High Mobility Group Proteins of Amphibian Oocytes: A Large Storage Pool of a Soluble High Mobility Group-1-like Protein and Involvement in Transcriptional Events

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ABSTRACT Oocytes of several amphibian species (*Xenopus laevis*, *Rana temporaria*, and *Pleurodeles waltlii*) contained a relatively large pool of nonchromatin-bound, soluble high mobility group (HMG) protein with properties similar to those of calf thymus proteins HMG-1 and HMG-2 (protein HMG-A; A, amphibian). About half of this soluble HMG-A was located in the nuclear sap, the other half was recovered in enucleated ooplasms. This protein was identified by its mobility on one- and two-dimensional gel electrophoresis, by binding of antibodies to calf thymus HMG-1 to polypeptides electrophoretically separated and blotted on nitrocellulose paper, and by tryptic peptide mapping of radioiodinated polypeptides. Most, if not all, of the HMG-A in the soluble nuclear protein fraction, preparatively defined as supernatant obtained after centrifugation at 100,000 *g* for 1 h, was in free monomeric form, apparently not bound to other proteins. On gel filtration it eluted with a mean peak corresponding to an apparent molecular weight of ~25,000; on sucrose gradient centrifugation it appeared with a very low S value (2–3 S), and on isoelectric focusing it appeared in fractions ranging from pH ~7 to 9. This soluble HMG-A was retained on DEAE-Sephacel but could be eluted already at moderate salt concentrations (0.2 M KCl). In oocytes of various stages of oogenesis HMG-A was accumulated in the nucleus up to concentrations of ~14 ng per nucleus (in *Xenopus*), corresponding to ~0.2 mg/ml, similar to those of the nucleosomal core histones. This nuclear concentration is also demonstrated using immunofluorescence microscopy. When antibodies to bovine HMG-1 were microinjected into nuclei of living oocytes of *Pleurodeles* the lateral loops of the lampbrush chromosomes gradually retracted and the whole chromosomes condensed. As shown using electron microscopy of spread chromatin from such injected oocyte nuclei, this process of loop retraction was accompanied by the appearance of variously-sized and irregularly-spaced gaps within transcriptional units of chromosomal loops but not of nucleoli, indicating that the transcription of non-nucleolar genes was specifically inhibited by this treatment and hence involved an HMG-1-like protein.

These data show that proteins of the HMG-1 and -2 category, which are usually chromatin-bound components, can exist, at least in amphibian oocytes, in a free soluble monomeric form, apparently not bound to other molecules. The possible role of this large oocyte pool of soluble HMG-A in early embryogenesis is discussed as well as the possible existence of soluble HMG proteins in other cells.

The high mobility group (HMG)[1] proteins are a family of nonhistone components of chromatin that are of relatively low molecular weight (M_r 7,200–28,000), contain high proportions of both positively and negatively charged residues distributed in a polar fashion, can be extracted from chro-

[1] *Abbreviations used in this paper:* HMG, high mobility group; PCA, perchloric acid; TCA, trichloroacetic acid.

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matin with 0.35 M NaCl or 5% perchloric acid, and are soluble in 2% trichloroacetic acid (TCA; for reviews see 1 and 2). They occur in diverse eucaryotic species and many of them have been purified and have been sequenced at least in parts (3–6). In mammals and birds, two predominant pairs of HMG proteins have been distinguished, i.e., the closely related relatively large polypeptides (M_r 26,000–29,000) HMG-1 and HMG-2 on the one hand, and the smaller proteins HMG-14 and HMG-17 (M_r 9,000–10,500) on the other hand. The HMG proteins are less abundant than the histones but they are still among the major proteins of chromatin. It has been estimated that there are, in a typical somatic cell nucleus, e.g., of a calf thymocyte, $\sim 10^6$ molecules of each HMG protein class and that the total HMG protein is ~3% by weight of the DNA (2, 7).

The presence of HMG-14 and -17 has been correlated with the preferential sensitivity of transcriptionally competent chromatin towards digestion with DNAse I (8, 9), and there is now widespread consensus that they are somehow involved in the maintenance of the specific structural organization of transcriptionally active or potentially transcribable chromatin (for reviews see 10–12). Hypotheses on the possible physiological role of the HMG proteins 1 and 2 are on less solid experimental ground. Unlike HMG-14 and -17, a substantial portion of HMG-1 and -2, which have been reported to bind preferentially to single-stranded DNA (13–16; for reviews see 11 and 12), are rather loosely bound to chromatin. They can be solubilized during cell fractionation (17), in particular after repeated exposure of isolated nuclei to 0.14 M NaCl (18), and in some cells they seem to occur, as suggested from immunofluorescence microscopy, in both compartments, nucleus and cytoplasm (19, 20). Whether certain subsets of the HMG proteins 1 and 2, and the homologous protein HMG-T of trout testis, are specifically associated with replicating and/or transcribable regions of chromatin is so far not clear. Whereas some authors have reported a specific association with "active" chromatin fractions obtained from various cell types and species (15, 21–24), other authors could not confirm this observation (25, 26). There is no doubt, however, that proteins HMG-1 and -2 are also integral components of chromatin.

In pilot experiments in which we have microinjected antibodies to certain proteins into germinal vesicles of living amphibian oocytes to examine their possible involvement in the transcription of lampbrush chromosomes (e.g., 27, 28) we have noted that antibodies to proteins HMG-1 from calf thymus induce a collapse of the transcription loops of these chromosomes. This had led us to examine the HMG proteins present in amphibian oocytes, specifically whether they are accumulated and stored in these nuclei in a similar way as found for histones (e.g., 29, 30). The relatively large pool of soluble, i.e., not chromatin-bound histones of amphibian oocytes is thought to be a prerequisite for the extremely rapid chromatin formation in the early embryonic cleavage stages (31). Therefore, it is conceivable that other nonhistone chromatin components may also be stored in the oocyte for use in the future embryonic development.

In the present study we show that oocytes of various amphibian species contain large amounts of a soluble form of one HMG protein, HMG-A, which is accumulated in the nucleus but is also found in the cytoplasm. We further show that antibodies against mammalian HMG-1 and -2 (32–34), when microinjected into these nuclei, interfere with transcriptional events in the lampbrush chromosomes.

MATERIALS AND METHODS

Animals and Oocytes: African clawed toads of the species *Xenopus laevis* were obtained from the South African Snake Farm (Fish Hoek, South Africa). Females of the frog species, *Rana temporaria*, and the salamander, *Pleurodeles waltlii*, were collected and kept as described (35). Oocyte proteins were labeled with [^{35}S]methionine as previously described (36).

Isolation of Nuclei and Nuclear Subfractions: Oocyte nuclei were isolated either manually in the 3:1 medium specified elsewhere (27), except that 1 mM $MgCl_2$ was added. Alternatively, germinal vesicles were isolated using a modification (30) of the large-scale method according to Scalenghe et al. (37). Low speed pellets (3,500 *g*, 10 min) and high speed supernatant fractions obtained after homogenization of isolated nuclei and centrifugation at 100,000 *g* for 1 h at 4°C were either used directly or kept frozen at −20°C (for details see 30).

