Biochemical and Biophysical Research Communications **260,** 143–149 (1999)
Article ID bbrc.1999.0782, available online at http://www.idealibrary.com on IDEAL®

Constitutive hsp70 Is Essential to Mitosis during Early Cleavage of *Paracentrotus lividus* Embryos: The Blockage of Constitutive hsp70 Impairs Mitosis

Gabriella Sconzo,[1] Franco Palla,* Cecilia Agueli, Giovanni Spinelli, Giovanni Giudice, Domenico Cascino, and Fabiana Geraci

*Dipartimento di Biologia Cellulare e dello Sviluppo 'Alberto Monroy,' Università di Palermo, Palermo, Italy; and *Istituto di Biologia dello Sviluppo CNR, Palermo, Italy*

Received April 28, 1999

Localization of constitutive hsp70 in eggs and early embryos of sea urchin *Paracentrotus lividus* is shown by means of *in situ* immunostaining. An accumulation of this protein is shown in the mitotic structures (asters, spindles and centrosomes). Microinjection of anti-hsp70 antibodies into eggs causes impairment of formation of mitotic structures and of cell division. This impairment goes from a complete mitotic block, to irregular mitotic apparatus formation with irregular cleavage, depending upon the antibody concentration. The localization of hsp70 after antibody microinjection is also described. Blockage of mitotic apparatus formation by nocodazole also blocks the concentration of hsp70 molecules observed in non-treated eggs. That the constitutive hsp70 plays a role in sea urchin mitosis is indicated.

Key Words: **constitutive hsp70; mitosis; sea urchin embryos.**

Constitutive hsp70 proteins are one of the major components of the molecular chaperone system in prokaryotic and eukaryotic cells (1, 2, 3). They belong to a larger family generally referred to as 70 kD 'heat shock' or 'stress' proteins, hsps70, which are synthesized under a wide variety of environmental stress conditions (4, 5). The hsps70, synthesized as a wave under cellular stress, are essential for cell survival (6, 7, 8) and are found in the cytosol and in the nucleus and, as ascertained in some cases, in the nucleolus of eukaryotic cells (9, 10). They protect some proteins and some proteic complexes, probably in molten globule state, from denaturation and disassembly (11–14) due to heat stress. In addition, the constitutive hsps70 work in non stressed cells carrying out physiological functions (15, 16). They have been found to be active in keeping polypeptides in an unfolded state and carrying them towards and across mitochondrial or endoplasmic reticulum membranes (17–20). Other members localized within these organelles bind the translocating polypeptides to prevent improper aggregation before the folding (21, 22). A constitutive hsp70 member is also able to disassemble clatrin cages by binding the clatrin light chains (23), while another hsp70 form named prp73 is involved in the lysosomal degradation of intracellular proteins (24). Besides, a constitutive hsp70 binds nascent polypeptide chains by holding them in the unfolded conformation until chain termination (25, 26). The ability to bind exposed hydrophobic parts of other proteins by the COOH-terminal domain was proposed as common to both constitutive and stress induced hsps70 (27). The amino-acid sequence of this family members are phylogenetically conserved and their three-dimensional structure of hsp70 has two different parts, an ATPase core-domain in the amino-terminal region (28) and a peptide-binding domain in the COOH-terminal region followed by an α-helical subdomain functioning as a lid (29), as determined by crystal structure analysis. The ATP-containing hsp70 in the deep cleft of N-terminal domain in closed conformation is able to bind the polypeptide substrate but also to loose it quickly, because the affinity is low. It was suggested that ATP-binding produces conformational changes in the protein binding domain opening the lid and causing substrate release; therefore, a model was proposed by Rudiger (30). ATP hydrolysis stimulated by a regulatory motif in the terminal part of the molecule (31) or by the hsp40 co-chaperone (32) catalyses in turn a more stable substrate binding closing the lid.

Constitutive hsp70 expression has also been studied during embryogenesis of some embryos as *Drosophila*,

[1] Corresponding author. Dipartimento di Biologia Cellulare e dello Sviluppo 'Alberto Monroy,' Università di Palermo, Viale delle Scienze, 90128 Palermo, Italy. Fax: (39) 91-420897. E-mail: gabriella.sconzo@unipa.it.

