The distribution of a 'mitosis-specific' antigen during *Drosophila* development

SARAH E. MILLAR, MATTHEW FREEMAN and DAVID M. GLOVER

Cancer Research Campaign, Eukaryotic Molecular Genetics Research Group, Department of Biochemistry, Imperial College, London SW7 2AZ, UK

Summary

We have used MPM-2, a monoclonal antibody raised against mitotic HeLa cells, to stain a *Drosophila* cell line, whole mounts of *Drosophila* embryos, and sectioned tissue from embryonic and larval stages of development. MPM-2 recognizes a major phosphoprotein of approximately 125×10^3 M_r in *Drosophila* tissue culture cells that, like the mammalian MPM-2 antigen, appears to be recognized only in mitotic cells. During early embryogenesis, when the embryonic nuclei divide as a syncytium with a very short nuclear division time, MPM-2 antigen is observed within the spindle compartment at all stages of the nuclear division cycle. Upon cellularization of the embryo and lengthening of the duration of the cycle, the antigen is predominantly seen in mitotic cells. *Drosophila* larvae contain both diploid and polytene tissues: in diploid tissue MPM-2 staining is specifically observed over mitotic cells, as expected from its distribution in cellularized embryos. Surprisingly, antigen is also detected in the nuclei of polytene cells that replicate their DNA but do not undergo mitosis.

Key words: mitosis, *Drosophila*, development.

Introduction

Mitosis is a process of fundamental importance in all organisms, yet little is known about its mechanism and regulation. One approach to the study of mitosis is to identify proteins whose presence or state of modification is specific to a particular stage of the cell cycle. Several lines of evidence suggest that certain proteins necessary for mitosis are present in mitotic but not interphase cells: fusion of mitotic cells with interphase cells can induce nuclear envelope breakdown and chromosome condensation in the interphase nucleus (Rao & Johnson, 1970; Matsui *et al.* 1972); chromosome condensation and germinal vesicle breakdown can be induced in *Xenopus* oocytes by injection of extracts from mitotic but not interphase mammalian cells (Sunkara *et al.* 1979); the maturation promoting factor (MPF) activity of *Xenopus* oocytes peaks during mitosis in fertilized eggs (Gerhart *et al.* 1984); MPF induces mitotic events when injected into *Xenopus* eggs arrested at the end of *S* phase by cycloheximide treatment (Miake-Lye *et al.* 1983; Halleck *et al.* 1984). The presence of mitosis-specific proteins has also been reported by Al-Bader *et al.* (1978).

Davis *et al.* (1983) raised monoclonal antibodies to synchronized mitotic HeLa cells and demonstrated that two antibodies, designated MPM-1 and MPM-2, recognize a series of phosphoproteins present only in mitotic cells. Immunofluorescence of HeLa cells using these antibodies reveals that the highest levels of antigen are found at metaphase, with slightly lower levels (60 % of metaphase levels) in anaphase and early telophase cells. Removal of phosphate groups from the antigens abolishes their recognition by MPM-1 and MPM-2. Thus it is possible that these proteins are present at all stages of the cell cycle but are phosphorylated only at mitosis. MPM-1 and MPM-2 recognize mitotic cells from a wide variety of organisms, including mouse, mosquito and slime mould (Davis *et al.* 1983).

In this paper we demonstrate that MPM-2 recognizes a *Drosophila* phosphoprotein whose distribution in tissue culture cells is similar to that of mammalian antigens. We also investigate the distribution of the antigen during development. The *Drosophila* embryo is a syncytium for the first 13 nuclear divisions, which

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have a cycle time of about 10 min (Foe & Alberts, 1983). Upon cellularization the cell cycle lengthens. Polyploid cells develop during embryogenesis and polytenization becomes extensive during larval development. We show that during both embryonic and larval development the MPM-2 antigen is not restricted to diploid cells, but is also found in polyploid nuclei that no longer undergo mitosis.

Materials and methods

Origin and culture of cell lines

Kc cells, from a *Drosophila* embryonic cell line (Barigozzi, 1971), were maintained in monolayer culture at 25°C in Schneider's medium (Gibco) supplemented with 10% (v/v) foetal calf serum (FCS), 500 units ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin sulphate. P19 cells, from a mouse C3H embryonal carcinoma cell line (Jones-Villeneuve *et al.* 1982), were maintained at 37°C in an incubator gassed with 5% carbon dioxide (v/v) in air of relative humidity greater than 95%. The cells were cultured in Dulbecco's Modification of Eagle's Medium (Flow Laboratories) supplemented with 10% FCS, 500 units ml^{-1} penicillin and 100 $\mu g\ ml^{-1}$ streptomycin sulphate.

Immunostaining of cells

Cultured cells were prepared for immunostaining by washing in phosphate-buffered saline (PBS) (130 mM-NaCl, 7 mM-Na_2HPO_4, 3 mM-NaH_2PO_4), fixing for 2 min in 1:1 (v/v) acetone/methanol, and washing in PBS. Cells were incubated in an appropriate dilution of MPM-2 ascites fluid in PBS/10% FCS for 12–16 h at 4°C. After washing in PBS, the cells were incubated for 12–16 h at 4°C in peroxidase-conjugated goat anti-mouse immunoglobulin G (IgG) (Jackson Immunoresearch Laboratories) diluted 1/1000 in PBS/FCS. After washing as before, the cells were incubated in a substrate solution containing 70 mM-*o*-dianisidine (Sigma), 0·003% (w/v) H_2O_2 in PBS. Cells were counterstained with the fluorescent DNA-specific dye Hoechst H33258 (Reidel De Haen AG, Hanover, FDR) at 1 $\mu g\ ml^{-1}$ in PBS. Stained cells were viewed with a Zeiss standard microscope fitted with epifluorescent optics with a filter suitable for Hoechst, and were photographed using Kodak Ektachrome ASA 400 colour slide film.