Radiolabeling of Oocyte Proteins and Extraction of HMG Proteins: Ten manually defolliculated oocytes (stages V-VI, [38]) were incubated in 0.1 ml modified Barth's medium to which 5 μl [^{35}S]methionine (final concentration 0.5 mCi/ml; New England Nuclear, Boston, MA) was added for 24 h at room temperature (35). Nuclei and ooplasms were manually separated and extracted with 5% perchloric acid (PCA) essentially as described by Nicolas and Goodwin (39). Alternatively, the procedure was simplified in the following way: The specimens, pellet or solution, were extracted for 30 min at 4°C with 3 or 5% PCA, centrifuged (15 min, 3,500 *g*), and the supernatant was precipitated with 15% TCA (final concentration) or by adding 6 vol of cold acetone. Final precipitates were dried and used for analysis.

Chromatography, Isoelectric Focusing and Sucrose Gradient Centrifugation: Gel permeation chromatography on Sephacryl S-300 and ion-exchange chromatography on DEAE-Sephacel were performed as previously described (30). For isoelectric focusing of proteins 5 ml of the 100,000 *g* supernatant fraction from isolated nuclei were applied to the isoelectric focusing column (LKB; 110 ml) by mixing it with the solution containing pH 2-11 ampholines (SERVA Feinbiochemica Gmbh & Co., Heidelberg, Federal Republic of Germany). Focusing was performed at 13 W (E_{max} = 1,600 V) for 16 h at 4°C, and finished at a final constant current of 1.9 mA. Fractions of 3 ml were eluted, the pH was measured, and the protein was precipitated with 30% TCA (final concentration), washed with acetone, dried, and analyzed using gel electrophoresis (see below).

Conditions for analysis of soluble nuclear proteins from oocytes by centrifugation in sucrose gradients were as recently described (30), except that for this study 5–20% (wt/vol) sucrose gradients were used and centrifugation times were 15 h. TCA-precipitated fractions were washed in acetone only.

Gel Electrophoresis, Peptide Map Analysis, and Immunological Detection of Polypeptides: Conditions for one- and two-dimensional gel electrophoreses were exactly as described (30). For "immunoblotting" experiments according to the method of Towbin et al. (40) as modified by Gigi et al. (41), polypeptides were usually separated on gels made from 15% acrylamide. Gels were stained with SERVA blue (equivalent to Coomassie Blue R250; SERVA Feinbiochemica Gmbh & Co.) or using silver stain technique according to Switzer et al. (42; for modifications see 43).

For two-dimensional peptide map analysis of tryptic fragments of polypeptide bands or spots excised from gels, the procedure of Elder et al. (44) was used. HMG proteins used for comparison were prepared from calf thymus as described (32).

Antibodies and Immunofluorescence Microscopy: Rabbit antibodies to HMG proteins from calf thymus have been described (19, 32–34). Immunofluorescence microscopy using cryostat sections through frozen ovaries of adult females of the species studied was essentially as described (28). Total IgG as well as affinity-purified IgG were used with identical results (compare 34). For comparison, rabbit antibodies against tubulin (generously provided by Dr. B. Jockusch, University of Bielefeld, Federal Republic of Germany), chicken antibodies, and monoclonal murine antibodies to ribonucleoproteins (cooperative work with Dr. T. Martin, University of Chicago, IL; for cross-reactivity with amphibian antigens see 45), as well as guinea pig antibodies to nucleoplasmin from *Xenopus laevis* ([35, 46]; either as IgG fractions or affinity-purified according to reference 47) were used.

Microinjection and Electron Microscopy of Nuclear Contents: Injection of small volumes of solutions containing antibodies (total IgG fraction or affinity-purified IgG; 5–20 ng protein per nucleus) into the germinal vesicles of *Xenopus laevis* or *Pleurodeles waltlii* has been described (27, 28). The preparation of lampbrush chromosomes and spread preparations for electron microscopy of transcriptional structures have also been described in detail (48, 49).

RESULTS

Gel Electrophoresis

When total ovaries of amphibia were extracted with 3% PCA, the extracts contained relatively large amounts of a polypeptide with similar mobility, on SDS PAGE, as purified HMG-1 protein from bovine thymus, i.e., appearing under our conditions at a position corresponding to an approximate M_r value of 26,000 (Fig. 1 *a*). Evidence that this band contained a HMG-1-like protein (tentatively designated HMG-A) was obtained by its reaction, after blotting on nitrocellulose paper, with antibodies against bovine thymus HMG-1, which also cross-reacted with HMG-2 (Fig. 1 *b*; compare 34). When total proteins of isolated oocyte nuclei from *Xenopus* and *Pleurodeles* were examined by the same immunoblotting technique, again only this band reacted with the HMG-1 antibodies (Fig. 1, *c* and *d*). Characteristically, the HMG-A polypeptide of *Xenopus* migrated slightly faster than that of *Pleurodeles* (Fig. 1, *a–d*). That the band reacting with HMG-1 antibodies was among the major polypeptide bands of whole germinal vesicles indicated that HMG-A was present in these nuclei in amounts far in excess over the amount of total chromatin present (for quantitative considerations of ratios of proteins to nucleic acids see 50 and 51).

PCA-extracts from isolated nuclei of *Xenopus laevis* and *Pleurodeles waltlii* resulted in a remarkable enrichment of the HMG-A polypeptide which, next to nucleoplasmin, was the

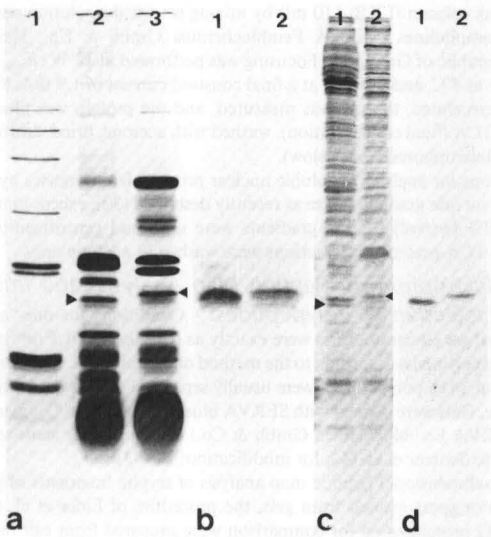


FIGURE 1 SDS PAGE of proteins extracted with PCA from total ovaries and from isolated oocyte nuclei of *Xenopus laevis* (a, lane *2*; c, lane *1*) and from whole ovaries and isolated oocyte nuclei of *Pleurodeles waltlii* (a, lane *3*; c, lane *2*) visualized by staining with Coomassie Blue (*a* and *c*) and by antibody reaction on nitrocellulose paper blots (*b* and *d*). (a) Lane *1*, reference proteins (from top to bottom): β-galactosidase (116,000), phosphorylase a (93,000), BSA (68,000), actin (43,000), and calf thymus histones H1, H3, H2B, H2A, and H4; lane *2*, 3% PCA-soluble polypeptides from total ovary from *Xenopus laevis*; lane *3*, 3% PCA-soluble polypeptides of total ovary from *Pleurodeles waltlii*. (*b*) Lane *1*, reaction of antibodies to HMG-1 with the polypeptides shown in *a*, lane *2*; lane *2*, reaction of HMG-1 antibodies with the polypeptides shown in *a*, lane *3*. (*c*) Total nuclear polypeptides of *Xenopus laevis* oocytes (lane *1*) and *Pleurodeles waltlii* (lane *2*) transferred to nitrocellulose filters. (*d*) Reaction of HMG-1 antibodies with the polypeptides shown in *c*, lanes *1* and *2*. Note specific antibody reaction with only one polypeptide band (arrowheads) in both species.

