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Original Article

Immunolocalization of HSP 70-related proteins constitutively expressed during *Xenopus laevis* oogenesis and development

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ABSTRACT Using immunocytochemical and biochemical methods, we analyzed the localization of HSP 70-related proteins constitutively expressed during oogenesis and embryogenesis in the amphibian *Xenopus laevis*. Our results provided evidence for a regional localization in oocytes. In embryos, the regional distribution observed in oocytes was found to be maintained from fertilization up to late blastula. It is noteworthy that, at the beginning of gastrulation, nuclear transfer of such proteins had already occurred by the time of internalization in the involuting marginal zone (IMZ), whereas cells of the vegetal area exhibited only a perinuclear localization of these proteins. These results suggest that HSP 70-related proteins might be involved in the control of the process of cellular internalization.

KEY WORDS: *Xenopus laevis, HSP 70-related proteins, oocytes, early embryogenesis, nuclear proteic transfer*

Introduction

After being subjected to heat shock, i.e. a transient increase in temperature, most prokaryotic and eukaryotic cells develop a «heat shock response» characterized by a decrease in synthesis of most cellular proteins and rapid production of a specific set of heat shock proteins (HSPs). Their high degree of conservation throughout evolution suggests that these HSPs are of vital importance in cell functions.

Heat shock proteins belong to a multigenic family; among them, HSP 70 are the most widely studied (for review, see Lindquist, 1986; Lindquist and Craig, 1988). In many types of organisms, their occurrence under stress conditions has been examined, but heat shock proteins are also involved in several processes essential for cellular function under normal conditions.

In somatic cells, constitutive forms of HSP 70 (HSC 70) are involved in a number of important biological pathways (reviewed in Ellis *et al.*, 1991), such as protein maturation and assembly (Beckmann *et al.*, 1990) and protein translocation across biological membranes of cellular organelles (Deshaies *et al.*, 1988; Murakami *et al.*, 1988; Kang *et al.*, 1990; Morishima *et al.*, 1990; Scherer *et al.*, 1990) and from the cytoplasm to the nucleus (Shi *et al.*, 1992).

With regard to amphibian development, such HSP 70-related proteins have been identified in *Xenopus laevis* oocytes. These proteins are transported from the cytoplasm to the nuclear envelope and are able to recycle bidirectionally across the nuclear membrane (Mandell and Feldherr, 1990). However, there is no information concerning the precise localization of these proteins during oogenesis.

Moreover, although evidence for constitutive expression of HSP 70 transcripts during the course of *Xenopus laevis* embryogenesis has been reported (Heikkila *et al.*, 1987; Horrel *et al.*, 1987), nothing is known concerning the localization of the corresponding proteins in the embryo.

The present paper focuses on the localization of HSP 70-related proteins constitutively expressed in oocytes and embryos in the amphibian *Xenopus laevis*. Using immunocytochemical and biochemical methods, we analyzed the distribution of HSP 70-related proteins during oogenesis. The HSP 70 regional distribution observed in stage 6 oocytes was maintained from fertilization through cleavage stages and up to gastrulation. At that time, nuclear transfer progressively occurred in the cells of the marginal zone.

Results

In order to analyze the localization of HSP 70-related proteins in oocytes and embryos, we checked the specificity of monoclonal antibody H3F18 on Western blot from two-dimensional electrophoresis of stage 6 oocyte proteins. H3F18 revealed two

Abbreviations used in this paper: A.H., animal hemisphere; BSA, bovine serum albumin; Cy, cytoplasm; EDTA, ethylene diaminetetraacetic acid disodium salt; FITC, fluorescein isothiocyanate-conjugated; FGFs, fibroblast growth factors; GFAP, glial fibrillary acidic protein; HSC 70, constitutive form of heat shock protein; HSPs, heat shock proteins; IMZ, involuting marginal zone; MBS, modified Barth solution; MMR, modified Ringer medium; N, nucleus; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; V.H., vegetal hemisphere.

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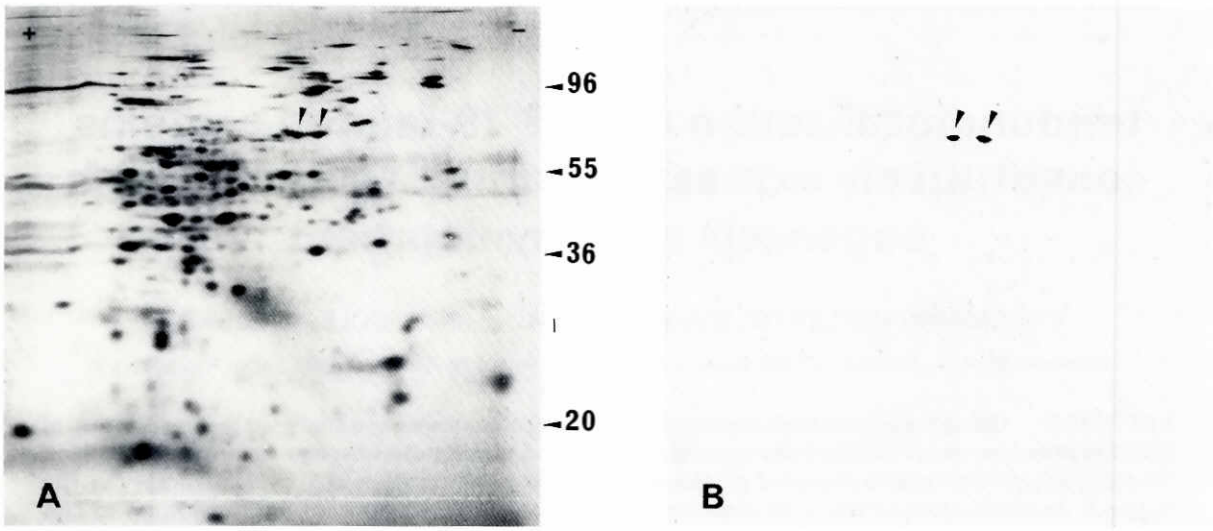