Preparation of extracts from Kc cells

Kc cells were extracted with 8 M-urea and diluted at least sixfold in Tris buffer (10 mM-Tris·HCl, pH 8·0, 1 mM-phenylmethylsulphonyl fluoride, 0·1 mM-L-1-tosylamide-2-phenylethylchloromethyl ketone, 0·1 trypsin-inhibitor-units ml^{-1} aprotinin) to give a protein concentration of approximately 3 mg ml^{-1}. Extracts were then either boiled for 5 min after the addition of an equal volume of 2× sodium dodecyl sulphate (SDS) sample buffer (4% SDS, 20% glycerol, 0·002% Bromophenol Blue, 0·2 M-dithiothreitol, 0·125 M-Tris·HCl, pH 6·8) before loading on an SDS–polyacrylamide gel, or were treated as described below.

Phosphatase treatment of Kc cell extracts

Extracts prepared as described above were incubated in Tris buffer for 2 h at 37°C after adding 3·5 units ml^{-1} *Escherichia coli* alkaline phosphatase (Sigma). The reaction was stopped by adding 2× SDS sample buffer and boiling for 5 min. In control experiments the incubation with alkaline phosphatase was carried out in the presence of 50 mM-NaH_2PO_4. To test for the activity of endogenous phosphatases in the Kc cell extracts, incubations were carried out without adding *E. coli* alkaline phosphatase, both in the presence and in the absence of 50 mM-NaH_2PO_4.

Preparation of extracts from whole salivary glands and salivary gland nuclei

Salivary glands from crawling third instar larvae were dissected in buffer A (15 mM-Tris·HCl, pH 7·4, 60 mM-KCl, 15 mM-NaCl, 0·5 mM-EDTA, 0·1 mM-EGTA, 1·5 mM-spermine, 0·5 mM-spermidine) containing 15 mM-β-mercaptoethanol (Sigma). Extracts of whole glands were prepared by transferring glands to SDS sample buffer containing 8 M-urea, vortexing at maximum speed for 5 min and boiling for 5 min. Salivary gland nuclei were prepared by suspending dissected glands in buffer A containing 15 mM-β-mercaptoethanol, 0·1% digitonin (Sigma) and vortexing at 30-s intervals for up to 4 min. Phase-contrast microscopy was used to check that the cells had been broken open. The nuclei were briefly centrifuged, washed in buffer A, suspended in SDS sample buffer containing 8 M-urea, and boiled for 5 min before loading.

SDS–polyacrylamide gel electrophoresis and immunoblotting procedures

SDS–polyacrylamide (8·5%) gels were run as described by Laemmli (1970). High molecular weight protein standards (40–250 ($\times 10^3$) M_r) from Bio-Rad were used as markers. The polypeptides were transferred electrophoretically to nitrocellulose filters as described by Burnette (1980). Filters were stained with Ponceau S (Serva) to reveal the positions of the molecular weight standards and to check efficiency of transfer. The filters were then transferred to 3% (w/v) bovine serum albumin (BSA) (Sigma) in PBS/0·02% NaN_3 for 4 h to block non-specific protein binding sites on the nitrocellulose. The filters were incubated for 4 h in a mixture of MPM-2 ascites fluid, and tissue culture supernatant containing Bx69, a monoclonal antibody against *Drosophila* tubulin (Frasch, 1985). These antibodies were diluted 1/500 and 1/5, respectively, in PBS/10% FCS. Filters were washed four times over 1 h in PBS containing 0·1% (v/v) Nonidet P40 (BDH) and then incubated for 2 h in peroxidase-conjugated goat anti-mouse IgG (Jackson) diluted 1/500 in PBS/FCS. After washing as before, filters were developed in substrate solution containing 2·8 mM-4-chloro-1-napthol (Aldrich Chemical Company) in PBS with 0·003% H_2O_2 added.

Immunofluorescence of embryo whole mounts

Embryos were prepared for antibody staining as described by Freeman *et al.* (1986). Fixed and devitellinized embryos were incubated in buffer A containing 10% FCS for 1 h at room temperature, blotted dry and then incubated for

12–16 h at 4°C in MPM-2 ascites fluid diluted 1/500 in buffer A/FCS. After washing four times for 1 h in buffer A, embryos were incubated for 12–16 h at 4°C in rhodamine-conjugated goat anti-mouse IgG (Jackson) diluted 1/500 in buffer A/FCS. The embryos were washed as before, then stained for 10 min with Hoechst H33258 at 1 $\mu g\,ml^{-1}$ in buffer A. After rinsing in buffer A the embryos were mounted in 85 % glycerol containing 2·5 % *n*-propyl gallate (Giloh & Sedat, 1982). Slides were viewed using a Zeiss standard microscope with epifluorescent optics with appropriate filters for Hoechst and rhodamine. Embryos were photographed on Kodak 2415 Technical film and developed with Kodak D-19 developer. The level of background was determined by incubating embryos in the second antibody alone. Although a low level of background fluorescence was detected, no staining of specific structures was observed.

Immunofluorescence of third instar larval brain squashes

Brains from crawling third instar larvae were dissected in buffer A. Brains were transferred to a drop of buffer A containing 3·7 % formaldehyde, 0·1 % (v/v) Triton X-100 on a siliconized coverslip, and left for 1 min. The coverslip was picked up on a glass slide treated with Denhardt's solution (450 mM-NaCl, 45 mM-trisodium citrate, pH 7·0, 0·02 % (w/v) PVP 360 (polyvinylpyrrolidone), 0·02 % (w/v) Ficoll, 0·02 % (w/v) nuclease-free BSA (Sigma)) and the brains were squashed by pressing hard on the coverslip. The slide was frozen in liquid nitrogen, and the coverslip flicked off with a razor blade. The tissue was postfixed for 1 min in 3·7 % formaldehyde, 0·1 % Triton in buffer A, and then transferred to methanol for 10 min. The material was rehydrated for 2×10 min in buffer A and then incubated in buffer A/10 % FCS for 1 h. Incubations with antibodies, mounting and photography were as described for embryo whole mounts.