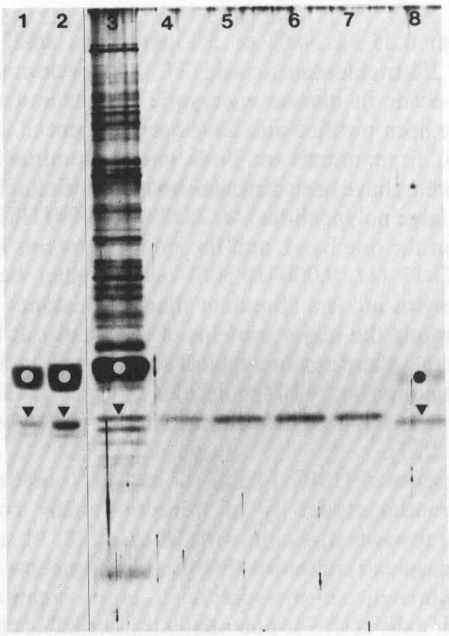


FIGURE 2 Gel electrophoresis of PCA-extractable HMG-A from oocyte nuclei and estimation of PCA-soluble HMG-A, after staining with Coomassie Blue (lanes *1* and *2*) and silver (lanes *3–8*). Lane *1*, 75 mass-isolated nuclei from *Xenopus laevis* extracted with 3% PCA, run on 18% polyacrylamide gels and stained with Coomassie Blue R250. Lane *2*, same as lane *1* but protein from 150 mass-isolated nuclei has been applied. Lane *3*, 3% PCA-soluble proteins from 18 oocyte nuclei of *Pleurodeles waltlii* after silver staining. Lanes *4–7*, 0.1, 0.2, 0.3, and 0.4 μg of calf thymus HMG-1 after silver staining. Lane *8*, 3% PCA-soluble proteins of 20 oocyte nuclei of *Xenopus laevis* after silver staining. The HMG-A polypeptide band of *Xenopus* is denoted by arrows; dots denote nucleoplasmin. Note that HMG-A from *Pleurodeles* co-migrates with bovine thymus HMG-1 whereas *Xenopus* HMG-A migrates slightly faster.

second most frequent protein in this fraction (Fig. 2). Careful calibration of staining of authentic bovine thymus HMG-1 with Coomassie Blue (not shown) and silver nitrate (Fig. 2, lanes 4–7) allowed for an estimate, by densitometry tracing, of the minimal amount of HMG-A detected in isolated nuclei of full-grown amphibian oocytes that came out as ~20 ng per nucleus in *Pleurodeles* and ~14 ng in *Xenopus laevis*. Similar amounts were found in nuclei of oocytes in *Rana temporaria* (not shown).

The quantitative importance of HMG-A among the total nuclear proteins as well as the soluble nuclear protein fraction, defined as supernatant obtained after centrifugation at 100,000 *g* for 1 h (for details see Materials and Methods), was directly seen from two-dimensional gel electrophoresis using nonequilibrium pH gradient electrophoresis in the first dimension (Fig. 3, *a* and *b*). Such separations suggested that the amount of soluble HMG-A present was in the same range as that of histones H2A, H2B, H3, and H4, i.e., proteins known to exist in a large storage pool in these nuclei (29–31, 52–54). In such two-dimensional separation of polypeptides the component HMG-A identified as closely related to bovine HMG-1 by peptide mapping (e.g., Fig. 3, *c–f*) appeared in a streak-like fashion extending from a position corresponding to a pH range of 7–9 (Fig. 3, *a* and *b*), similar to the broad range of isoelectric focusing previously described for undenatured calf thymus HMG-1 and -2 (55). Detailed comparison of tryptic peptide maps of HMG-A with those of bovine HMG-1 and