Fig. 1. Specificity of monoclonal antibody H_3F_{18}. (A) *Two-dimensional electrophoresis of a 10,000 g supernatant from stage 5-6 oocytes of Xenopus laevis. Arrows indicate position of HSP 70-related proteins. Silver staining according to Morrissey (1981). On the left, molecular mass markers (Daltons).* **(B)** *Corresponding immunoblot after incubation with anti-Plasmodium falciparum HSP 70 monoclonal antibody (mAb H3F18).*

spots of Mr 70,000 pI 5.58 and 5.75, respectively (Fig. 1). Distribution of HSP 70-related proteins, both in oocytes and embryos, was then investigated by immunofluorescence on sections using H3F18. In order to demonstrate that the immunofluorescence we observed was not due to non-specific binding of the antibody, two kinds of controls were performed. First, we treated oocyte and embryo sections with a different (presumably non-reactive) mouse monoclonal antibody: the anti-mouse glial fibrillary acidic protein (GFAP, Boehringer Mannheim). No fluorescence was ever detected when those sections were revealed by the labeled secondary goat anti-mouse IgG, except an autofluorescence from erythrocyte hemoglobin (Abbadie *et al.*, 1987). In addition, we observed no effects when the secondary antibody was used alone (not shown).

Distribution of HSP 70-related proteins in Xenopus laevis oocytes

HSP 70-related proteins were observed throughout the course of oocyte development, but their intracellular localization depended on the oocyte stage considered.

In previtellogenic oocytes (Stages 1 and 2; Fig. 2A,B), the occurrence of HSP 70-related proteins was noted in the cytoplasm and, to a lesser extent, in the germinal vesicle. The mitochondrial mass was strongly labeled, suggesting substantial accumulation of these proteins in such organelles (Fig. 2B).

During the course of vitellogenesis (Stages 3 to 5), the localization of HSP 70-related proteins scarcely differed from that previously described; they were mainly cytoplasmic. At stage 3, preferential fluorescence was observed at the periphery of the oocyte between the yolk bodies, whereas the yolk-free cytoplasm showed less labeling. In contrast, the mitochondrial mass near the nucleus exhibited very intense fluorescence (Fig. 2D). By the end of vitellogenesis (Stages 5-6), the distribution of HSP 70-related proteins appeared polarized in the cytoplasm, with a very slight increase in their concentration in the nucleus (Fig. 2F,H). Indeed, from stage 5 on, there occurred a decreasing gradient in the distribution of HSP 70-related proteins from the animal to the vegetal region of the oocyte. Similar distribution of these proteins was observed in unfertilized eggs from stimulated but unfertilized females. Indeed, decreasing fluorescence from the animal to the vegetal region was also detected with yolk-free patches clearly labeled in the animal part of the egg (Fig. 4A).

In order to confirm these immunofluorescence results, HSP 70 content from whole stage 6 oocyte, cytoplasmic and nuclear compartments was analyzed by immunoblotting of 2D electrophoresis separated polypeptides, with each sample corresponding to one oocyte. Immunoblots were developed with ^{125}I-labeled anti-mouse antibody to increase the sensitivity of the assay and enable direct quantification (Fig. 3). Results are summarized in Table 1.

These results from quantification studies provided evidence for a nuclear/cytoplasmic HSP 70 labeling ratio of about 15%. Since in full grown oocytes the ratio between nucleus and yolk-free cytoplasm volumes is known to be about 10% (Hausen and Riebesell, 1991), such results clearly indicated that the HSP 70 concentration was higher in the nucleus than in the cytoplasm. These results, compared to those from immunocytochemical localizations, suggested that HSP 70 epitopes may be masked in nuclei.

The same biochemical approach was used to determine whether the decreasing HSP 70 gradient really existed from the animal to the vegetal regions. Results from HSP 70 labeling quantification studies (Table 1) showed that HSP 70 was present in almost equal amounts in both the vegetal and the enucleated animal hemispheres. Such results were not evident in our immunofluorescence observations.

Distribution of HSP 70-related proteins during early embryogenesis

After fertilization, *Xenopus laevis* eggs underwent a series of rapid and synchronous divisions. Throughout these cleavage stages, the regional distribution of HSP 70-related proteins previously described for stage 5-6 oocytes was observed.

Thus, in an animal-vegetal section of a two-cell embryo, HSP 70-related proteins exhibited, in the animal part of the blastomeres, a perinuclear localization and a concentric cytoplasmic distribution