0006-291X/99 $30.00

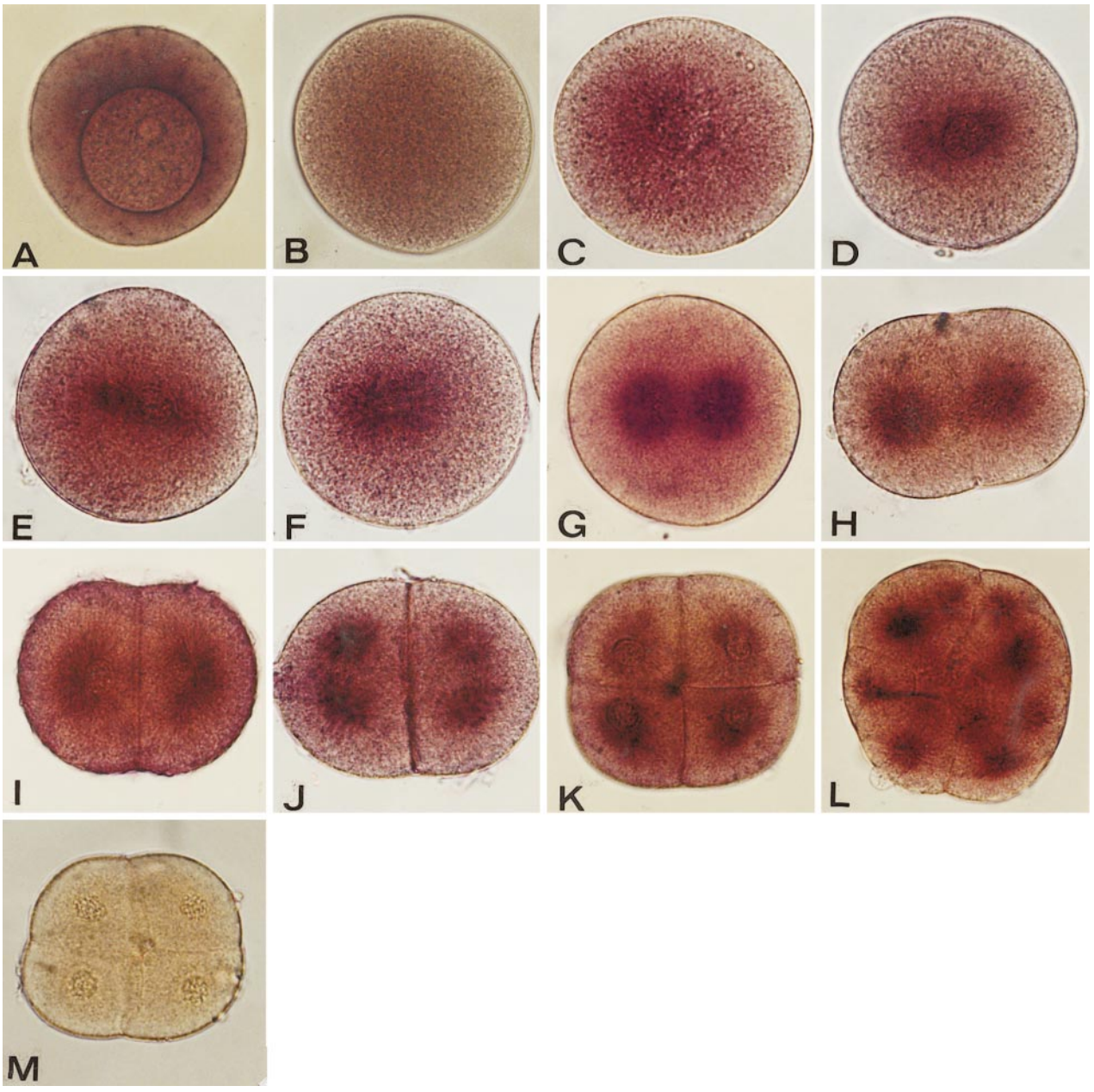


FIG. 1. Immunolocalization of constitutive hsp70 in whole mount sea urchin oocytes, eggs and early embryos. Immunophosphatase staining was performed on Bouin-fixed whole embryos using anti-hsp70 mAb. (A) Oocyte; (B) unfertilized egg; (C) fertilized eggs at 10 minutes fertilized egg; (D) 20 minutes; (E) 30 minutes; (F) 40 minutes; (G) 50 minutes; (H) 60 minutes; (I) 1 h and 10 minutes after fertilization (2 cells); (J) 1 h and 20 minutes after fertilization (towards 4 cells); (K) 2 h (8 cells); (L) 2 h and 30 minutes (16 cells). Staining provides evidence of the mitotic apparatus in which there is the constitutive hsp70. (M) An example of control embryos in which the anti-hsp70 mAb was omitted in the primary antibody reaction step: the embryo is not stained. This shows that the diffused color spread through the other cells of the figure is not due to background.