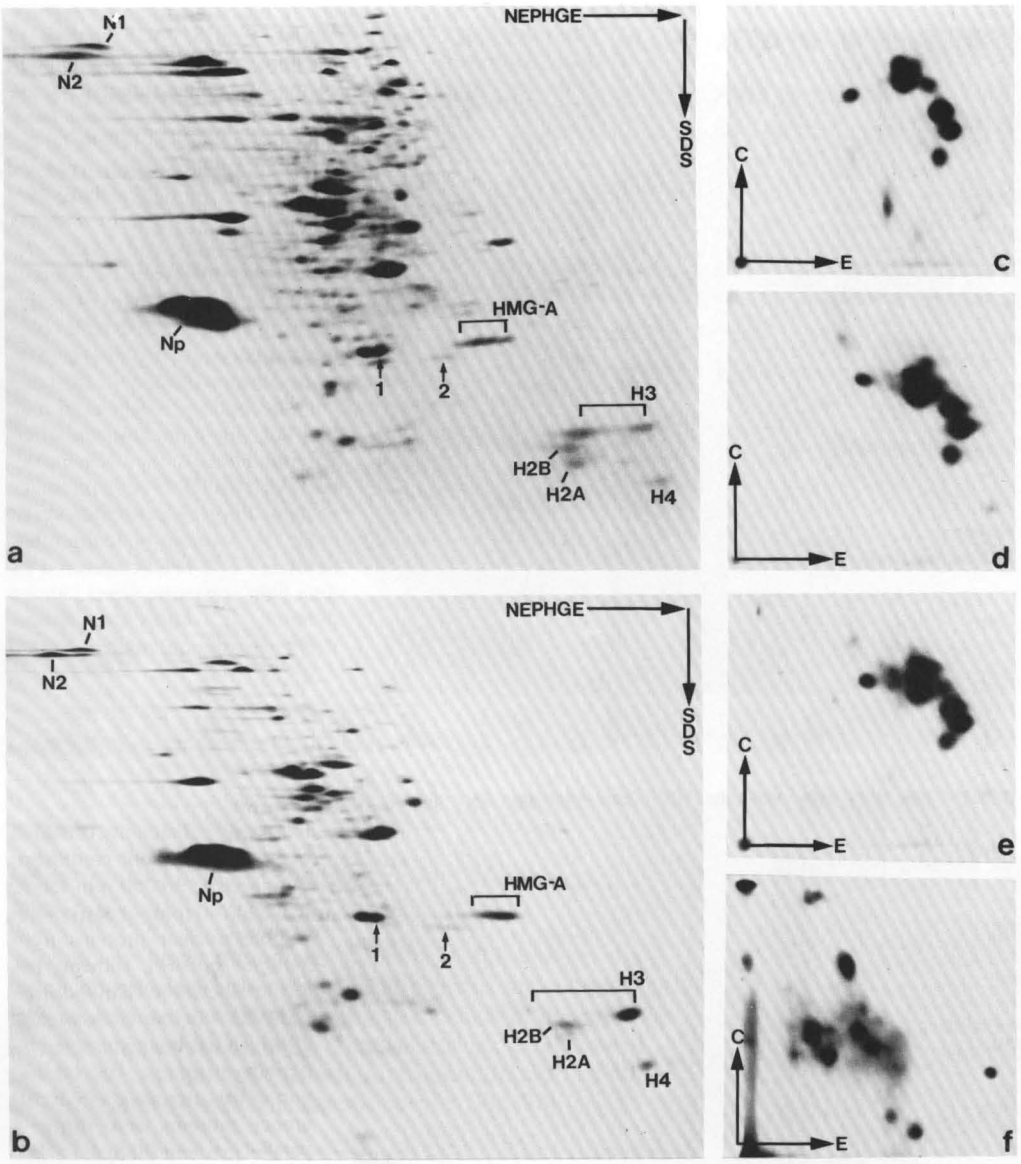


FIGURE 3. Identification of HMG-A after two-dimensional gel electrophoresis of total nuclear proteins from *Xenopus laevis* oocytes (*a*) and of 100,000 *g* supernatant proteins (*b*) by peptide mapping (*c–f*). (*a*) Two-dimensional gel electrophoresis (first dimension, nonequilibrium pH gradient electrophoresis, [*NEPHGE*]; second dimension, electrophoresis in the presence of SDS) of 300 ethanol-fixed, mass-isolated oocyte nuclei from *Xenopus laevis*. (*b*) Two-dimensional gel electrophoresis (as in *a*) of the 100,000 *g* supernatant fraction from mass-isolated oocyte nuclei. (*c*) Tryptic peptides from radioiodinated calf thymus HMG-1 (*E*, electrophoresis; *C*, chromatography); (*d*) Tryptic peptide mapping of the HMG-A spot (*bracket*) excised from the gel shown in *b*: (*e*) Mixture of *c* and *d*; (*f*) Tryptic peptide mapping of a prominent spot (denoted by arrow *1* in *b*) with similar electrophoretic mobility in the presence of SDS as HMG-A. Arrow *2* in *b* denotes another polypeptide with a peptide map fingerprint different from that of HMG-A (not shown). *N1* and *N2*, karyophilic acidic polypeptides N1 and N2; *Np*, nucleoplasmin.

HMG-2 also revealed some minor differences that distinguished HMG-A from bovine HMG-1 and -2 (Fig. 3, *d* and *e*), but not bovine HMG-1 from bovine HMG-2.

Polypeptides similar in their electrophoretic mobilities to proteins HMG-14 and 17 have not been detected among the nuclear proteins in comparably high amounts, in agreement with recent findings of Weisbrod et al. (56). This emphasizes the selective importance of polypeptide HMG-A in oocytes.

We also examined low speed pellets containing most of the chromosomal and nucleolar material but could not detect significant amounts of HMG proteins. However, our method would not have allowed us to determine <1 pg of HMG-A per pelletable material of one nucleus.

Determinations of Sizes and Isoelectric Points of Native Proteins

On gel filtration of soluble nuclear proteins (Fig. 4) HMG-A was recovered in fractions with a mean peak maximum corresponding to an apparent M_r of 25,000, clearly separated from the prominent larger complexes such as those of nucleoplasmin and the fractions containing histones and the karyophilic polypeptides N1 and N2 (see Fig. 4; compare 30). This demonstrated that the soluble HMG-A protein of amphibian oocytes was not contained in larger complexes but was, most likely, individual "free" molecules.

A similar observation was made on sucrose density gradient centrifugation of the nuclear supernatant fraction (Fig. 5). The HMG-A protein was among the smallest components, sedimenting with 2–3 S, compatible with the interpretation that they exist as individual molecules and do not form aggregates under these ionic conditions (for tendency of HMG-1 to aggregate at low ionic strength see 55).

To determine the isoelectric point(s) of the soluble native HMG-A molecules we performed an isoelectric focusing in a sucrose-containing column. Again, HMG-A was recovered in fractions widely separated from nucleoplasmin as well as from polypeptides N1 and N2 and the bulk of the histones (Fig. 6). HMG-A did not focus sharply but was spread over a range from ~7.0 to 9.0 pH, indicative of some charge heterogeneity (charge heterogeneity is also suggested by the results with extracted calf thymus HMG-1 and -2; [55]). In contrast to most of histones H3 and H4, HMG-A protein did not seem to exist in stable complexes with acidic proteins. Despite its slightly basic character, the *Xenopus* oocyte HMG-A was bound, at neutral pH, to a potent anion exchange substrate

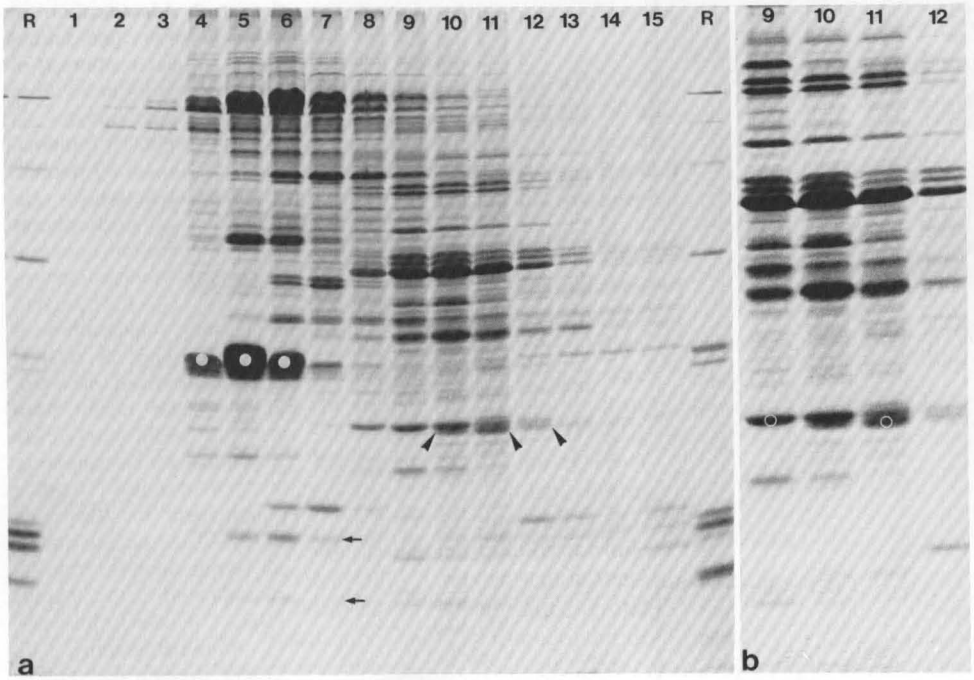


FIGURE 4. SDS PAGE analysis of fractions obtained by gel filtration of the 100,000-*g* supernatant nuclear proteins from *Xenopus laevis* oocytes on Sephacryl-S300. (*a*) Positions of reference proteins for gel filtration for ferritin, catalase, BSA, and chymotrypsin are 440,000, 230,000, 68,000, and 25,000, respectively. Arrowheads denote HMG-A. (*b*) Enlargement of the gel showing the fractions used for peptide mapping analysis (dissected regions denoted by circles). The band denoted by the left circle (in fraction slot 9) is a polypeptide different from HMG-A; the circle in fraction slot *11* is HMG-A. *R*, reference proteins for gel electrophoresis (as in Fig. 1). Positions of *Xenopus* histones H3 and H4 as detected by their light scattering effect (30) are denoted by horizontal arrows. White dots denote nucleoplasmin.