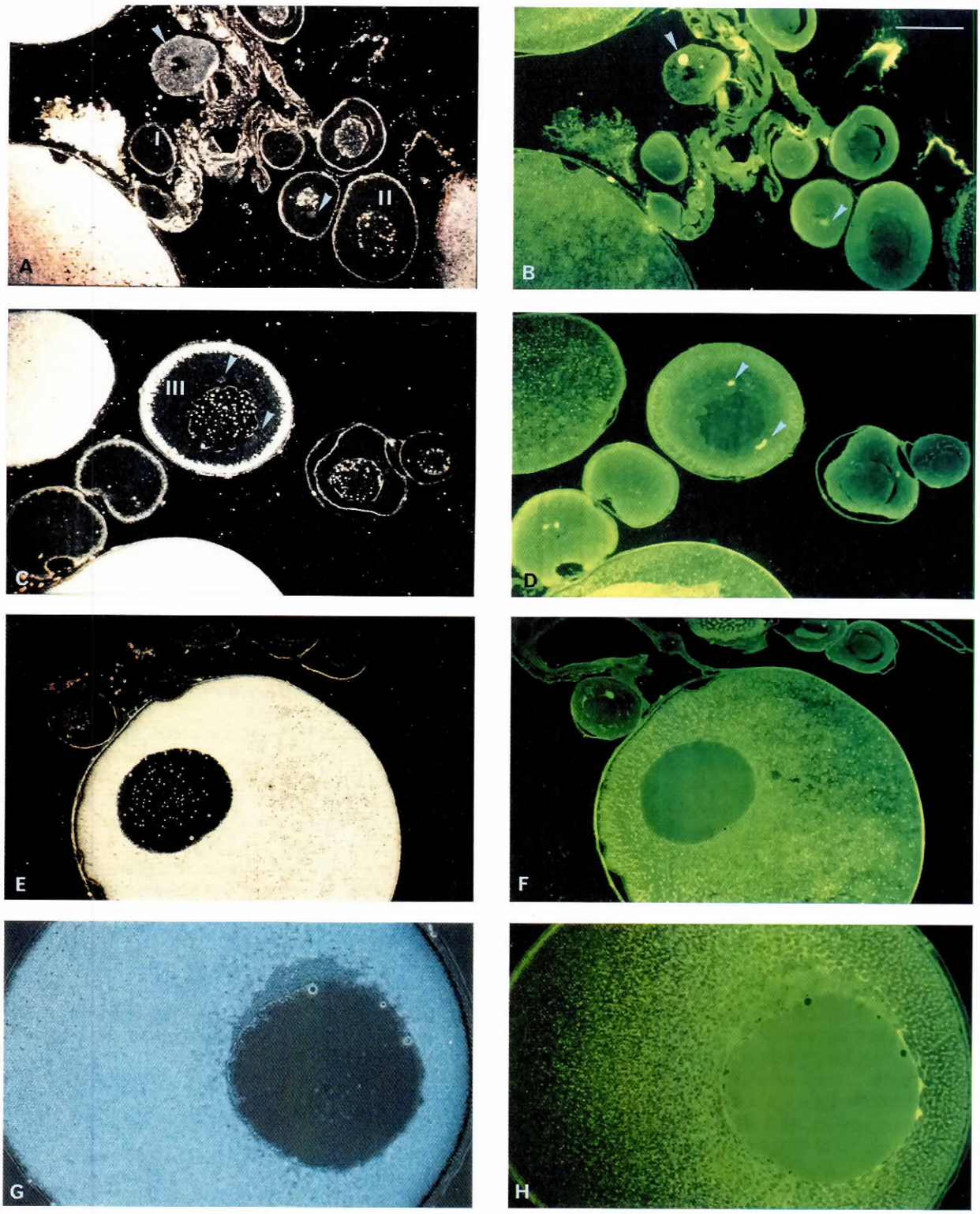

Fig. 2. Immunolocalization of HSP 70-related proteins on sections through *Xenopus laevis* ovary. (A,C,E and G) *Phase contrast.* **(B,D,F and H)** *Corresponding immunofluorescence.* **(A)** *Previtellogenic oocytes (Stages 1 and 2).* **(B)** *HSP 70-related proteins are preferentially cytoplasmic. Note the strong labeling of mitochondrial mass (arrows).* **(C)** *Stage 2 and 3 oocytes.* **(D)** *HSP 70-related proteins are localized distally in cytoplasm. Mitochondrial masses are strongly labeled (arrows); faint staining of nucleus.* **(E)** *Stage 5 oocyte.* **(F)** *Labeling in cytoplasm between yolk platelets.* **(G)** *Stage 6 oocyte; the excentrical position of nucleus indicates the animal part of oocyte.* **(H)** *Note the labeling according to a decreasing gradient from animal to vegetal oocyte region. Bar, 200 μm.*

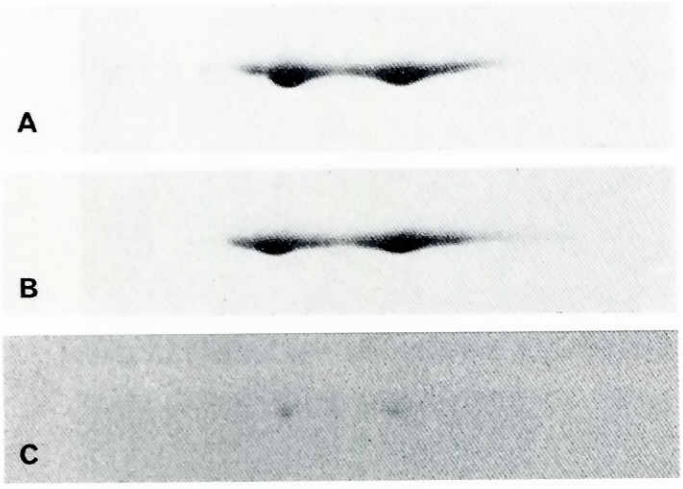

Fig. 3. *Xenopus laevis* stage 5-6 oocytes. *Autoradiography of HSP 70-related protein immunoblots revealed with ^{125}I-labeled second antibody.* **(A)** *whole cell;* **(B)** *cytoplasm, and* **(C)** *nucleus extracts from one oocyte.*

(Fig. 4B). A latitudinal section in the animal region of a four-cell embryo showed that HSP 70-related proteins were distally scattered in the cytoplasm of blastomeres, which appeared uniformly labeled in such a section (Fig. 4C). A detail in the nuclear region of a small animal blastomere from an eight-cell embryo provided evidence for the radiating aspect of the labeling around the nucleus, itself faintly labeled (Fig. 4D_1 and D_2).

During further stages of segmentation (stage 6), we observed the same type of regional distribution of HSP 70-related proteins. Animal blastomeres seemed consistently more fluorescent than vegetal ones (Fig. 4E). Moreover, in some vegetal blastomeres, regional distribution was clearly observed (Fig. 4E, arrow). For animal blastomeres, the intracellular distribution of HSP 70-related proteins was similar to that observed on latitudinal sections of the animal part of a four-cell embryo (Fig. 4C,E). Furthermore, whatever the cleavage stage considered, HSP 70-related proteins were also detected at the cellular limits (Fig. 4B,C).

During blastula transition, the regional distribution of HSP 70-related proteins previously described during cleavage stages was always apparent. At the cellular level, these proteins were concentrated in the perinuclear area of all cells, both in the animal and in the vegetal hemispheres. However, it should be noted that such a concentration was particularly obvious in cells from the marginal zone and in blastomeres of the vegetal region (Fig. 5A). Since the nuclei number is higher in the animal part of the embryo, fluorescence appeared to be concentrated mainly in animal blastomeres, in contrast to vegetal blastomeres, which exhibited weaker labeling.