sea urchin, *Xenopus* and mouse (33–36). In *Paracentrotus lividus* sea urchin embryos, one of the Mediterranean species, a constitutive hsp70 mRNA is basally present at a very low level (37). Constitutive hsps70 have been localized in the cytoplasms much more than in the nuclei by immunofluorescences on sections of embryos, and appear more concentrated in some embryonic territories as in the portion facing the lumen of the cells which invaginate at gastrulation (34). In the present study we focalized our attention on constitutive hsp70 localization soon after fertilization and during early cleavage. Whole mount embryos have been used to observe constitutive hsp70 distribution inside their cells. We found the basally expressed hsp70 located in mitotic apparatus and proposed a new role by blocking its function. These results represent the first

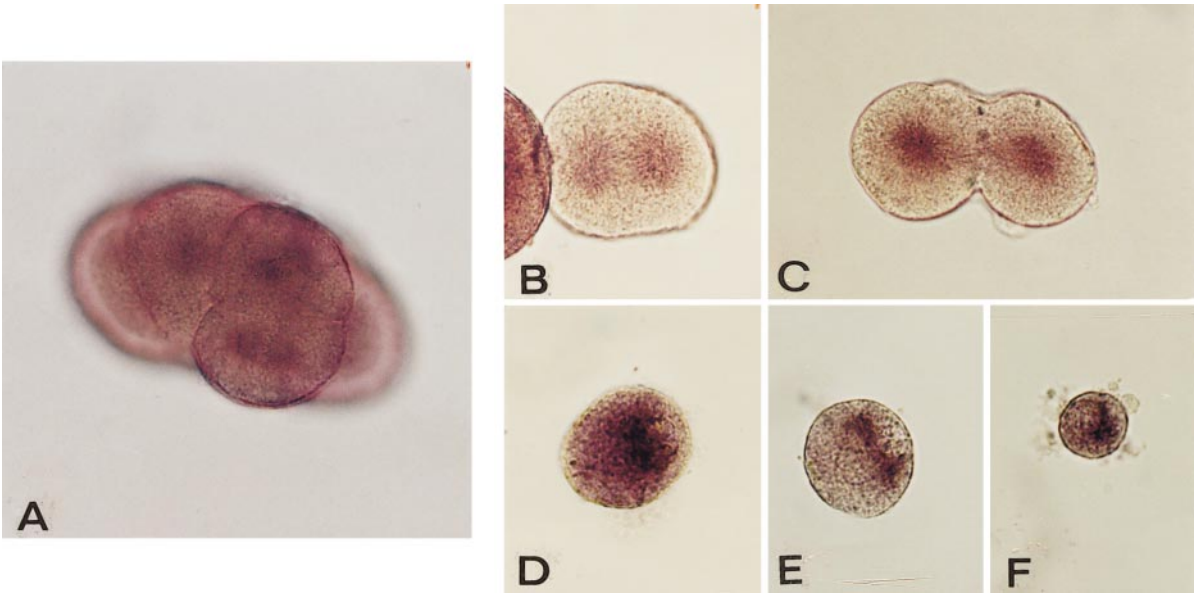


FIG. 2. Immunolocalization of constitutive hsp70 in dissociated sea urchin early embryos. Immunophosphatase staining was performed on Bouin-fixed partially dissociated embryos (A) or dissociated cells (B-F). Cells of a partial decompacted embryo (A) and completely dissociated cells show: staining of mitotic spindle (A) or staining of two poles corresponding to asters (B and C) or staining of the cytoplasm with a higher concentration close to the nucleus (D) or outside the nucleus (E and F).

evidence for a role of hsp70 in cell division in sea urchin, and and the second in a developmental system.

MATERIALS AND METHODS

Embryo culture. Paracentrotus lividus embryos were reared from fertilization on at 10,000/ml in thermostatic chamber at the physiological temperature of 20°C by stirring with a rotating propeller (25 rpm) in millipore filtered sea water (mfsw) containing 10 mM Tris pH 8.0, 50 μg/ml streptomycin sulfate (Sigma) and 30 μg/ml penicillin G potassium salt (Merck). Fertilization was carried out in mfsw containing 3 mM of p-aminobenzoic acid, PABA, (Sigma) which is used to avoid fertilization envelop hardening. Fertilized eggs were soon passed through a 80 μm mesh to remove fertilization envelopes and then reared as described above.

Embryo fixation at different developmental stages. Embryos, collected at the desired developmental stages from fertilization on, were fixed for 12-16 h by dropping them in Bouin fixative (15:5:1 of picric acid solution, 37% formaldehyde, glacial acetic acid respectively). They were kept at 4°C in 70% ethanol after serial dehydration.