Immunostaining of sectioned Drosophila *tissue*

Drosophila embryos or larvae were mounted in Tissue-Tek II O.C.T. compound (Raymond A. Lamb, London, UK) on a cork block. The tissue was frozen in liquid nitrogen and 15-μm or 20-μm sections were cut at −20°C using a Reichert-Jung Cryocut E cryostat. Sections were collected on slides subbed in 0·5 % (w/v) gelatin/0·005 % (w/v) chromic potassium sulphate, and were fixed immediately for 20 min in 3·7 % formaldehyde in PBS. The slides were washed for 40 min in four changes of PBS and then incubated for 1 h in PBS containing 10 % FCS. Incubation with MPM-2 ascites fluid and peroxidase-conjugated indicator antibody, counterstaining with Hoechst, and photography were as described for tissue-culture cells. The level of background staining was determined by incubation of sections in indicator antibody alone. A low level of background stain was observed; the only specific structure to be stained was the larval cuticle.

Results

Distribution of MPM-2 antigen in mouse P19 cells and in Drosophila *Kc cells*

MPM-2 antibody, which was raised against mitotic HeLa cells (Davis *et al.* 1983), recognizes an epitope present in mitotically dividing cells of many species. Fig. 1A,B shows cultured P19 cells, from a mouse C3H embryonal carcinoma cell line, that have been immunostained with MPM-2 antibody and counterstained with the fluorescent DNA-specific dye Hoechst H33258. The three cells showing dark brown immunoperoxidase staining contain condensed mitotic chromosomes as revealed by Hoechst fluorescence. The distribution of MPM-2 antigen in *Drosophila* Kc cells parallels its distribution in mouse P19 cells: the most strongly immunoreactive cells are those in mitosis (Fig. 1B,D). In both the *Drosophila* and mouse cells lines staining is fairly uniform throughout the mitotic cells; cells at other stages of the cell cycle generally exhibit little or no staining, although weak nuclear staining is seen in some interphase cells.

Drosophila *MPM-2 antigen is phosphorylated*

If the *Drosophila* MPM-2 antigen is functionally analogous to the mammalian antigens, as suggested by its similar distribution in tissue-culture cells, one would predict that, like mammalian antigens, it is a phosphoprotein whose recognition by MPM-2 is abolished on removal of phosphate groups. Immunoblots of Kc cell extracts show that a major antigen of approximately $125\times10^3 M_r$ is recognized in Kc cells (Fig. 2, track a). A larger antigen of approximately $250\times10^3 M_r$ is also detected in some extracts, as well as minor bands of approximately 150, 93 and 75 ($\times10^3$) M_r. In order to test whether the *Drosophila* antigen is phosphorylated, we incubated Kc cell extracts for 2 h at 37°C with or without adding bacterial alkaline phosphatase. In both cases this treatment abolishes recognition of the *Drosophila* protein by MPM-2 (see Fig. 2, tracks b, c). This does not appear to be a consequence of proteolytic degradation since incubation of the blot with anti-tubulin antibody reveals a sharp band of tubulin (Fig. 2). The appearance of the blot on staining with Ponceau S immediately after electroblotting to visualize all the transferred protein bands also indicates that little or no proteolytic degradation has occurred. Destruction of the epitope is prevented by inclusion in the incubations of 50 mM-NaH_2PO_4, a concentration of phosphate ions known to inhibit phosphatase action (Reid & Wilson, 1971) (see Fig. 2, tracks d, e). This suggests that MPM-2 recognizes a phosphorylated epitope that is destroyed by endogenous phosphatase,

or by a combination of endogenous and added phosphatase, on incubation in the absence of phosphate ions.

The pattern of distribution of MPM-2 antigen changes during embryogenesis upon cellularization

The *Drosophila* embryo is a syncytium for the first 13 nuclear divisions. The nuclei initially divide in the interior of the embryo but after the ninth cleavage most of them migrate to the periphery. At this stage it is possible to observe parasynchronous waves of mitosis that originate at the embryo poles. This allows one to study nuclei at different stages of mitosis within a single embryo.

We have carried out indirect immunofluorescence experiments on whole mounts of *Drosophila* blastoderm embryos to examine the distribution of MPM-2 antigen at different stages of the nuclear division cycle. The embryos were counterstained with Hoechst H33258 to allow accurate assessment of both the developmental stage of each embryo, and the nuclear or cell cycle stage of its chromosomes. In contrast to our findings with tissue-culture cells, we observe that the MPM-2 antigen is present at all stages of the mitotic cycle in all nuclei prior to cellularization. Fig. 3 shows regions of representative blastoderm embryos that are in interphase (A,B), metaphase (C,D) and anaphase (E,F). It is clear that the antigen