As gastrulation began, HSP 70-related proteins gradually became visible in nuclei from the clump of cells found in the internal marginal zone (Fig. 5C, E, F1 and F2). In contrast, in the blastoporal region, bottle cells, the nuclei of which presented a more decondensed chromatin, exhibited only perinuclear labeling (Fig. 5C,D).

In midgastrula, bottle cells that moved inwards on the dorsal side at the anterior tip of the archenteron showed preferential nuclear labeling. Such labeling was also observed in the cells of the archenteron roof. Cells of the involuting and involuted dorsal mesoderm, axial mesoderm and, to a lesser extent, the sensorial layer of the neurectoderm, also presented nuclear labeling, whereas those of the epithelial layer of the neurectoderm remained weakly labeled (Fig. 6A,B,C). Ventrally, the nuclei of bottle cells were less fluorescent than those from the cluster of involuting and involuted ventral mesoderm (Fig. 6D,E,F). By the end of gastrulation (small yolk plug stage; Fig. 6G and H), nuclei from the endodermal yolk mass cells and from the involuting cells forming the proximal part of the archenteron roof, as well as those from the blastoporal collar, were clearly labeled. Throughout gastrulation, HSP 70-related proteins were clearly visible in the cytoplasm and at the level of cellular membranes (Figs. 5C, 6A,D,G).

In conclusion, at gastrulation, it is noteworthy that HSP 70-related proteins that exhibited a perinuclear localization during midblastula transition became nuclear in cells of the marginal zone.

Discussion

By immunoblotting, we demonstrated that monoclonal antibody H3F18 is specific for two 70-kD proteins in *Xenopus laevis* oocytes. Indeed, this antibody clearly reacts with only two prominent 70-kD *Xenopus laevis* oocyte proteins previously designated B_3 and B_4 (pI 5.58 and 5.75 respectively). These polypeptides are known to be constitutively expressed heat shock proteins (Horrell *et al.*, 1987; King and Davis, 1987; Mandell and Feldherr, 1990). They are also similar to the heat shock cognate proteins (HSC 70s) characterized in *Drosophila* (Craig *et al.*, 1983).

Regional distribution of Xenopus laevis HSP 70-related proteins in oocytes and early embryos

On the basis of cytological analysis, we have shown that HSP 70-related proteins B_3 and B_4 are preferentially localized in cytoplasm throughout oogenesis, with a particularly strong concentration in mitochondria. These HSP 70-related proteins B_3 and B_4 were previously described as present in equivalent concentrations both in the nucleus and in the cytoplasm of *Xenopus* oocytes (De Robertis *et al.*, 1978; Feldherr and Ogburn, 1980; Mandell and Feldherr, 1990). However, these results concerned only the newly

TABLE 1

QUANTIFICATION OF HSP 70-RELATED PROTEINS IN DIFFERENT CELLULAR COMPARTMENTS OF STAGE 6 OOCYTES

	HSP70 labeling CPM	Volume nl	HSP70 labeling CPM/nl (yolk-free)
Total oocyte		1472	1000
Cytoplasm	1254	500 without yolk platelets	2.5
Nucleus	187	50	3.74
Enucleated animal hemisphere	599	365 without yolk platelets	1.64
Vegetal hemisphere	556	135 without yolk platelets	4.11

Total oocyte, nuclear, cytoplasmic and yolk volume values are from Hausen and Riebesell (1991), Danilchik and Gerhart (1987).