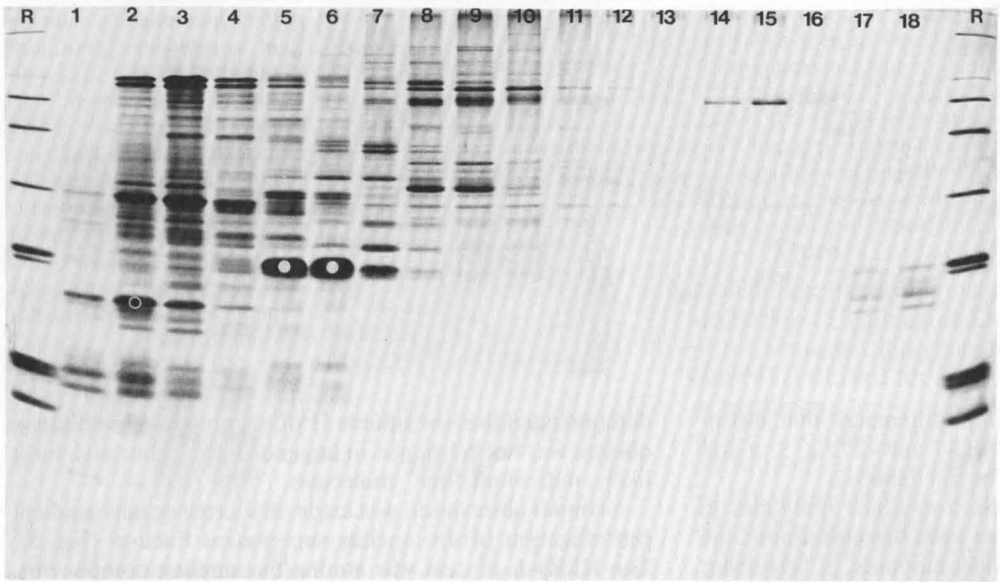


FIGURE 5. Fractions obtained by sucrose gradient centrifugation (5-20%; 36,000 rpm for 15 h in a SW40 rotor at 4°C) of the soluble nuclear proteins from *Xenopus* oocytes, showing the low molecular weight position of HMG-A (white circle in lane *2*), which has been identified by peptide mapping. For calibration of the sucrose gradient reference proteins were run in a parallel gradient (BSA, 4.3 S; immunoglobulin, 6.5 S; catalase, 11.3 S; and β-galactosidase, 16.5 S. Sedimentation direction is from left to right (fractions *1–18*). Reference proteins for gel electrophoresis include myosin heavy chain (uppermost band in slots denoted *R*; all other proteins are as described in Fig. 1). The position of the *Xenopus* histones at this concentration is hardly visible under translucent illumination but can be visualized by the light scattering effect in fractions *3–6*. Nucleoplasmin is denoted by white dots.

such as DEAE-Sephacel (Fig. 7). As expected, however, this binding was rather loose and the protein could be eluted at relatively low salt concentrations (Fig. 7). This unexpected behavior of HMG-A could be due to its amphipolar molecular structure characterized by a very acidic C-terminal region, the "HGA region" (6). In none of these separations did we note an enrichment of other HMG proteins in any of the fractions (56), again indicating that only HMG-A was present in such excessive amounts.

Demonstration and Determination of Cytoplasmic HMG-A

Since the literature on the nuclear vs. cytoplasmic distribution of HMG-1 in various diverse cell types has been somewhat controversial (17, 19, 20. 57–60), we manually dissected nuclei and enucleated ooplasms from the same oocytes and analyzed the proteins recovered in PCA-extracts from these cell fractions. The results (Fig. 8, *a* and *b*) showed that about equal amounts of HMG-A could be recovered in nucleus and cytoplasm, thus presenting a clear example of storage of soluble HMG-A in the cytoplasm of one cell, the oocyte. Considering the volume ratio of oocyte nucleus and ooplasm, however, the same results also indicated that HMG-A was more than tenfold concentrated in the nuclear compartment. This nuclear accumulation was also seen using immunofluorescence microscopy of sections through oocytes of stages V and VI (data not shown).

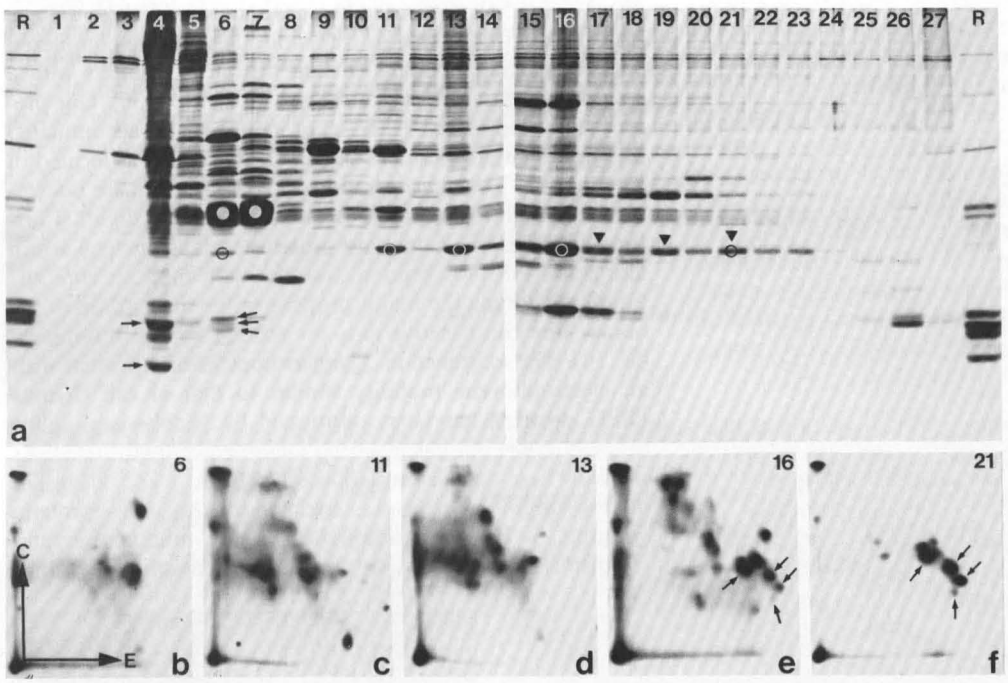


FIGURE 6. SDS PAGE of fractions eluted from an isoelectric focusing column loaded with soluble nuclear proteins of *Xenopus* oocytes (*a*) and identification of protein HMG-A by peptide mapping analysis (*b–f*) of selected peptides (indicated by circles in *a*; *b* corresponds to fraction 6, *c* to fraction *11*, *d* to fraction *13*, *e*, to fraction *16*, and *f* to fraction *21*). Positions of HMG-A are denoted by arrowheads; positions of the core histones are indicated by horizontal arrows (most of histones H3 and H4 is recovered at pH 4.6, the other core histones are spread between 4.7 and 5.3; some histone H3 and H2B is contained in neutral and even basic fractions). Histones have been detected by the light scattering effect (30) and identified by two-dimensional gel electrophoresis. Reference proteins for gel electrophoresis are as described in Fig. 1. Arrows in *e* and *f* denote typical tryptic marker peptides of HMG-A, which are not recognized in *b–d*.

Presence of HMG-A Pools in Earlier Stages of Oogenesis

Relatively large amounts of protein HMG-A were also found on gel electrophoresis of total proteins and of PCA-extracts from nuclei isolated from earlier stages of oogenesis. Fig. 9*a* presents an example. The nuclear accumulation of this protein was recognized, using immunofluorescence microscopy, already in previtellogenic oocytes (Fig. 9*b*).