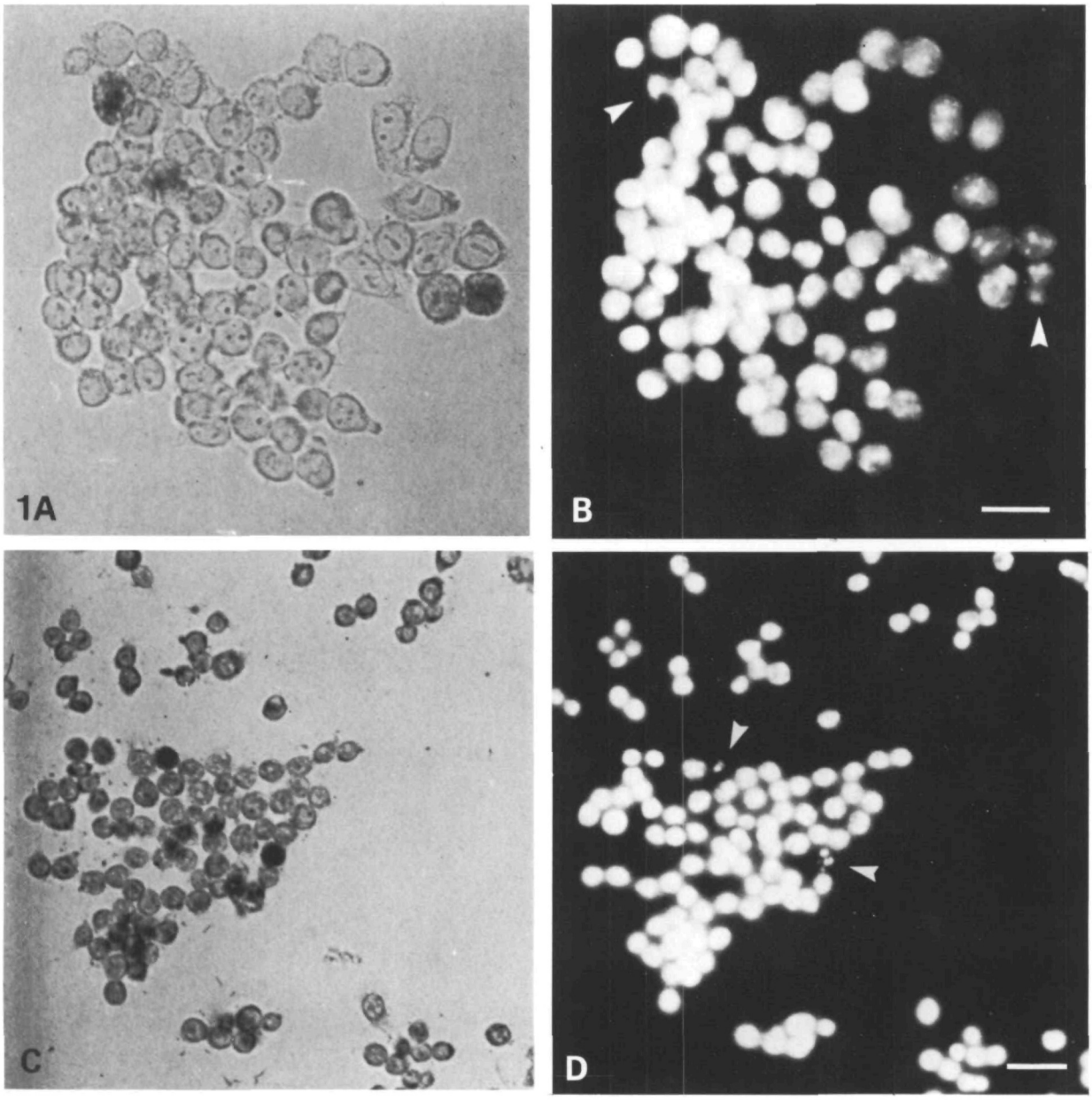


Fig. 1. Mouse P19 embryonal carcinoma cells (A,B) and *Drosophila* Kc cells (C,D) stained with MPM-2 (A,C) and counterstained with Hoechst (B,D). MPM-2 staining is seen over the chromosomes and cytoplasm of mitotic cells, indicated by arrowheads. Bars, 20 μm.

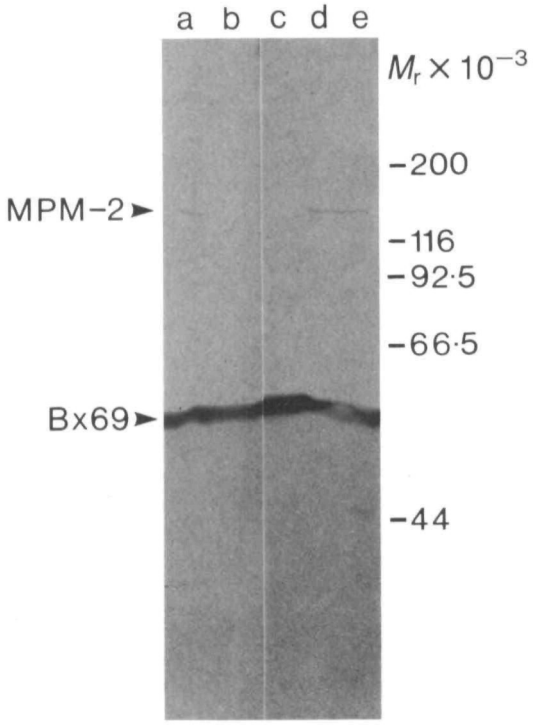


Fig. 2. Immunoblot of *Drosophila* Kc cell extracts stained with MPM-2 and Bx69 (anti-tubulin). Kc cells were extracted with 8 M-urea and diluted with Tris buffer, as described in Materials and Methods, before being treated as follows: track a, no treatment; track b, incubated for 2 h at 37°C; track c, incubated for 2 h at 37°C after adding 3·5 units ml^{-1} *E. coli* alkaline phosphatase; track d, incubated for 2 h at 37°C in the presence of 50 mM-NaH_2PO_4 and 3·5 units ml^{-1} alkaline phosphatase; track e, incubated for 2 h at 37°C in the presence of 50 mM-NaH_2PO_4. After treatment, an equal volume of 2×SDS sample buffer was added and the extracts were boiled for 5 min. Approximately 200 μg total protein was loaded per track.

is present in interphase nuclei at levels comparable with those observed during mitosis. At metaphase the antigen is present over an area roughly corresponding to the position of the former interphase nucleus with some punctate staining over the metaphase chromosomes. At anaphase the antigen lies over a diamond-shaped area within which is found the spindle. In some cases there is also weak staining over the region of the spindle poles.