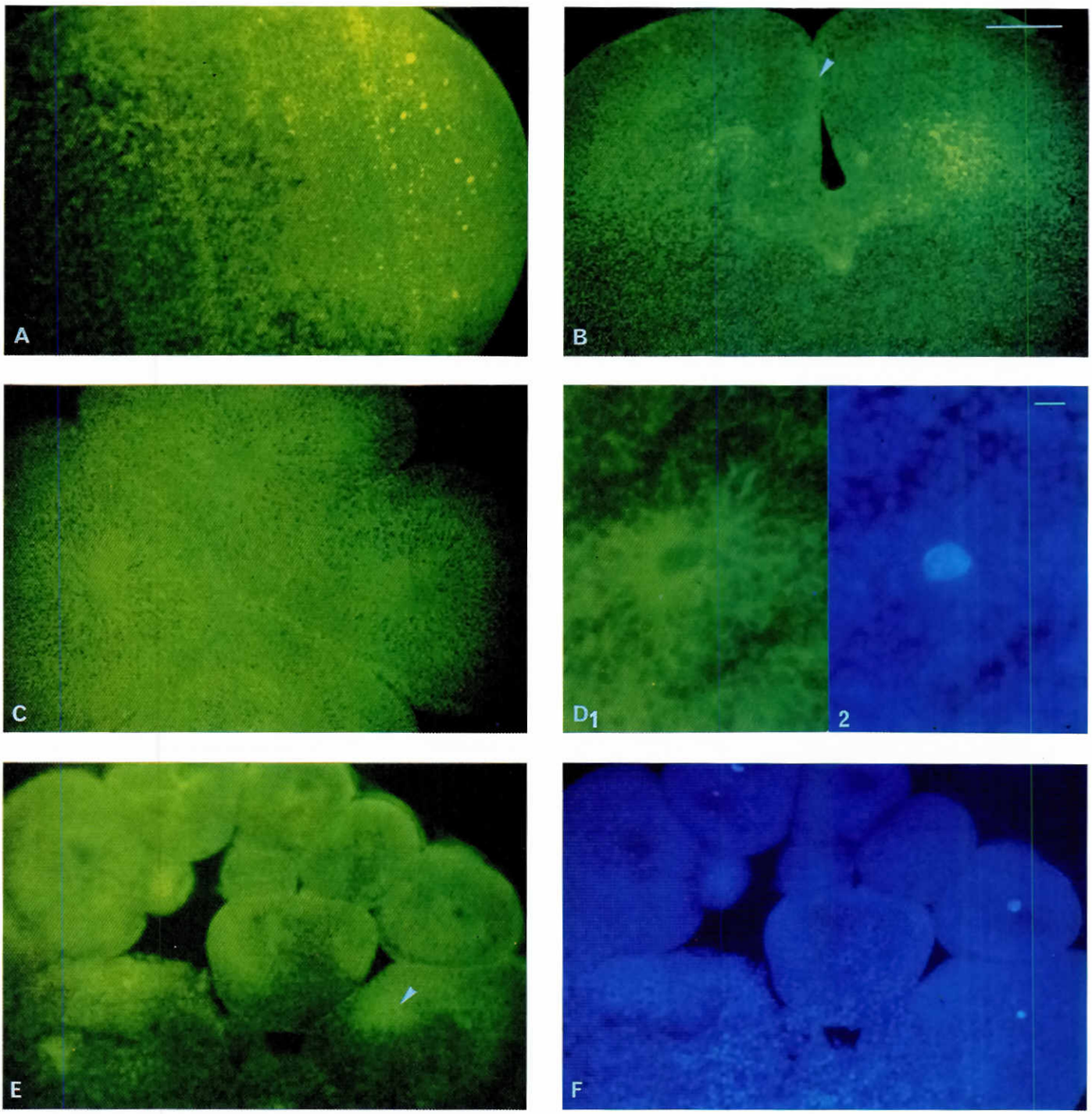

Fig. 4. Immunolocalization of HSP 70-related proteins in *Xenopus laevis* mature oocyte and during early embryogenesis (segmentation). (A) *Mature oocyte showing decreasing fluorescence from the animal cap (right) to the vegetal region. Note yolk-free patches clearly labeled.* **(B)** *Two-cell embryo. Note the preferential labeling in the animal part of the two blastomeres. Arrow shows the cleavage furrow.* **(C)** *Four-cell embryo (latitudinal section). In the animal region, labeling is distally scattered in the cytoplasm of blastomeres.* **(D)** *Eight-cell embryo.* **(D_1)** *Detail of the nuclear region of a micromere. Note the important labeling around the nucleus.* **(D_2)** *DAPI nuclear counterstain.* **(E and F)** *Xenopus laevis morula.* **(E)** *Note the polar distribution of labeling from the animal to the vegetal part of the embryo. Labeling in vegetal blastomeres close to animal blastomeres is also distributed in a polarized manner (arrow). In animal blastomeres, the intracellular distribution is distally scattered from the nuclear region, which itself is faintly labeled.* **(F)** *DAPI nuclear counterstain. Bar, 200 μm in (A), (B), (C), (E) and (F); 20 μm in (D).*