Microinjection of HMG-1 Antibodies into Oocyte Nuclei

When antibodies to bovine HMG-1 were injected into nuclei of living oocytes of lampbrush chromosome stages of *Pleurodeles waltlii*, followed by preparation of the chromosomes at certain times after injection, progressive retraction of the transcribed chromosomal loops was recognized, which did not occur when immunoglobulins from nonimmunized rabbits, guinea pigs, mice, or chickens were injected (as a representative example chromosome IV of this organism is shown in Fig. 10, *a–d*; for identification of chromosomes see 61). This antibody-induced loop retraction was accompanied by a maximal eight- to tenfold contraction of the chromosomal axis (Fig. 10, *c* and *d*). Electron microscopic examinations of spread chromatin from such injected nuclei, using the technique of Miller et al. (62) as modified by Scheer et al. (27), showed that early stages of antibody-induced loop retraction were characterized by the appearance of variously-sized, irregularly-distributed gaps within the transcriptional units, i.e., regions devoid of lateral fibrils containing nascent ribonucleoprotein (Fig. 11, *a–c*). No such interruptions occurred in the pre-rRNA genes of nucleolar chromatin detected on the same grid (Fig. 11*a* and *inset*). In more advanced

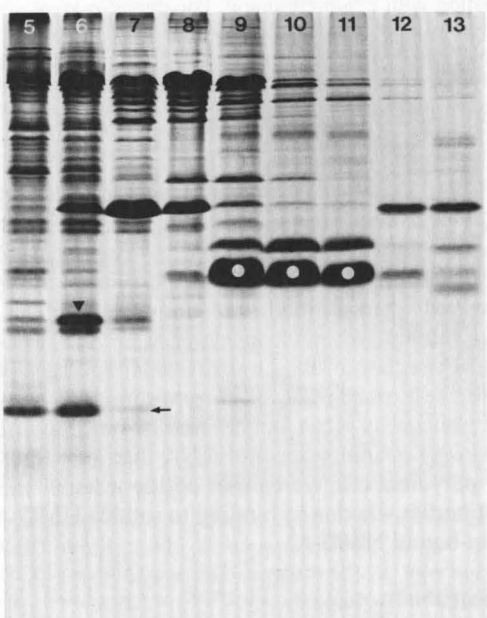


FIGURE 7 Anion exchange chromatography of soluble nuclear proteins from *Xenopus* oocytes, showing proteins bound to DEAE-Sephacel that have been eluted by an increasing salt gradient from 0–0.4 M KCl. HMG-A protein (arrowhead) has been identified by two-dimensional gel electrophoresis and by peptide mapping (not shown). The position of histone H3 is denoted by a horizontal arrow; nucleoplasmin is denoted by white dots. Fraction numbers are indicated on the top margin. HMG-A elutes between 0–0.2 M KCl.

stages of loop retraction such spread preparations were difficult to obtain and only occasionally remaining short loop strands with very sparse transcriptional complexes were de-

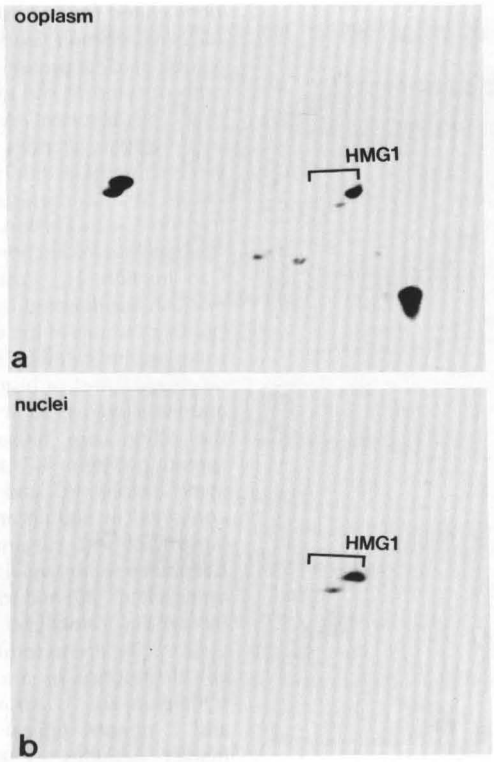


FIGURE 8 Autoradiofluorography of PCA-extracted polypeptides from 10 ooplasms (*a*) and 10 nuclei (*b*) of *Xenopus* oocytes labeled by incubation with [^{35}S]methionine. Polypeptides were separated on two-dimensional gel electrophoresis (NEPHGE and SDS, as in Fig. 3). Brackets denote the position of added nonradioactive calf thymus HMG-1. Note that similar amounts of labeled HMG-A are detected in both cell compartments.

tected. In this respect the observations made with antibodies to HMG-1 were very similar to those made upon injection of antibodies to histone H2B (27) and RNA polymerase II (28). No significant effects on lampbrush chromosome structure were observed when antibodies to ribonucleoproteins (compare 28), tubulin, or nucleoplasmin were injected. These findings suggest that antibodies to HMG-1 specifically interfered with the transcription of chromosomal genes, at least those transcribed by RNA polymerase II, but not with transcription of genes coding for pre-rRNA that were transcribed by RNA polymerase I. We cannot decide whether the effect of the antibodies was due to binding to soluble HMG-A or to chromatin-bound HMG-A.

DISCUSSION

Constitutive proteins of chromatin have a high tendency to bind to DNA and in most cells are found as integral parts of the chromatin structure, i.e., in an insoluble state. However, these proteins can also occur in soluble forms in both the cytoplasm and the nucleoplasm and, at least in certain cell types, they are accumulated in the nucleus. The existence of at least some soluble histones or HMG proteins may be deduced simply from the fact that they are synthesized in the cytoplasm and from findings that they can distribute in a common cytoplasm between an endogenous nucleus and a nucleus experimentally introduced into the cell (58). Direct evidence of the existence of soluble pools of such proteins has been presented for the amphibian oocyte, which contains large amounts of histones in the cytoplasm as well as in the nucleus, with a remarkably high concentration in the latter (29–31, 51–54). Considerable proportions of these oocyte histones does not occur as free molecules but are complexed with very acidic proteins so that histone complexes with a resulting negative electrical charge are formed (30). Our present study shows that HMG-A protein was also accumulated in amphibian oocytes and that about half of it was located in the nucleus. The concentration of HMG-A in these oocytes was very high, comparable with those of the individual histones: Besides very abundant proteins such as nucleoplasmin and actin, HMG-A protein, together with the four core histones, are among the major proteins of the nuclear sap of the oocyte (for comparative data on protein concentrations see 46, 54). The existence in *Xenopus* oocytes of a protein with an electrophoretic mobility similar to that of calf thymus HMG proteins has been mentioned by Nicolas and Johns (quoted in 63) but has not been identified or quantitated. As we show here the accumulation of HMG-A in the oocytes of diverse amphibian species is selective for this HMG protein; amounts of HMG-14 and HMG-17 are much lower and contribute little, if any, to the intranuclear protein storage