The average duration of the nuclear division cycle lengthens from approximately 10 min for the first 12 cycles, to 21 min for cycle 13 and at least 65 min for cycle 14 (Foe & Alberts, 1983). During the fourteenth nuclear cycle the surface nuclei become cellularized, and gastrulation begins. Not all nuclear divisions occur synchronously after cellularization of the embryo. Fig. 4 shows part of the ventral furrow in a whole mount preparation of a gastrulating embryo stained with MPM-2 antibody. This furrow contains a number of mitotically dividing cells, whereas the cells flanking the furrow are in interphase. It can be seen that the antigen is now present predominantly in mitotic cells. Furthermore, it is no longer restricted to the spindle compartment, but is distributed throughout the cell. The flanking cells in interphase show a very much lower level of staining that is restricted to the nuclei. This distribution of the antigen more closely resembles that observed in tissue culture cells.

MPM-2 antigen is found in the polyploid yolk nuclei of embryos at syncytial blastoderm

Whilst most of the nuclei migrate to the cortex of the embryo after the ninth cleavage, a number remain in the centre of the embryo. These are known as the yolk nuclei. After the tenth cycle, they cease dividing and begin to become polyploid. Examination of frozen sections of embryos at nuclear cycles 12–14 reveals that some of the yolk nuclei contain MPM-2 antigen. Fig. 5A,B shows a transverse section of a stage 13 embryo in which MPM-2 antigen is detected by immunoperoxidase staining (A), and nuclei are visualized using Hoechst (B). This shows that the antigen is not restricted to mitotically dividing diploid cells in the embryo, but is also found within non-dividing polyploid cells.

During larval development MPM-2 stains the chromosomes and cytoplasm of mitotic diploid cells and the nuclei of polytene cells

During the period between gastrulation and hatching of the first instar larva, many of the embryonic cells cease dividing and become polyploid. These cells are the precursors of larval structures including fat body, intestine and salivary glands. Much of subsequent larval development involves polytenization and cell growth. Other embryonic cells remain diploid; these are precursors for larval brain and imaginal discs, whose cells continue to divide throughout larval life (Bodenstein, 1950). The distribution of MPM-2 antigen in larval tissue was determined by immunoperoxidase staining of frozen sections of larvae from all three instars. Additionally, the staining pattern of MPM-2 in brain tissue was examined using indirect immunofluorescence on squashes of brains dissected from third instar larvae.

Fig. 5C,D shows part of a section from the anterior region of a first instar larva, in which tissues have reached only low levels of polyploidy. The strongest staining observed is over clumps of cells in the proliferating diploid tissues of the larva. Weak staining of the nuclei, but not the cytoplasm, of some polyploid cells is also detected.

Fig. 6 shows an example of a third-instar larval brain squash stained with MPM-2 (A) and counterstained with Hoechst (B). The Hoechst stain reveals

that most of the cells are in interphase and contain little or no MPM-2 antigen. There is one example of a metaphase cell in the field; this stains strongly with MPM-2, the antigen being present over both chromosomes and cytoplasm. Thus the distribution of MPM-2 antigen in diploid larval tissue is similar to that observed in the cellularized embryo.

Fig. 5E,F shows the distribution of MPM-2 antigen in a section of a third instar larva, in which some tissues, particularly the salivary glands, have reached high levels of polytenization. As expected, the antibody gives strong staining of diploid tissue, but it is also observed that some polytene nuclei, particularly those of the salivary gland, stain strongly with MPM-2. Some variation in the intensity of staining is observed between different nuclei of the same salivary gland. Examination of serial sections of many glands reveals that MPM-2 antigen is present in all the gland nuclei, but appears to be concentrated towards the centre of the nucleus; the strongest staining with MPM-2 is observed when the knife has bisected a nucleus.

The presence of MPM-2 antigen in salivary gland nuclei was confirmed by immunoblotting extracts