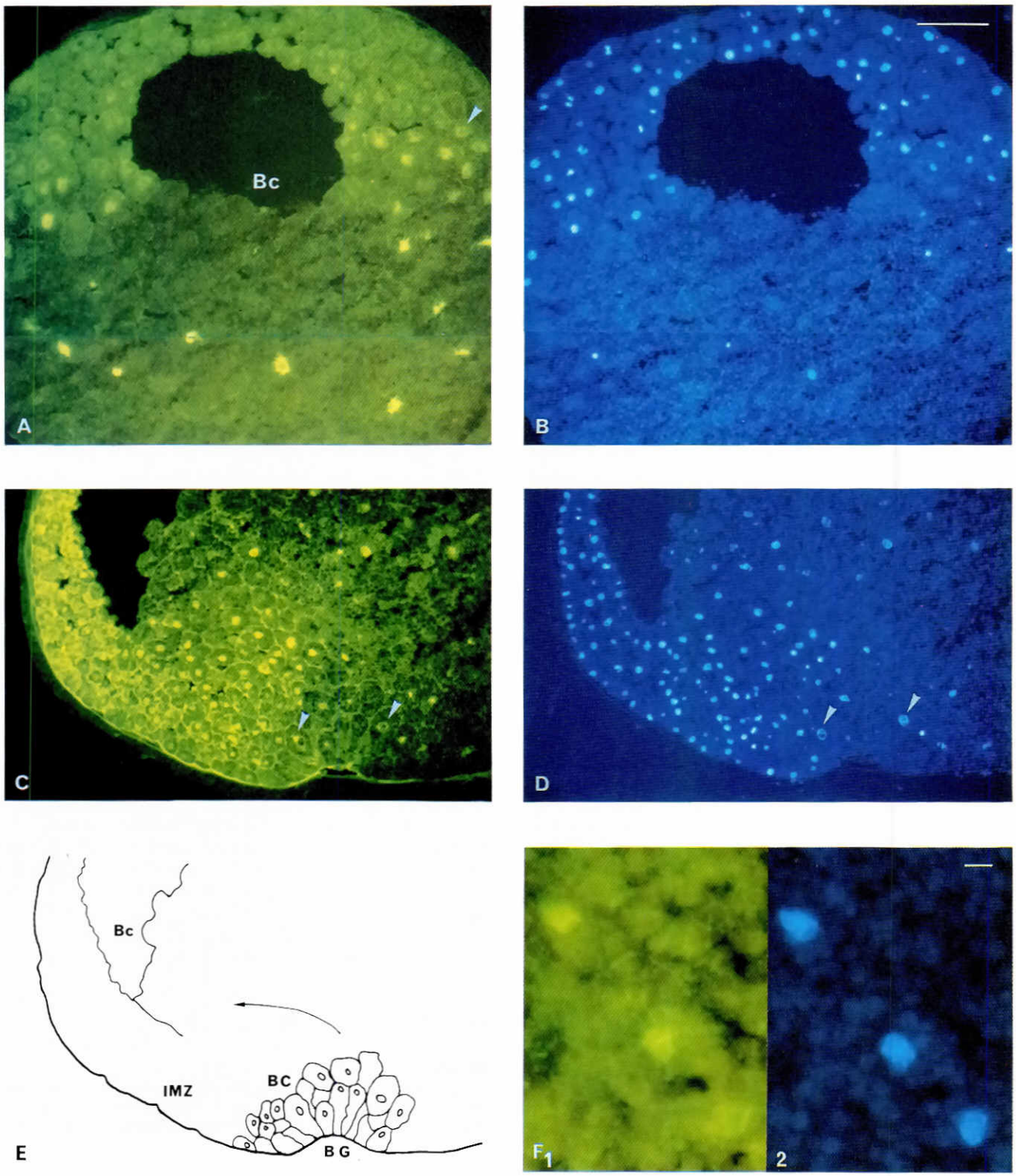

Fig. 5. Immunolocalization of HSP70-related proteins in *Xenopus laevis* during early embryogenesis (blastula and early gastrula). (A and B) *Midblastula transition (MBT). Bc: blastocoel.* **(A)** *Polarized distribution of labeling; animal blastomeres are more fluorescent than vegetal blastomeres. At the cellular level, perinuclear labeling is observed in blastomeres. This concentration is obvious in cells of the marginal zone (arrow) and in vegetal blastomeres.* **(B)** *DAPI nuclear counterstain.* **(C and D)** *Xenopus laevis early gastrula (dorsal side).* **(C)** *Note the nuclear labeling of cells from the involuted internal marginal zone. In involuting bottle cells (BC) from the blastoporal region, only perinuclear labeling is observed.* **(D)** *DAPI nuclear counterstain.* **(E)** *Schematic interpretation of C. Bc: blastocoel; BC: bottle cells; BG: blastoporal groove; IMZ: involuting marginal zone.* **(F)** *Detail of blastomeres from IMZ.* **(F_1)** *Note the nuclear fluorescence.* **(F_2)** *DAPI nuclear counterstain. Bar, 200 μm in (A), (B), (C) and (D); 20 μm in (F).*

synthesized polypeptides and microinjected labeled polypeptides. They never took into consideration polypeptides stored in oocytes.

In order to unambiguously quantify the amount of HSP 70 protein in oocyte cellular compartments, we performed immunoblots of total proteins from stage 6 oocytes, rather than the more classical immunoprecipitation of labeled proteins, which would have limited our analysis to that of proteins synthesized during the labeling period.

Results from such quantification studies provided evidence for a higher HSP 70-related protein concentration in oocyte nuclei than in cytoplasms. When compared to results of immunolocalizations on oocyte sections, which show a weak nuclear signal, such results suggest that HSP 70 protein epitopes may in fact be masked in nuclei. According to such a hypothesis, only a portion of the HSP 70 proteins would be reactive in nuclei. It is interesting to note that in stage 6 oocytes, the HSP 70 concentration was higher in nuclei than in cytoplasms.

Taking into account these data and the well known role of HSP 70 proteins in transferring other proteins through different organelles or cellular compartments (Chirico *et al.*, 1988; Deshaies *et al.*, 1988), our results concerning the regionalization of HSP 70-related proteins in stage 6 oocytes suggest that these proteins may be indirectly involved in gene control related to late oogenesis events.

Concerning the animal-vegetal distribution of the HSP 70 protein, immunolabeling quantification studies strongly suggested that the decreasing gradient of HSP 70-related proteins from the animal to the vegetal part of full grown oocytes, as revealed by immunocytochemical localizations, was related only to the asymmetric distribution of yolk platelets in oocytes. Indeed, in oocytes, due to the yolk distribution, there are more free spaces in the animal half than in the vegetal one, namely 37% and 15% respectively (from Danilchik and Gerhart, 1987 and Hausen and Riebesell, 1991). Since the radioactivity detected by immunolabeling of HSP 70-related proteins was found to be equivalent in both hemispheres, these HSP 70 proteins are likely to be concentrated in the small free area of the vegetal part, where fluorescence appears as small, bright, scattered dots. In contrast, in the animal region, the fluorescent spaces are more spread out, so that HSP 70 animal-vegetal distribution appears to conform to a decreasing fluorescent gradient.

In embryos, the regional distribution observed in oocytes was found to be maintained after fertilization during cleavage stages at the level of whole embryos. In this case, we can again assume that the preferential labeling of animal blastomeres is due to asymmetric distribution of the yolk in embryos. Furthermore, this apparent gradient is increased by the perinuclear area fluorescence of blastomeres which are much more numerous in the animal region.

Nuclear transfer of HSP 70-related proteins and cell internalization

We have pointed out that progressive perinuclear concentration and nuclear transfer of HSP 70-related proteins occur by the time of internalization in the involuting marginal zone (IMZ), whereas cells of the vegetal area exhibit only perinuclear localization of such proteins.

During gastrulation, as involution progresses, the nuclear occurrence of HSP 70-related proteins becomes evident in internalized cells, which is not the case in external layers. We therefore assume that such a nuclear transfer might be related to the active internalization process.

The preferential nuclear localization of HSP 70-related proteins that we observed in embryonic cells during the course of development has never been reported under normal thermal conditions, except during the S phase of the cell cycle (Milarsky and Morimoto, 1986). In contrast, such a localization was described in somatic cells submitted to stress (Velasquez and Lindquist, 1984). Under normal thermal conditions, during amphibian embryogenesis, the nuclear transfer of proteins is related to particular events which occur at definite times of development. For example, Dreyer *et al.* (1982), and then Dreyer (1987) described a shift in oocyte nuclei proteins from the cytoplasm to the nucleus of embryonic cells at stages specific for each individual protein. It is noteworthy that the nuclear entry of these proteins occurs synchronously in different parts of the embryo and may therefore be related to a signal common to all regions of the embryo. From our observations, this was not the case for the nuclear transfer of HSP 70-related proteins, which only involved specific cell types at definite moments in embryogenesis.