Immunocytochemistry. Samples of about one-two thousand embryos of each considered stage were analyzed for each assay. After washings with slow waving in 100% ethanol and then in 100% methanol containing 2 mM EDTA, the embryos were rehydrated with serial ethanol, washed 3 times with PBST (phosphate-buffered saline containing 0.1% Tween 20) and allowed to incubate overnight with PBST containing 3% BSA containing monoclonal anti-hsp70 antibodies (1:3000 dilution; Sigma). In control embryos anti-hsp70 antibodies were omitted. The secondary antibody, an anti-mouse anti-IgG alkaline phosphatase (AP) conjugated (1:1000 dilution; Promega), was added to the samples for 1 h after 3 washes with PBST. The secondary antibody surplus was eliminated by washing 3 times with PBST and last with TBS. Staining was developed for 1-3 h by NBT/BICP (Boehringer Mannheim) in a buffer, containing 100 mM NaCl, 100 mM Tris-HCl, pH 9.5, 50 mM $MgCl_2$. The staining reaction was stopped by two washes with 50% ethanol. The whole embryos resuspended in 80% glycerol were finally mounted on coverslips, observed by an Axioskop MC80 microscope (Zeiss) and photographed with 100 Asa Kodak Gold film.

Embryonic cell dissociation. Embryos at 8-cell and 16-cell stages were rapidly washed with Ca^{2+} free sea water and then with glycine EDTA solution only for few minutes by gently pipetting. After microscopic observation partial or total dissociation was stopped and the cells were fixed as done for whole embryos.

Microinjections into unfertilized eggs. Unfertilized eggs were microinjected with 50 pl PBS containing different monoclonal anti-hsp70 antibody concentrations (Sigma) following the procedures indicated by Palla *et al.* (38) and Berg *et al.* (39). The antibody concentrations used in the assays were 4 μg/μl, 3 μg/μl, 2 μg/μl and 0.2 μg/μl. A monoclonal anti-mouse anti-IgG (Promega) was injected at the same time in serial concentrations from 4 μg/μl to 0.2 μg/μl in the control embryos. Before microinjections the eggs were dejelled and stuck on protamine sulfate treated Petri dishes. At least 10 batches of 50 eggs were microinjected for each antibody concentration, fertilized in the presence of 3 mM PABA and allowed to develop at 18°C in MFSW. In the hsp70 localization experiments 4 batches of 50 eggs were injected with 0.2 μg/μl of anti-hsp70 antibodies, fertilized and when the established time was reached the sea water was gently removed and 2 ml of Bouin solution were immediately added in order to completely cover the embryos. After 5 minutes the embryos were gently removed by a siliconized pulled pipette and left in 1 ml of Bouin solution for fixation. The immunodetection assays were as previously described omitting only the anti-hsp70 step.

RESULTS

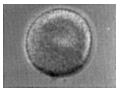
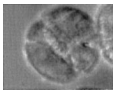
hsp70 Localization Soon after Fertilization on and during Early Cleavage

Transparency of sea urchin *P. lividus* embryos makes them suitable for immunocytochemistry assays and their sizes allow the observation of up to one thou-

sand whole mounts of embryos synchronously developed at the same time. We studied the hsp70 localization by an antibody staining reaction in whole eggs and early embryos from soon after fertilization on. Unfertilized eggs were compared with 10 min-fertilized eggs to individuate constitutive hsp70 by anti-hsp70 antibodies (Fig. 1, B and C, respectively). Diffused staining appears either in unfertilized or fertilized eggs indicating the presence of hsp70 in the whole egg cell, nevertheless, a slight difference between the unfertilized egg and the 10 min-fertilized egg can be observed. In the latter the hsp70 is indeed slightly less concentrated in the cell periphery and more concentrated in the cell center where the pronuclei are coming across. The oocytes contain a storage of constitutive hsp70 (Fig. 1A) which is clearly present in unfertilized eggs confirming a previous result obtained on oocyte sections by immunofluorescence (Sconzo *et al.*, 1997). Twenty minutes after fertilization most of the hsp70 is always diffused in the whole egg cell, but some of it can be observed condensed outside the nucleus at two opposite sides (Fig. 1D). The same localization is more clearly observed 30 min after fertilization (Fig. 1E) suggesting that hsp70 is located in the same sites of the arising mitotic apparatus. Forty minutes after fertilization, when the nuclear envelope disappears, the hsp70 localization appears to stain the astral microtubules of the spindle poles (Fig. 1F) achieving on this side the highest concentration from 50 minutes after fertilization (Fig. 1G) till 60 minutes when the egg cell is dividing in the two daughter cells (Fig. 1H). Constitutive hsp70 is again located outside the nucleus more concentrated at opposite sides of the first division plane (Fig. 1I). In the following developmental stages, at 4-cells just before the division, at 8-cells and at 16-cells the hsp70 is localized near the nucleus in the asters (Fig. 1J, K and L respectively). The control embryos, lacking anti-hsp70 antibody in the primary antibody reaction step, never showed staining, as shown, for example, in the 4-cell embryo of Fig. 1M.