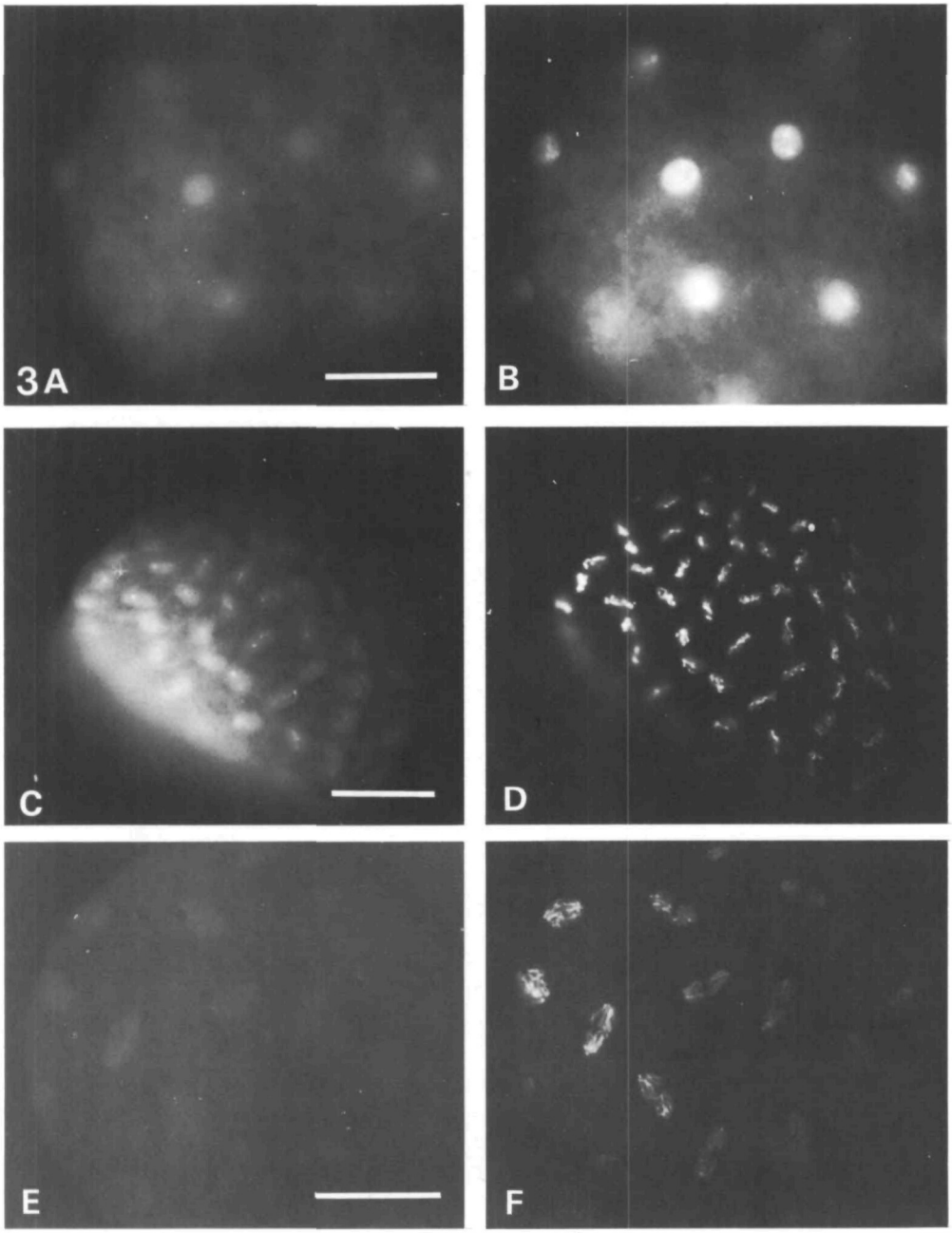
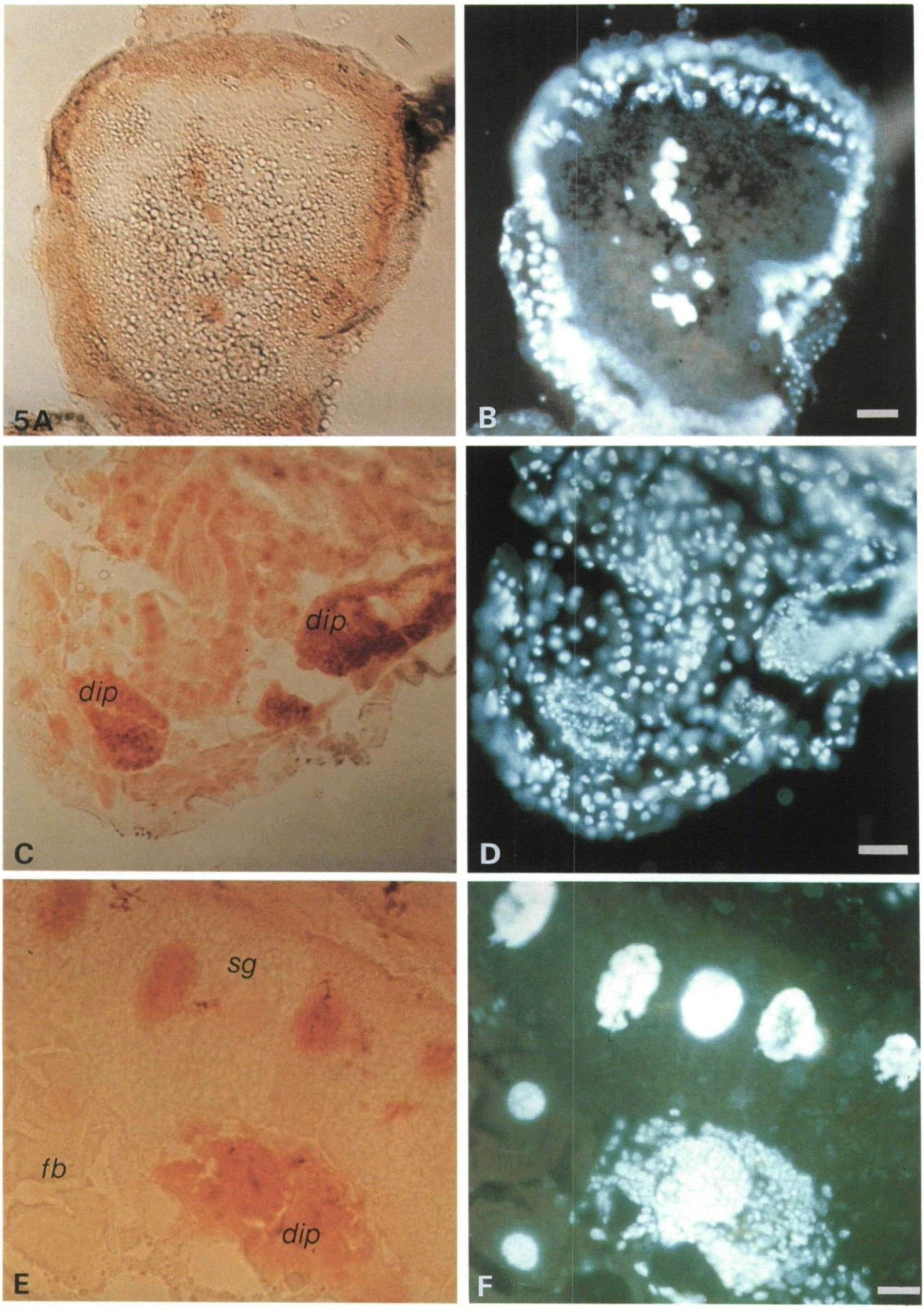