In other respects, the c-myc protein, which accumulates in the oocyte cytoplasm in late oogenesis, migrates to the nucleus after fertilization and in early stage embryos, suggesting its participation in early events of development during segmentation (Gusse *et al.*, 1989). Similar observations have been reported for fibroblast growth factors (FGFs), which show regional distribution in the vegetal hemisphere of oocytes, and maintain this localization during early cleavage stages. Following midblastula transition, their nuclear transfer in cells of the marginal zone has been observed at the time of mesodermal induction, suggesting that FGFs are prepositioned in mesoderm-forming regions and are actively involved in mesoderm induction *in vivo* (Shiurba *et al.*, 1991).

Our own results with nuclear transfer of HSP 70-related proteins at the time of gastrulation in involuting or involuted cells of the marginal zone suggest that these proteins might be involved in the control of gene expression at that time. Taking into account their role of «chaperone proteins» in transporting other proteins to the nucleus (Shi and Thomas, 1992), we can assume that HSP 70-related proteins are involved in the nuclear transfer of specific proteins directly involved in the internalization process.

In conclusion, from our immunolocalization results, the distribution of HSP 70-related proteins during development appears to be indirectly involved in the processes of cellular internalization. Further analysis of their association with other proteins implicated in such processes should provide information on their possible contribution to these developmental events.

Materials and Methods

Animals

Adult *Xenopus laevis* were purchased from the Centre National de la Recherche Scientifique, Domaine de la Valette, Montpellier, France, and raised in our laboratory under standard conditions (20°C, photoperiod 12-12) for several months before use.

Oocytes and embryos

Pieces of ovary dissected from anesthetized females (MS 222 0.1% Sandoz) were isolated in modified Barth solution (MBS): 88 mM NaCl, 1 mM KCl, 2.4 mM CO_3 HNa, 0.82 mM SO_4 Mg, 0.33 mM Ca $(NO_3)_2$, 0.41 mM $CaCl_2$ and 10 mM HEPES, pH 7.4. Follicle cell-free oocytes were then obtained as follows; pieces of ovary were incubated for 15 min in Steinberg EDTA: 116 mM NaCl, 1.3 mM KCl, 4.6 mM TRIS and 10 mM EDTA, pH 7.1. Next, they were manually defolliculated and transferred to MBS. Follicle cell

