lower level of polyteny (from one of the cells at the base of the salivary duct). (*F*) Corresponding fluorescence micrograph. Lines connecting corresponding chromosomal regions were drawn by use of the technique described in the legend to Fig. 4. Bar, 10 μm.

slides. The variations in fluorescence distribution may reflect either genetic differences between individual larvae or small differences in the stage of development of the individual larva.

Quantitative differences during the development of *Chironomus* larvae were investigated by the solid-phase radioimmunoassay. Salivary glands from second, third, or fourth instars were isolated, homogenized, and analyzed. Using calf thymus HMG-1 and H4 as standards, the amounts of HMG-1 and H4 were determined for the various stages of development. Fig. 7 shows that, during the second and third instars, the amounts of the proteins that bind antibodies increase at a moderate rate; however, with the onset of the fourth instar, rapid increase of both proteins takes place.

Microscopic observation of chromosomes isolated from the third and fourth instars revealed that, as the chromosomes mature and the banding pattern becomes more distinct (3), there is an overall increase in the intensity of the fluorescence obtained with anti-HMG-1 serum. In addition, the fluorescence becomes consolidated into defined regions, thereby producing a more distinct, well-defined, banding pattern (data not shown).

DISCUSSION

In the present manuscript we investigate the *in situ* organization of a defined nonhistone chromosomal protein in polytene chromosomes. The feasibility of these studies is based on the availability of well-characterized, affinity-purified antibodies to chromosomal protein HMG-1.

The results clearly demonstrate that *C. thummi* salivary glands contain one type of molecular species that binds HMG-1 antibodies. Because the molecular weight of this species is indistinguishable from that of HMG-1 protein isolated from calf thymus, and because previous structural and serological studies (33, 38) suggest that the primary sequence of HMG-1 protein is relatively conserved during evolution, we conclude that *C. thummi* salivary glands contain a protein identical or very similar to HMG-1 isolated from calf thymus. Apparently, this protein performs an important cellular role for which it requires its entire structure. The solid-phase radioimmunoassay and the immunofluorescence studies also indicate the presence of extranuclear HMG-1 in the salivary glands. These findings are in agreement with previous studies in which HMG-1 was detected in the cytoplasm of mammalian cells by immunofluorescence (6). Microinjection of ^{125}I-labeled HMG-1 also demonstrated that although HMG-1 tends to accumulate into nuclei, it can rapidly cross the cytoplasm to equilibrate between nuclei (29).

Visualization by immunofluorescence of HMG-1 protein in the polytene chromosomes of *Chironomus* reveals the following points: HMG-1 protein is abundant in polytene chromosomes, it is distributed in a distinct banding pattern along the entire axis of the chromosomes, its distribution generally correlates with the distribution of chromosomal material (but many exceptions are apparent), its distribution is absolutely reproducible only among chromosomes obtained from the salivary glands of one individual (its apparent location varies among individual larvae and may be dependent on the developmental stage of the larvae); and, during the development of the larvae, the amount of HMG-1 per chromosome seems to increase. Concomitant with this increase, there is a consolidation of the apparent location of the HMG-1 protein into distinct regions of the chromosomes.

The immunofluorescence studies have been done on chromosomes fixed with formaldehyde (23, 36). This procedure minimizes the possibility of rearrangement and extraction of HMG-1 during manipulation of the chromosomes. (Indeed, we have observed that, in the absence of formaldehyde fixation, the acidic solutions used to spread the chromosomes extract the HMG-1 from the chromosomes.) The reproducible patterns, obtained in sister salivary glands treated separately on different slides, argue against nonspecific contamination of chromosomes by cytoplasmic HMG-1 or redistribution of the protein during chromosome preparation. Thus, the immunofluorescence experiments reflect the true distribution of the exposed antigenic determinants residing in chromosome-bound HMG-1. We emphasize that the fluorescence distribution patterns and the intensity of fluorescence observed are not an absolute indication of the location or quantity of HMG-1 on any locus in the chromosomes. Obviously, the binding of the antibodies is dependent on the availability of antigenic determinants present in the immunogen. Thus, if the association of HMG-1 with other chromosomal components brings about conforming changes in part of the determinants, or if part of the determinants are sterically hindered, the antibodies will not recognize these regions. Therefore, the only definite conclusion