As the cleavage goes on some cells become superimposed with each other and the hsp70 localization can be less clear. To confirm hsp70 localization in mitotic apparatus during cleavage we dissociated embryos at 8-cell stage and 16-cell stage then fixed the dissociated cells. A view of partially dissociated embryos or completely dissociated cells is shown in Fig. 2. The figure shows a partially dissociated embryo in which the mitotic spindles of two blastomeres are made clear by immunostaining (A); a dividing cell dissociated from 8-cell embryos in which the asters begin to move away from each other (B) and another cell in which part of the mitotic spindle is still visible when the furrow is forming (C); and also some cells from 16-cell embryo in which hsp70 is clearly located outside the nucleus and also spread throughout the cell (D); centered in two poles (E); and at last a micromere in which the hsp70 is localized in a pole outside the nucleus. These images clearly indicate a specific localization of constitutive hsp70 in the mitotic apparatus during early cleavage, and that the constitutive hsp70 appears to move inside the cell depending upon the cell cycle.

TABLE I

Embryos	Anti-hsp70 4 μg, 3 μg, 2 μg/μl	Anti-hsp70 0.2 μg/μl	Anti-mouse IgG (4 μg-0.2 μg/μl)
No cleavage 	76%	4.3%	0
Irregular cleavage 	18.6%	78.2%	1%
Regular cleavage 	5.4%	17.5%	99%

Impairment of Cell Cycle Following Anti-hsp70 Antibody Microinjections

The localization of constitutive hsp70 in the mitotic apparatus raises the question about its function. It can be hypothesized that hsp70 is in the spindle poles and near the nucleus because it has a specific chaperone function in this structure.

To verify this possibility, experiments of loss of hsp70 function were carried out by microinjecting anti-hsp70 antibodies in living unfertilized eggs and by following the embryonic development from fertilization on. We reasoned that if the hsp70 had not a role in the mitotic spindle, cellular divisions would proceed normally; otherwise, its block would cause defects in cellular divisions. Different amounts, corresponding to 4 μg, 3 μg, 2 μg and 0.2 μg/μl of monoclonal anti-hsp70 antibodies were microinjected in the unfertilized eggs and the effects on development of these eggs were observed and summarized in Table I. Only 5.4% of the embryos developed normally if antibodies from 4 μg to 2 μg/μl were microinjected, while the remainder, 18.6% of the embryos were irregularly segmented and most of the embryos, 76%, did not divide (see figures within Table I as examples). If the microinjected amount was 0.2 μg/μl, i.e., 10-20 times lower, the percentage of normal embryos increased to 17.5%, nevertheless, most of the embryos, 78.2%, cleaved irregularly, while 4.3% embryos remained unsegmented. The highest amount of unrelated antibodies, for example anti-mouse IgG microinjected in embryos as control assays, did not produce any adverse effect, and only 1% of the embryos developed irregularly. The embryonic development was followed until the control non microin-

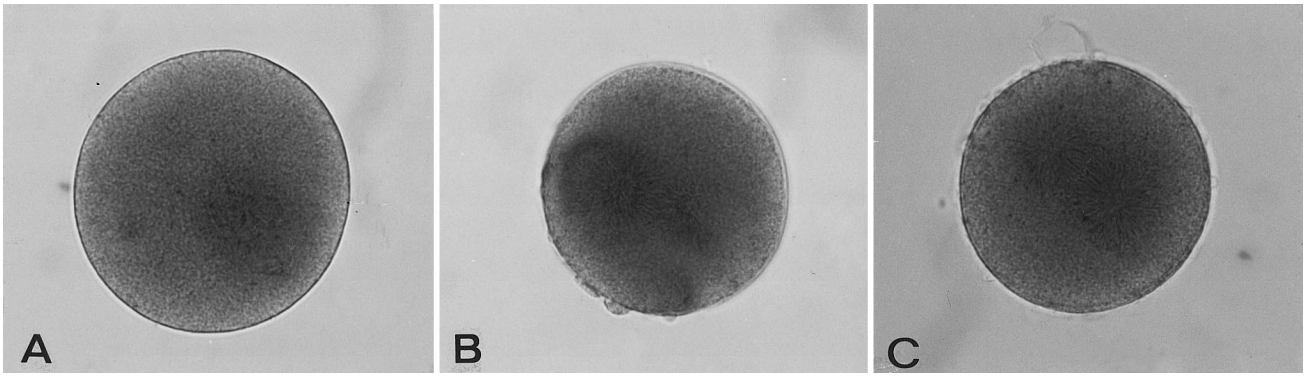


FIG. 3. Constitutive hsp70 localization after anti-hsp70 mAb microinjection. Unfertilized eggs were microinjected with 0.2 μg/μl of anti-hsp70 mAb and after fertilization were allowed to develop until control embryos (not shown) reached two- or four-cell stage. In the immunolocalization assay the primary antibody reaction step was omitted because the anti-hsp70 mAb had already been microinjected into the living eggs. (A) First type of hsp70 localization: immunostaining is in part condensed in eccentric zone of the zygote egg. (B) Second type of hsp70 localization: immunostaining is condensed around and in the irregular asters of the zygote. (C) Third type of hsp70 localization: immunostaining is faintly condensed in the asters of zygote. The microinjected anti-hsp70 mAb block the constitutive hsp70 depending on the amount of antibody used.