Fig. 3. Whole mounts of *Drosophila* embryos at syncytial blastoderm showing nuclei at interphase (A,B), metaphase (C,D), and anaphase (E,F), stained with MPM-2 (A,C,E) and counterstained with Hoechst (B,D,F). Bars, 20 μm.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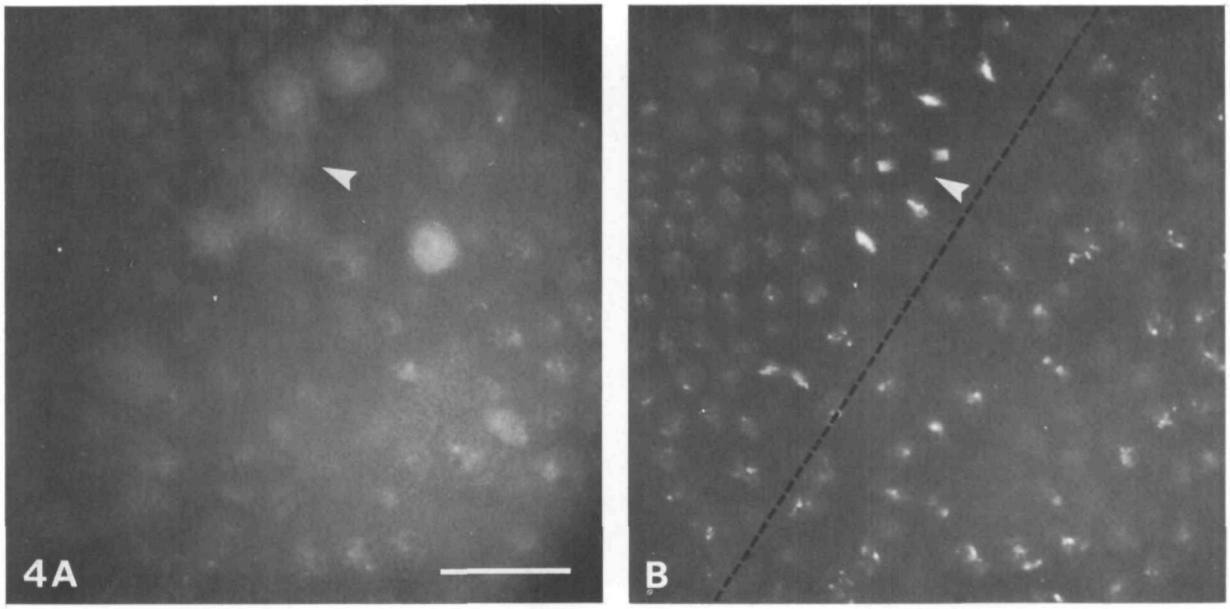

Fig. 4. Whole mount of a *Drosophila* embryo at nuclear division cycle 15, stained with MPM-2 (A) and counterstained with Hoechst (B). The embryo has undergone cellularization and is beginning to gastrulate. The position of the ventral furrow is marked by a broken black line. An anaphase figure is indicated by an arrowhead. Bar, 20 μm.

of whole salivary glands and isolated gland nuclei. Two major polypeptides, of approximately 250 and 125 $(\times 10^3) M_r$, and three minor antigens of approximately 150, 93 and 75 $(\times 10^3) M_r$ are detected by MPM-2 in the nuclear extract (Fig. 7, track b). These antigens are of the same molecular weight as those found in Kc cells; however, their relative abundance is different: the $250 \times 10^3 M_r$ antigen predominates in salivary gland nuclei, whereas the $125 \times 10^3 M_r$ antigen is by far the most abundant of those detected in Kc cells. No antigen is detectable in an equivalent loading of total gland protein (Fig. 7, track a), indicating that the antigen is confined to the nucleus and that it constitutes only a small fraction of total salivary gland protein.

Fig. 5. A,B. A 15-μm transverse section of an embryo at nuclear division cycle 13 stained with MPM-2 (A) and counterstained with Hoechst (B). The yolk nuclei (in the embryo interior) are polyploid at this developmental stage. Bar, 20 μm. C,D. A 15-μm longitudinal section of the anterior region of a first instar *Drosophila* larva stained with MPM-2 (C) and counterstained with Hoechst (D). Most of the tissue in the section is polyploid or polytene, and shows weak nuclear staining; regions of diploid tissue (*dip*) contain clumps of strongly staining cells. Bar, 20 μm. E,F. A 20-μm longitudinal section of a third instar larva stained with MPM-2 (E) and counterstained with Hoechst (F). The section includes salivary gland (*sg*), fat body (*fb*) and diploid tissue (*dip*). Bar, 10 μm.

Discussion

We have described the distribution of MPM-2 antigen in *Drosophila* Kc tissue culture cells, in whole mounts of *Drosophila* embryos, and in frozen sections of tissue from embryonic and larval stages. In Kc cells MPM-2 recognizes a phosphoprotein that is predominantly found in cells undergoing mitosis. These characteristics of the antigen are similar to those of the mammalian antigen, suggesting that the *Drosophila* and mammalian antigens may be fuctionally analogous. As in the case of the mammalian protein, it is possible that the *Drosophila* protein recognized by MPM-2 is present throughout the cell cycle, but becomes phosphorylated only at mitosis.

In contrast, the MPM-2 antigen is present at comparable levels throughout the nuclear division cycle in the pre-cellularized embryo. By the time cellularization occurs, at division cycle 14, and the cycle time has lengthened to about 1 h, the distribution of antigen is much more like that in cultured cells, mitotic cells showing the strongest staining. The different pattern seen in the earliest embryos possibly reflects the extremely short cycle time of about 10 min. The nuclei could be primed for mitosis throughout this rapid cycle, which may be insufficiently long to allow dephosphorylation of the antigen.