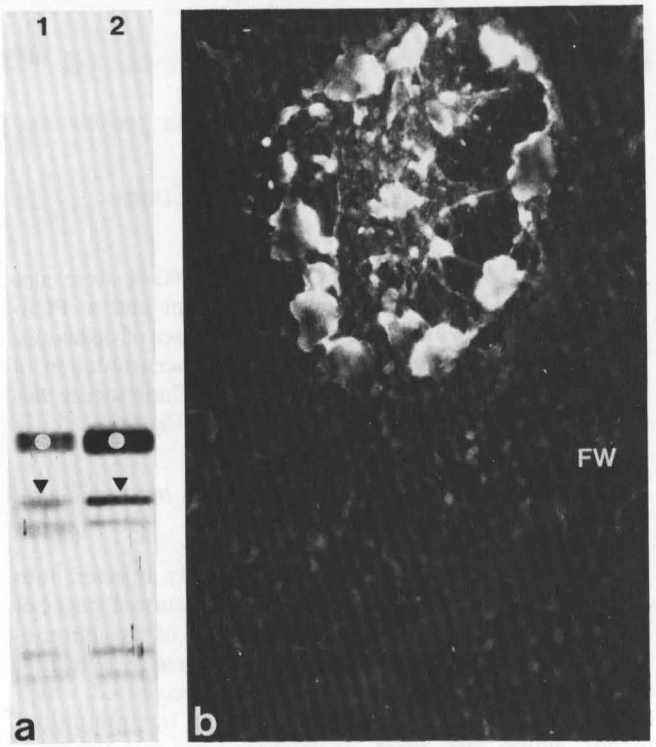


FIGURE 9 Presence of protein HMG-A in growing oocytes of *Xenopus laevis*. (*a*) 20 nuclei were manually isolated from lampbrush stage oocytes (0.8-mm diam) and from full-grown oocytes (1.3-mm diam). The PCA-soluble proteins were separated on a 18% polyacrylamide gel and silver-stained. Nucleoplasmin is indicated by white dots; HMG-A is indicated by arrowheads. Lampbrush stage oocyte nuclei contain ~1–1.5 ng HMG-A per nucleus (lane *1*) as opposed to ~14 ng HMG-A per nucleus of mature oocytes (lane *2*). Whether the faint bands below HMG-A are also HMG proteins remains to be examined. (*b*) Immunofluorescence microscopy of a frozen section through a previtellogenic oocyte stained with antibodies to HMG-1 protein from calf thymus. Although the structural preservation of the nuclear interior is rather poor in such freeze-sectioned and formaldehyde-fixed ovarian tissue, the nucleus shows a strong fluorescence. The cytoplasm also shows a faint but significant fluorescence. *FW*, follicle wall. Bar, 20 μm. × 800.

pool (56). The properties of amphibian oocyte HMG-A are very similar to those of mammalian HMG-1. In contrast to histones H3 and H4, HMG-A molecules are not bound to other proteins but appear as free monomeric molecules distributed throughout the nuclear sap and the cytosol. Moreover, in both nucleus and cytoplasm the HMG-A protein appears with similar sizes and electrical charges, indicating that the intracellular distribution is not regulated by different modifications of the protein.

Our demonstration of large amounts of HMG-A in microdissected nuclei and ooplasms in living oocytes also supports previous observations using immunofluorescence microscopy that a considerable proportion of the HMG-1 in cultured cells is located in the cytoplasm (19). Clearly, nuclei can accumulate HMG proteins and in many somatic cells a considerable portion of the total HMG proteins is bound to chromatin (57, 59). Rechsteiner and Kuehl (58) have proposed that the binding of HMG-1 to chromatin "is sufficient in itself to assure that it will concentrate in the nucleus." Our present data speak against this and present a case of accumulation of a HMG-1-like protein in the nucleoplasm without binding to chromatin. Moreover, in our opinion the failure of various authors to detect cytoplasmic HMG-1 is most likely due to preparative problems, primarily the almost inevitable loss of some soluble protein during incubation with antibody solutions and buffers (for problems in the immunofluorescent demonstration of even the most abundant soluble nuclear protein, nucleoplasmin, see 46). The sizes of the nuclear and cytoplasmic pools of free HMG proteins in cells other than oocytes are not known but the presence of some cytoplasmic

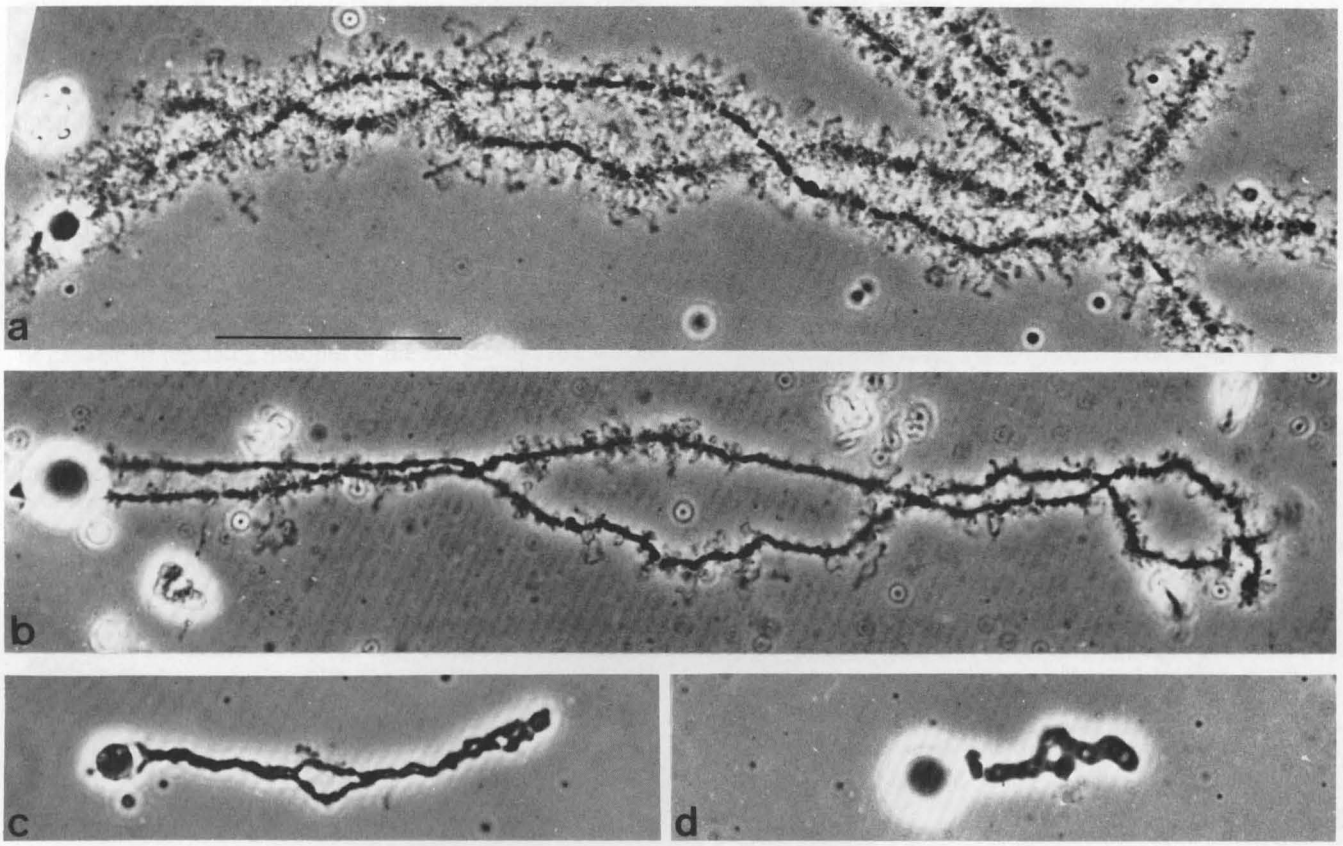
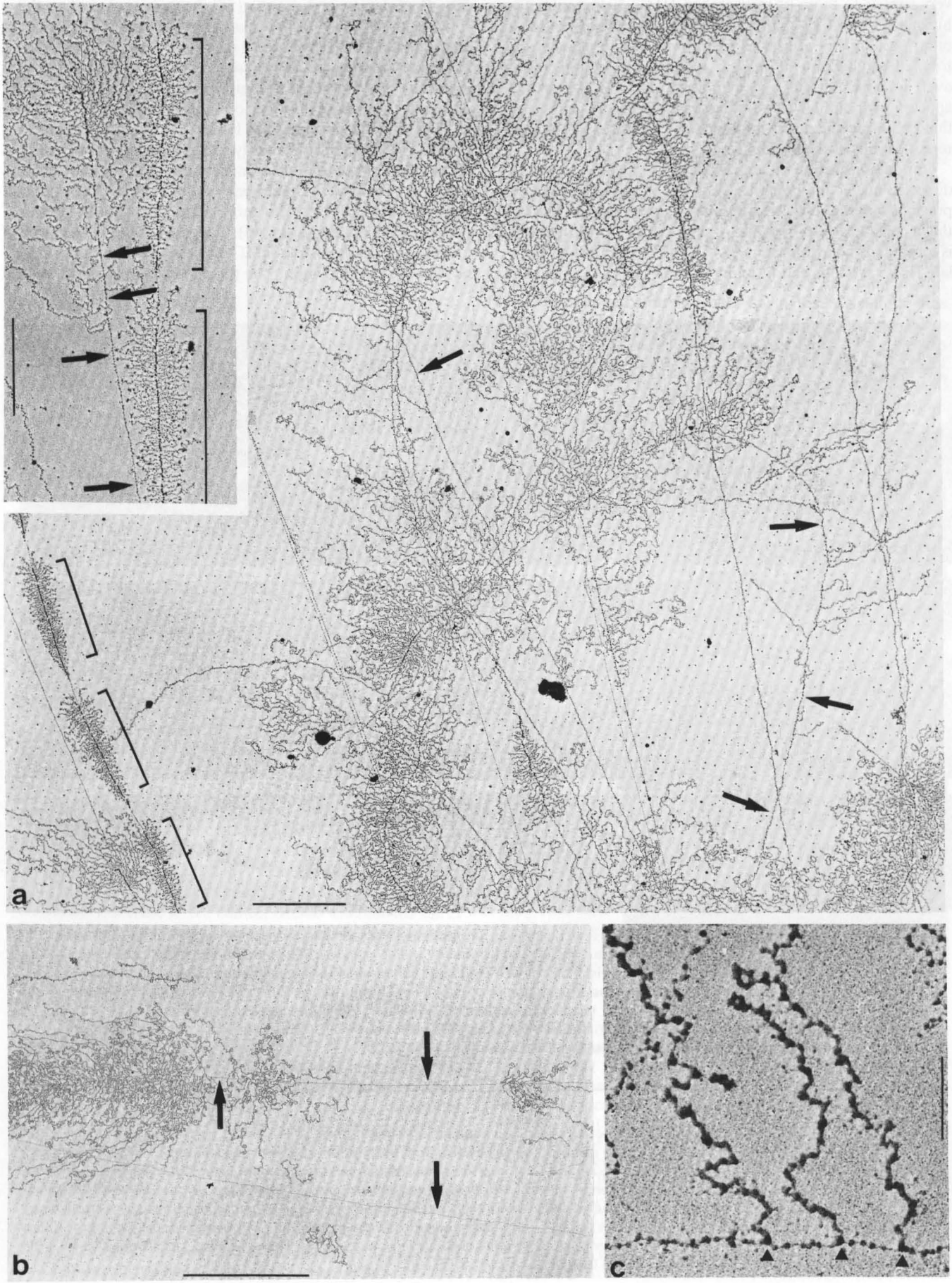