jected embryos developed up to pluteus. Embryos microinjected with anti-hsp70 antibodies were stopped or delayed and irregular in their development, while those microinjected with anti-mouse IgG antibodies were not and developed normally up to pluteus These data suggest that constitutive hsp70 is involved and has a role in mitosis during cleavage.

Constitutive hsp70 Localization after Anti-hsp70 Microinjections

With the aim to understand which is the role of the constitutive hsp70 in mitosis we located constitutive hsp70 in embryos which had been microinjected at the unfertilized egg stage with anti-hsp70 or with anti-mouse anti-IgG as control. The lowest amount of antibody concentration, 0.2 μg/μl, was used to obtain the highest number of irregular embryos and some regular ones. The microinjected eggs were fertilized in presence of PABA, observed during their development for 100 min, then fixed and analyzed by immunostaining. Figure 3 shows three eggs as examples of different consequences on cleavage following anti-hsp70 microinjection. In some cases part of hsp70 is more concentrated in an eccentric zone and there is not a specific localization (Fig. 3A); in some other cases hsp70 is localized in the asters, which are irregularly situated (one of the two has a regular shape and the other is incomplete) (Fig. 3B); in other cases hsp70 is localized in the spindle asters (Fig. 3C). The percentage of each cell type is the same reported in Table I for 0.2 μg/μl, corresponding to: (A) 'no cleavage'; (B) 'irregular cleavage'; (C) 'regular cleavage'. Embryos microinjected with anti-mouse IgG antibodies as controls developed normally, and reached the 4-cell stage (not shown) at the time when the mAb injected eggs were fixed for the immunostaining. These data indicate that spindle and asters growth is impaired when in living eggs constitutive hsp70 is blocked by anti-hsp70 and that anti-hsp70 antibodies partially or entirely block the constitutive hsp70 movement inside the cell, depending upon the concentration.

hsp70 Localization after Nocodazole Treatment

The question can then be asked whether constitutive hsp70 is able to move through the cell to the mitotic apparatus when spindle growth is prevented by some drug. To verify this possibility fertilized eggs were cultured in sea water containing 20 μg/ml nocodazole, which is known to be a spindle growth inhibitor. The development of the treated eggs stopped at the one cell stage, while 2 h after fertilization the control embryos cultured without nocodazole reached the 8-cell stage (not shown). In the treated eggs the constitutive hsp70 visualized by antibody staining reaction is spread throughout the cell without any particular localization (Fig. 4), but it seemed slightly more concentrated in the cell center around the nucleus. This result shows a correlation between the lack of hsp70 movement inside the cell and impairment of mitotic apparatus formation.

DISCUSSION

We studied constitutive hsp70 localization in whole sea urchin embryos from fertilization throughout early cleavage to identify a possible role as molecular chaperone during cell division. Indeed, some other studies have localized constitutive hsp70 in mammalian centrosomes (40, 42) or showed its association with the centrin in *Xenopus* oocyte centrosomes (43) but its role was not essential to cell division and its function not known. The cell division length is different among different cell types and above all in embryonic cells. In *P.*

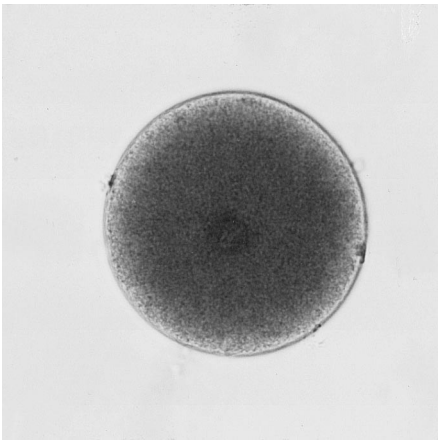


FIG. 4. Immunolocalization of constitutive hsp70 in nocodazole-treated embryos from 30 min fertilization on. Immunophosphatase staining is spread through the cell and is slightly condensed around the nucleus. Nocodazole blocks cell division and the embryos do not develop.