The pattern of MPM-2 staining of embryo whole mounts shows several interesting characteristics. At metaphase punctate staining is seen over the chromosomes; at anaphase weak staining over the region of

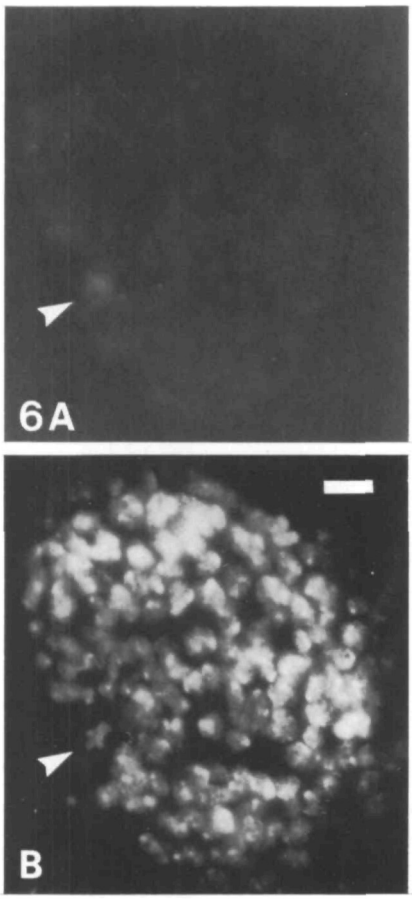

Fig. 6. Third instar larval brain squash stained with MPM-2 (A) and counterstained with Hoechst (B). The Hoechst counterstain shows that most of the cells in the field are in interphase. A single cell in metaphase (arrowhead) stains strongly with MPM-2. Bar, 10 μm.

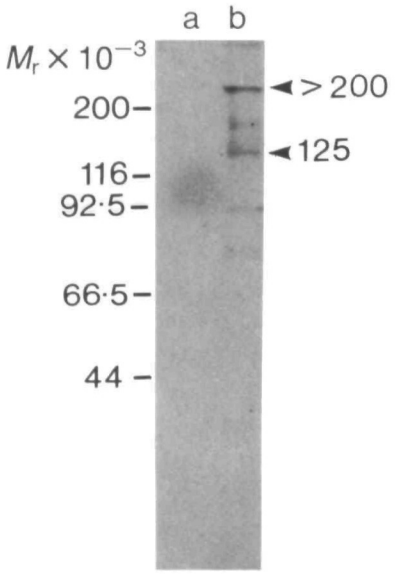

Fig. 7. Immunoblot of extracts of whole salivary glands (track a) and isolated salivary gland nuclei (track b) stained with MPM-2. Approximately 100 μg of total protein was loaded per track.

the spindle poles is occasionally observed in addition to the stronger staining seen over the general area of the spindle. These patterns are similar to those obtained when MPM-2 is used to stain mammalian cells treated with Triton X-100 prior to fixation (Vandre *et al.* 1984). The results of Vandre *et al.* indicate that the stained structures correspond to kinetochores and centrosomes. We do not know whether this is the case in *Drosophila* since we do not have a good antibody against the kinetochore. Furthermore, the distribution of antigen over the region of the centrosome in *Drosophila* embryos may simply reflect the partitioning of nuclear contents. Several *Drosophila* nuclear antigens show a tripartite staining pattern in anaphase corresponding to the position of the parental nucleus and its flanking regions around the spindle poles (Frasch *et al.* 1986).

MPM-2 antigen is found to be present in some of the polyploid yolk nuclei during syncytial blastoderm. Polyploid nuclei in some species are considered to undergo a nuclear cycle similar to the mitotic cycle, but lacking the events of chromosome separation and nuclear envelope breakdown (Pearson, 1974). The presence of antigen in yolk nuclei may reflect the existence of a similar cycle in these nuclei. Alternatively, the staining may simply be due to the presence of residual antigen left from the last mitotic cycle.

The presence of antigen in larval polytene nuclei, especially in the highly polytenized salivary gland nuclei where MPM-2 staining is particularly strong, is harder to explain. The presence of an antigen in both diploid and polytene tissues might be taken to suggest that it is required for a function common to both types of tissue; for instance, one might expect antigens necessary for DNA replication or for the maintenance of chromatin structure to be present in polytene as well as diploid nuclei, whereas antigens involved in nuclear envelope breakdown and cell division might be expected to be specific to diploid cells. Since we observe some variation in the intensity of MPM-2 staining of different nuclei within the same salivary gland, we thought it possible that the presence of antigen might correlate with a particular stage of the DNA replication cycle in these nuclei. We investigated this possibility by incubating glands in the thymidine analogue bromouridine, and then performing double-labelling experiments with MPM-2 and a monoclonal antibody against bromouridine. No correlation between MPM-2 staining and the incorporation of bromouridine was observed (data not shown). Examination of serial sections of stained glands revealed that antigen is present in all the nuclei of each gland, but that the strongest staining with MPM-2 is observed when the nucleus is bisected by the sectioning knife. This is not due to the presence of nuclear membrane providing a barrier to the antibody, since the same

phenomenon is observed when Triton X-100 is included in fixation and antibody incubation steps to permeabilize the membrane. Rather, these results seem to suggest that antigen is concentrated towards the centre of the giant nuclei, and may indicate that at least some fraction of the antigen is associated with chromatin. One possible role for MPM-2 antigen in mitosis is in chromatin condensation, since several lines of evidence suggest that this is correlated with the phosphorylation of non-histone proteins (e.g. see Adlakha *et al.* 1982; Wu & Gerhart, 1980). The normal events of mitosis fail to occur in salivary gland nuclei, which are transcriptionally active and so may be considered to be in 'interphase'. However, the presence of MPM-2 antigen in these nuclei may suggest that the state of condensation of the highly ordered salivary gland chromosomes is actually more similar to that of the chromosomes of mitotic than of interphase diploid cells. Alternatively, the presence of MPM-2 antigen in salivary gland nuclei may simply be an extension of the weak staining seen in interphase diploid nuclei, the intensity of staining reflecting the larger size of the polytene nuclei. It may prove possible to resolve this question by determining whether the weak staining observed in interphase diploid nuclei correlates with the presence of the $250\times10^3 M_r$ antigen that predominates in salivary gland nuclei.

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