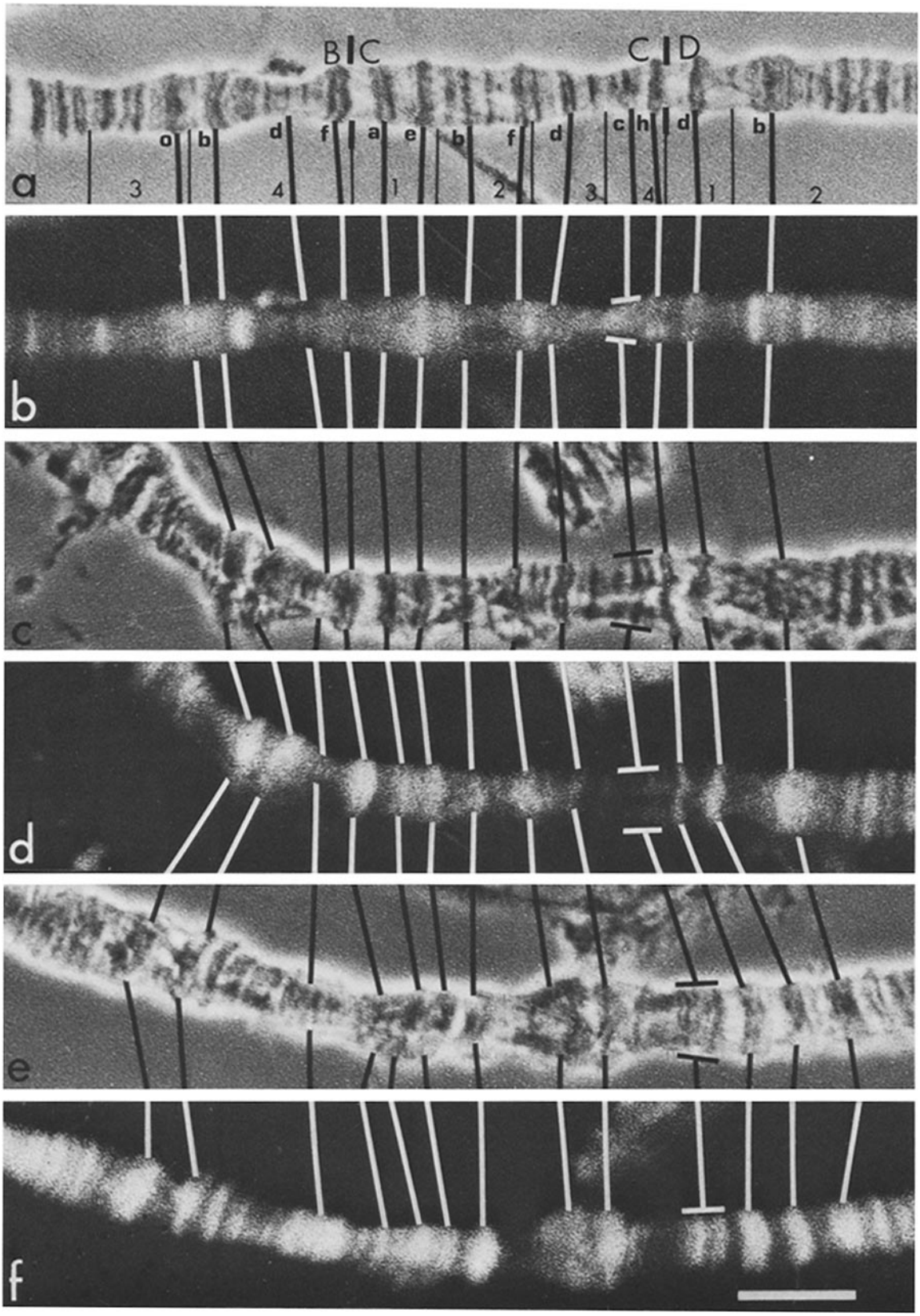

FIGURE 6 Individual variations in the banding pattern obtained with anti-HMG-1 antibodies. Chromosome III from three mid-fourth instar larva reacted with affinity-purified, rhodamine-labeled HMG-1 antibodies diluted 1:100. Panels *A*, *C*, and *E* are phase-contrast micrographs. Panels *B*, *D*, and *F* are corresponding fluorescence micrographs. Black and white lines have been drawn between corresponding chromosome areas by use of the technique described in the legend of Fig. 4. Chromosome regions were identified by means of the chromosome maps of Keyl (20) Hägele (16). Bar, 10 μm.