lividus embryos the cellular divisions proceed at a very fast rate, every 30 minutes during cleavage except for the first division which takes 1 h. Soon after fertilization constitutive hsp70 appears spread in the whole fertilized egg. Immunostaining by monoclonal antibody against the most conserved part of hsp70 indeed is able to detect the hsp70 present within the cells, while by contrast control embryos appear unstained when the same antibody was omitted in the immuno-reaction step. From 20 minutes on it can be seen that a sensible portion of the hsp70 appears concentrated around the nucleus, then in two poles coinciding later with the astral spindle poles. This is highlighted by hsp70 immunostaining, that indicates that some amount of constitutive hsp70 is associated with the mitotic apparatus during mitosis or is near the nucleus when it is again reassembled. It may be inferred that centrosomes collect an amount of hsp70 which is localized through the cell according to the cell cycle. Also, during early cleavage, a part of hsp70 is again clearly concentrated either in the mitotic structure or near the nucleus, while the remaining hsp70 is spread in the whole cell. To better visualize hsp70 localization when the embryonic cells become superimposed in a optical observation, we show figures of partially or totally dissociated embryonic cells. The results indicate that hsp70 may have a function in sea urchin embryo mitosis although in other species this has not been shown yet.

To test the hypothesis of such a function in sea urchins we have *in vivo* blocked constitutive hsp70 function by microinjecting anti-hsp70 antibody into the eggs. After fertilization we obtained either a block of cellular divisions or irregular segmentations of most of the microinjected eggs depending on the antibody concentration used. The block of hsp70 by anti-hsp70 antibodies interferes with mitosis and with a correct embryonic development too. This result is in contrast with that reported for cultured mammalian cells in which survival is not impaired for at least 36 h after anti-hsp70 antibody microinjections (6). One explanation of this different behavior may reside in the fact that a developmental system is a special cell system, assembling and disassembling mitotic apparatus in a very short time compared to that of most cell systems.

The hsp70 localization in developing sea urchin eggs containing microinjected *in vivo* anti-hsp70 is altered respect to that observed in normal developing fertilized eggs. Three images of hsp70 localization patterns are found which show the possible developmental consequences of microinjected antibodies. The percentage of each image has been found to be the same as those presented in Table I. In the first case hsp70 is diffused throughout the egg with some of it concentrated in variable peripheral regions, the percentage of this localization corresponds to those for 'no cleavage' of Table I, when the consequences of antibody microinjection was a complete block of cell division. In a second case hsp70 is concentrated in incomplete asters irregularly positioned; the percentage corresponds to 'irregular cleavage' of Table I. In the third case hsp70 was again found throughout the cell but with a light concentration in correspondence of the asters; the percentage corresponds to 'regular cleavage' of Table I in which cell divisions appeared normal. These figures suggest a function of hsp70 in the mitotic structure. It may be supposed that the hsp70 stabilizes some proteins constitutive of the mitotic apparatus. The results indicate that if hsp70 is blocked, there is some difficult for the mitotic apparatus to achieve its regular structure and the right position in the cell, probably because most of hsp70 cannot exert its chaperone function in stabilizing the arising mitotic structures. When we prevented microtubule nucleation and mitotic apparatus formation, arresting the cell cycle and cleavage by nocodazole administration, the hsp70 localization appeared dispersed in the cell with some of it concentrated around the nucleus. In this case the hsp70 is not blocked, nevertheless, that part of constitutive hsp70 which follows in the cell cycle mitotic apparatus formation during cell cycle does not move within the cell. The appropriate hsp70 localization therefore depends on the presence and formation of the mitotic apparatus.

ACKNOWLEDGMENTS

We thank G. Morici for manuscript editing. This work was supported by grants of I. Murst (60% and 40%), CNR Stress Proteins 97.04810.ST74 and 96.04990.ST74, and by Regione Siciliana.

REFERENCES

1. Ellis, R. J., and Van der Vies, S. M. (1991) *Annu. Rev. Biochem.* **60,** 321–347.
2. Hendrick, J. P., and Hartl, F. U. (1993) *Annu. Rev. Biochem.* **62,** 349–384.