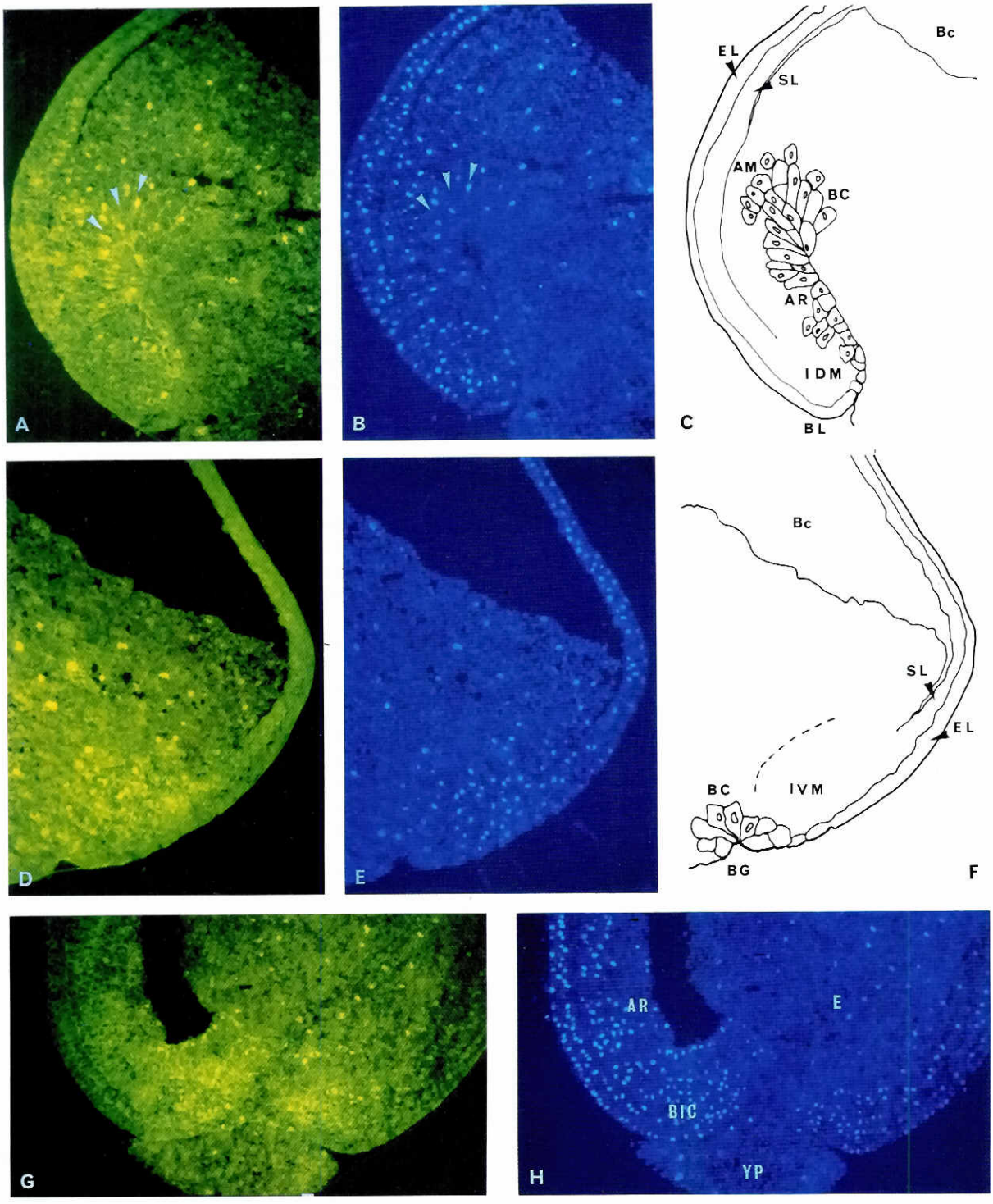

Fig. 6. Immunolocalization of HSP 70-related proteins in *Xenopus laevis* mid and late gastrula stages. (A, B and C) *Midgastrula, dorsal side on the left.* ***(A)*** *Note the clear nuclear labeling in bottle cells (BC), archenteron roof (AR), involuting dorsal mesoderm (IDM), axial mesoderm (AM) and sensorial layer (SL) of neurectoderm.* **(B)** *DAPI nuclear counterstain.* **(C)** *Schematic interpretation of (A). AM: axial mesoderm; Bc: blastocoel; BC: bottle cells; BL: dorsal blastoporal lip; EL: epithelial layer of dorsal neurectoderm; IDM: involuting dorsal mesoderm; SL: sensorial layer of dorsal neurectoderm.* **(D, E and F)** *Midgastrula ventral side with blastopore groove (BG) and bottle cells (BC).* **(D)** *Note the nuclear labeling of involuting ventral mesoderm (IVM).* **(E)** *DAPI nuclear counterstain.* **(F)** *Schematic interpretation of (D). Bc: blastocoel; BC: bottle cells; BG: ventral blastoporal groove; EL: epithelial layer of ventral ectoderm; IVM: involuting ventral mesoderm. SL: sensorial layer of ventral ectoderm.* **(G and H)** *Small yolk plug stage.* **(G)** *Nuclear labeling of cells from endodermal yolk mass (E), involuting cells from archenteron roof (AR), cells from blastoporal collar (Blc). YP: yolk plug.* **(H)** *DAPI nuclear counterstain. Bar, 200 μm.*

removal was monitored according to Horrell *et al.* (1987). Oocyte stages were characterized according to size and appearance (Dumont, 1972).

Eggs were obtained from frogs which were induced to mate by injection of 600 U chorionic gonadotropin (Sigma). Embryos were maintained in modified Ringer medium (MMR) pH 7.8, according to Ubbels *et al.* (1983). The jelly coat was removed in 2% cysteine hydrochloride brought to pH 8; after extensive washes in MMR, embryos were kept in 1/4 strength MMR with 100 U penicillin-streptomycin/ml. Embryos were staged according to Nieuwkoop and Faber (1967). From stage 22, the vitelline membrane was removed with forceps before the samples were fixed.

Antibodies

The molecular probe H3F18 used to detect *Xenopus laevis* HSP 70-related proteins was provided by Dr. D. Mattei, Institut Pasteur, Paris, France. H3F18 is a mouse monoclonal antibody raised against *Plasmodium falciparum* HSP 70-like protein (Blisnik, 1988; Mattei, 1989). The epitope recognized by H3F18 is a 16 aa sequence lying about 160 residues before the protein C-terminus. The specificity of the anti-HSP 70 antibody was checked by incubation with the corresponding antigenic peptide.

As a control, we used a mouse mAb GFAP (glial fibrillary acidic protein, GA-5, Boehringer Mannheim), which is presumed to be non-reactive and we also tested the effect of the second antibody alone: FITC (fluorescein isothiocyanate-conjugated) goat anti-mouse IgG (Miles Scientific, Paris).

Protein extraction, gel electrophoresis and immunoblotting

Defolliculated oocytes were homogenized in 125 mM TRIS, 12 mM disodium EDTA, pH 6.8. After centrifugation (10,000 g for 10 min), the clear supernatant was collected and stored at -20°C.

Two-dimensional electrophoresis was performed with equilibrium pH gradient electrophoresis using ampholines pH 3-11 in the first dimension, followed, in the second dimension, by electrophoresis on an SDS 10 % polyacrylamide slab gel (O'Farrell, 1975). The polypeptides separated by two-dimensional electrophoresis were blotted at 500 mA for 2 h in 25 mM Tris, 0.192 M glycine. The immobilon sheet (Immobilon PVDF, Millipore) was incubated for 1 h in 1% BSA in PBS at 40°C, followed by 30 min at room temperature with the monoclonal antibody H3F18, and then washed with several changes with 1‰ PBS-Tween 20. Peroxidase-conjugated sheep anti-mouse IgG (Institut Pasteur Production) was added for 30 min. After several washes in 1‰ PBS-Tween 20, the peroxidase activity was revealed using 4-chloro-1-naphtol as substrate.

HSP 70-related proteins quantification

Stage 6 oocytes were collected and then defolliculated as previously described.

The absence of follicle cells was checked by microscope examination of oocytes stained with Hœchst 33258 (Sigma). Proteins from ten whole oocytes, ten nuclei and ten cytoplasmic fractions were extracted and treated for electrophoresis as previously described (Moreau *et al.*, 1991). Bisections of oocytes were performed from quickly frozen cells. Ten animal and vegetal halves were treated for electrophoresis as described above.

Samples corresponding to a single unit from each fraction were separated by two-dimensional electrophoresis and electrophoretically transferred (see above). Blots were incubated for 1 h in 3% BSA in PBS at 40°C. They were washed with PBS 1‰ Tween 20 and then incubated for 45 min with mAb H3F18 at room temperature. After washing, they were incubated with a ^{125}I-labeled (specific activity 3000 Ci/mmol) anti-mouse antibody (Amersham). The proteins were localized by autoradiography and the labeled spots were excised from the blot and counted.

Immunocytochemistry

Pieces of ovary and embryos were fixed at 20°C with modified Romeis fixative (saturated mercuric chloride, 5% trichloracetic acid and 37% formaldehyde: 5/4/3/v/v/v; Hausen *et al.*, 1985) for 2 h. They were transferred to 100% ethanol for 2 h with one change, then to 50% absolute ethanol/polyester wax and 100% polyester wax (Steedman, 1957). Sections were overlaid with 1% PBS-BSA for 10 min followed by antibody H3F18 (1:100 dilution) for 45 min. They were then washed three times in PBS. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Miles Scientific, Paris) used at a dilution of 1:100 was added for another 45 min. This was followed by nuclear counterstain with DAPI (4'-6' diamidino-2-phenylindol-dihydrochloride: DAPI 0.5 µg/ml). After several changes in PBS, slides were mounted in Mowiol.

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