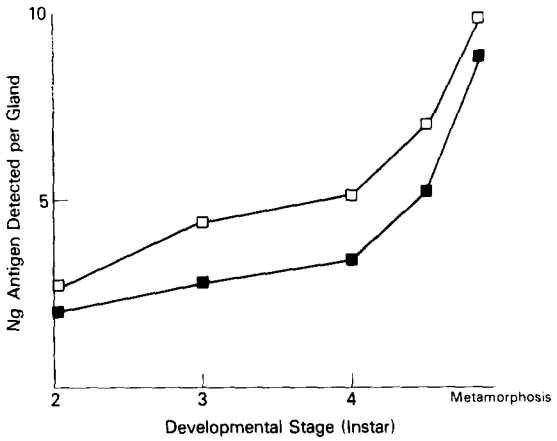

FIGURE 7 Solid-phase radioimmune assay determination of the amount of HMG-1 and H4 in salivary glands at different stages of larval development. □, HMG-1; ■, H4. Amounts of these proteins were determined by use of calf thymus HMG-1 and H4 standard curves.

that can be made is that, in different loci in the chromosome, protein HMG-1 is exposed to various degrees.

Recent evidence suggests that protein HMG-1 is associated with the linker regions between chromatin core particles, and that it performs a structural, rather than regulatory, role in chromatin (27). Its apparent distribution on the polytene chromosomes seems to be different from that of other well-defined proteins. For example, protein D1, which is specific to *Drosophila melanogaster*, is found in only four distinct regions of the genome (2). RNA polymerase B is present almost exclusively in puffs and interband regions of *D. melanogaster* polytene chromosomes (18). Studies applying antisera to specific purified histones on unfixed, or minimally fixed, chromosomes indicate a correlation between the mass of DNA in a region and the amount of histones present. Dark, dense bands tend to fluoresce brighter than the less dense bands or the diffuse interbands (23). The distribution of HMG-1 is reminiscent of a stiuation observed by Silver and Elgin (35, 36) in *Drosophila* polytene chromosomes using several antisera, each elicited against a mixture of nonhistone chromosomal protein of similar molecular weight. Each of these antisera stained selected regions of the chromosome. In some cases, the staining pattern changed upon subjecting the larvae to heat, suggesting rearrangement in the organization of chromosomal proteins. Because the antigens have not been rigorously characterized, it is not possible to determine whether the changes observed reflect rearrangement of structural or regulatory proteins, or whether the changes occurred in proteins specific to the organism studied or in proteins that are ubiquitous to chromosomes in various species. However, they demonstrate that immunofluorescence can serve as a potent tool to study structural changes associated with functional alterations in the genome.

In *Chironomus*, the developmental changes in the amounts of chromosomal and salivary gland proteins have been studied using various techniques (7, 24, 42). However, the chromosomal distribution of these proteins was not determined. The use of specific antisera on a well-characterized antigen, HMG-1, has permitted not only the quantifying and characterization of this chromosomal protein, but has also provided the necessary resolution for localizing it at various stages of development. The exposure of the HMG-1 antigenic determinants differs among various loci in the chromosome, and the loci at which these determinants are preferentially exposed may change during the development of the organism. It is noteworthy that the distribution of ecdysterone-binding sites in *Drosophila* polytene chromosomes changes during development (14). Although the presence of HMG-1 may affect the structural alteration of selected chromosomal regions, it is possible that the presence of HMG-1 may merely reflect areas of the chromosome that have been rearranged, thereby exposing HMG-1 determinants. At present, it is not known whether HMG-1 plays an active or passive role in the maturation of chromosome structure.

It is possible that other chromosomal proteins are arranged in the chromosome in a similar manner. Obviously, such an organization of a protein can serve to modulate the structure of selected regions in response to external stimuli.

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