FIGURE 10 Light micrographs (phase contrast optics) showing progressive loops retraction of lampbrush chromosomes after injection of antibodies to HMG-1 protein into nuclei of *Pleurodeles waltlii* oocytes. All photographs show, at the same magnification, chromosome bivalent IV, which is characterized by a large subterminal sphere (*left*; compare 61). (*a*) Injection of nonimmune IgG (1.5–2 mg/ml) does not alter the structural appearance of the chromosomes. (*b*) Partial retraction of lateral loops into the chromosome axis is seen 30 min after injection of antibodies to HMG-1. After prolonged exposure (*c*, 3 h; *d*, 4 h) all lateral loops are completely retracted into the chromomeric axis. Note the drastic size reduction of the chromosomes after antibody-induced transcriptional inactivation. Bar in *a*, 50 μm. × 640.

FIGURE 11 Electron microscopy of a spread preparation showing chromatin from a *Pleurodeles* oocyte nucleus 15 min after injection of antibodies to HMG-1 into the nucleus. Transcriptional arrays of lampbrush chromosome loops and pre-rRNA genes (the latter are denoted by brackets in *a* and in the *inset* in *a*) are recognized. The transcriptional units of the chromosomal loops are frequently interrupted by transcript-free regions of various lengths ("gaps", some are denoted by arrows in *a* and *b*), whereas pre-rRNA genes have retained their close packing density of nascent transcripts. The structure of individual transcriptional complexes and nascent ribonucleoprotein fibrils in such partly inactivated genes is shown in *c* (arrowheads). Segments of the loop chromatin axis that are free of transcripts exhibit a beaded, nucleosomal configuration (*c*). Bars, 2 (*a*), 1 (*inset* in *a*), 5 (*b*), and 0.2 μm (*c*). *a*, × 8,500; inset in *a*, × 18,000; *b*, × 4,600; *c*, × 75,000.


a
b
c

HMG-1 is also indicated in the study of Rechsteiner and Kuehl (58) who have recovered ~32% of ^{125}I-HMG-1 injected into HeLa cells in the cytoplasm. Artificial leaching out of the nucleus and redistribution of nuclear HMG as proposed by Bhullar et al. (59) and Wu et al. (60) for explaining the immunofluorescent data of Bustin and Neihart (19) is clearly excluded by our findings of relatively large amounts of HMG-A in manually enucleated ooplasms. Probably, the sizes of the specific nucleoplasmic and cytoplasmic pools of HMG proteins will have to be determined for each cell type (17). However, the amphibian oocyte clearly exemplifies the build up of large pools of a soluble HMG-1-like protein in both the nucleus and the cytoplasm.

What is the specific function of the masses of HMG-A accumulated in the nucleus and in the cytoplasm of the amphibian oocyte? This question is in a way similar to the question of the biological function of the large amounts of histones accumulated in oocytes of amphibia (for review see 31) and of sea urchin (64). The usual answer to the latter question is that these storage pools are needed to assemble the masses of DNA rapidly synthesized during early embryonic development until gastrulation into chromatin (31). This general hypothesis seems so plausible to us that we would like to apply it for the HMG-1-like proteins, which may also be needed for chromatin formation during amphibian embryogenesis. The existence of a large pool of HMG-A is especially interesting in this respect since HMG-1 has been reported to be capable of replacing histones of the H1 family (16), which is underrepresented in oocytes, eggs, and early stages of embryogenesis (31). Moreover, the reported capability of HMG-1 to bind to single-stranded DNA (13–15) points to a possible involvement of the oocyte HMG-A in the intensive replication characteristic of early amphibian embryogenesis.

Besides its possible function in early embryogenesis, the abundant HMG-A of the oocyte may also be involved in maintaining oocyte structures actually engaged in transcription. This is strongly indicated by our microinjection experiments but we recognize that presently there is no basis to further speculate about possible mechanisms of involvement of HMG-A in transcription.

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