3. Rassow, J., von Ahsen, O., Bomer, U., and Pfanner, N. (1997) *Trends Cell Biol.* **7,** 129–133.
4. Lindquist, S., and Craig, E. A. (1988) *Annu. Rev. Genet.* **22,** 631–677.
5. Morimoto, R. I., Tissieres, A., and Georgopoulos, G. (1990) Stress Proteins in Biology and Medicine. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
6. Riabowol, K. T., Mizzen, L. A., and Welch, W. J. (1988) *Science.* **242,** 433–436.
7. Palleros, D. R., Welch, W. J., and Fink, A. L. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5719–5723.
8. Parsell, D. A., and Lindquist, S. (1993) *Annu. Rev. Genet.* **27,** 437–496.
9. Velazquez, J. M., and Lindquist, S. (1984) *Cell.* **36,** 655–662.
10. Welch, W. J., and Feramisco, J. R. (1984) *J. Biol. Chem.* **259,** 4501–4513.
11. Welch, W. J., and Suhan, J. P. (1985) *J. Cell Biol.* **101,** 1198–1211.
12. Miffin, L. C., and Cohen, R. E. (1994) *J. Biol. Chem.* **269,** 15710–15717.
13. Stege, G. J. J., Li, L., Kampinga, H., Konings, A. W., and Li, G. C. (1994) *Exp. Cell. Res.* **214,** 279–284.
14. Kuwajima, K. (1996) *FASEB J.* **10,** 102–109.
15. Gething, M. J., and Sambrook, J. (1992) *Nature* **355,** 33–45.
16. Bukau, B., and Horwich, A. L. (1998) *Cell* **92,** 351–366.
17. Chirico, W. J., Waters, M. G., and Blobel, G. (1988) *Nature* **332,** 805–810.
18. Deshaies, R. J., Kock, B. D., Werner-Washburne, M., Craig, E. A., and Sheckman, R. (1988) *Nature* **332,** 800–805.
19. Beckmann, R. P., Mizzen, L. A., and Welch, W. J. (1990) *Science* **248,** 850–854.
20. Panzner, S., Dreier, L., Hartmann, E., Kostka, S., and Rapoport, T. A. (1995) Cell **81,** 561–570.
21. Hartl, F. U., Hlodan, R., and Langer, T. (1994) *Trends Biochem. Sci.* **19,** 20–25.
22. Stuart, R., Cyr, D. M., Craig, E. A., and Neupert, W. (1994) *Trends Biochem. Sci.* **19,** 87–92.
23. DeLuca-Flaherty, C., McKay, D. B., Parham, P., and Hill, B. L. (1990) *Cell* **62,** 875–887.
24. Chiang, H.-L., Terlecky, S. R., Plant, C. P., and Dice, J. F. (1989) *Science* **246,** 382–385.
25. Nelson, R. J., Ziegelhoffer, T., Nicolet, C., Werner-Washburne, M., and Craig, E. A. (1993) *Cell* **71,** 97–105.
26. Frydman, J., Nimmesgern, E., Ohtsuka, K., and Hartl, U. (1994) *Nature* **370,** 111–117.
27. Gebauer, M., Zeiner, M., and Gehring, U. (1997) *FEBS Lett.* **417,** 109–113.
28. Flaherty, K. M., DeLuca-Flaherty, C., and McKay, D. B. (1990) *Nature* **246,** 623–628.
29. Zhu, X., Zhao, X., Burkholder, W. F., Gragerov, A., Ogata, C. M., Gottesman, M. E., and Hendrickon, W. A. (1996) *Science* **272,** 1606–1614.
30. Rudiger, S., Buchberger, A., and Bukau, B. (1997) *Nature Struct. Biol.* **4,** 342–349.
31. Freeman, B. C., Myers, M. P., Schumacher, R., and Morimoto, R. I. (1995) *EMBO J.* **14,** 2281–2292.
32. Minami,Y., Hohfeld, J., Ohtsuka, K., and Hartl, F. U. (1996) *J. Biol. Chem.* **271,** 19617–19624.
33. Palter, K. B., Watanabe, M., Stinson, L., Mahowald, A. P., and Craig, E. A. (1986) *Mol. Cell Biol.* **6,** 1187–1203.
34. Sconzo, G., Amore, G., Capra, G., Giudice, G., Cascino, D., and Ghersi, G. (1997) *Biochem. Biophys. Res. Commun.* **234,** 24–29.
35. Herberts, C., Moreau, N., and Angelier, N. (1993) *Int. J. Dev. Biol.* **37,** 397–406.
36. Bevilacqua, A., Fiorenza, M. T., and Mangia, F. (1997) *Nucleic Acids Res.* **25,** 1333–1338.
37. Sconzo, G., Scardina, G., and Ferraro, M. G. (1992) *Gene* **121,** 353–358.
38. Palla, F., Bonura, C., Anello, L., Di Gaetano, L., and Spinelli, G. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 12322–12326.
39. Berg, L. K., Chen, S. W., and Wessel, G. M. (1996) *Development* **122,** 703–713.
40. Rattner, J. B. (1991) *Exp. Cell Res.* **195,** 110–113.
41. Brown, C. R., Doxsey, S. J., Hong-Brown, L. Q., Martin, R. L., and Welch, W. J. (1996) *J. Biol. Chem.* **271,** 824–832.
42. Uzawa, M., Grams, J., Madden, B., Toft, D., and Salisbury, J. L. (1995) *Dev. Biol.* **171,** 51–59.