PRO EXPERIMENTIS

Is there really actin around *Xenopus laevis* yolk platelets?[1]

R. Colombo

Laboratory of Cell Biology, Institute of Zoology, University of Milano, via Celoria 10, I-20133 Milano (Italy), 19 June 1981

Summary. Our previous immunofluorescence experiments (1) on actin localization in Xenopus development showed a fluorescent halo around Xenopus yolk platelets. This fact suggests the presence of a sort of actin covering of the yolk platelet; we have called this structure the 'actin-shell'. In this work, by the use of a DNase I-fluorochrome complex, we were able clearly to demonstrate the presence of the actin-shell around Xenopus yolk platelets. A proposal about the function of the actin-shell is made; its presence could mark the difference between autosynthetic and heterosynthetic eggs.

In a previous work[2] we described the presence of an actin-shell around the yolk platelets of *Xenopus laevis* eggs.
This structure was demonstrated by means of an indirect immunofluorescence technique, using an anti-Xenopus muscle actin rabbit serum. In spite of careful testing of serum specificity, some doubts remain as to whether or not undetected cross-reactions to structural proteins were present. In order to eliminate this ambiguity, we decided to formulate a technique to detect actin (and only actin) in cytoplasmic structures. Our approach was based on the particular affinity between actin and DNase I. The 1st reports on this interrelation were in 1967, with the Lindberg's findings regarding the presence of a cytoplasmic inhibitor of the enzyme in calf spleen cells[3]; they culminated in 1974 when Lazarides and Lindberg identified actin as the inhibitor[4]. We used their results as the theoretical basis for a practical method of cytoplasmic actin localization.
Lyophilized DNase I from bovine pancreas (Boehringer) was dissolved in cold phosphate-bicarbonate buffer (7 vol. phosphate buffer 0.01 M pH 7.2+3 vol. of 0.5 sodium bicarbonate solution pH 9.0) at a concentration of 10–15 mg/ml. The solution was then placed in the cold room, and magnetically stirred until completely cooled.
At this point, 0.5 mg of fluorescein isothiocyanate (FITC), were added per 30 mg of protein, and then the coupling mixture was left overnight in the cold room, under gentle magnetic stirring. The aforementioned fluorchrome/protein ratio proved to be the best way to prevent overlabeling. Unreacted FITC and small molecule derivates of FITC were easily removed by gel filtration through a Sephadex G 25 column.

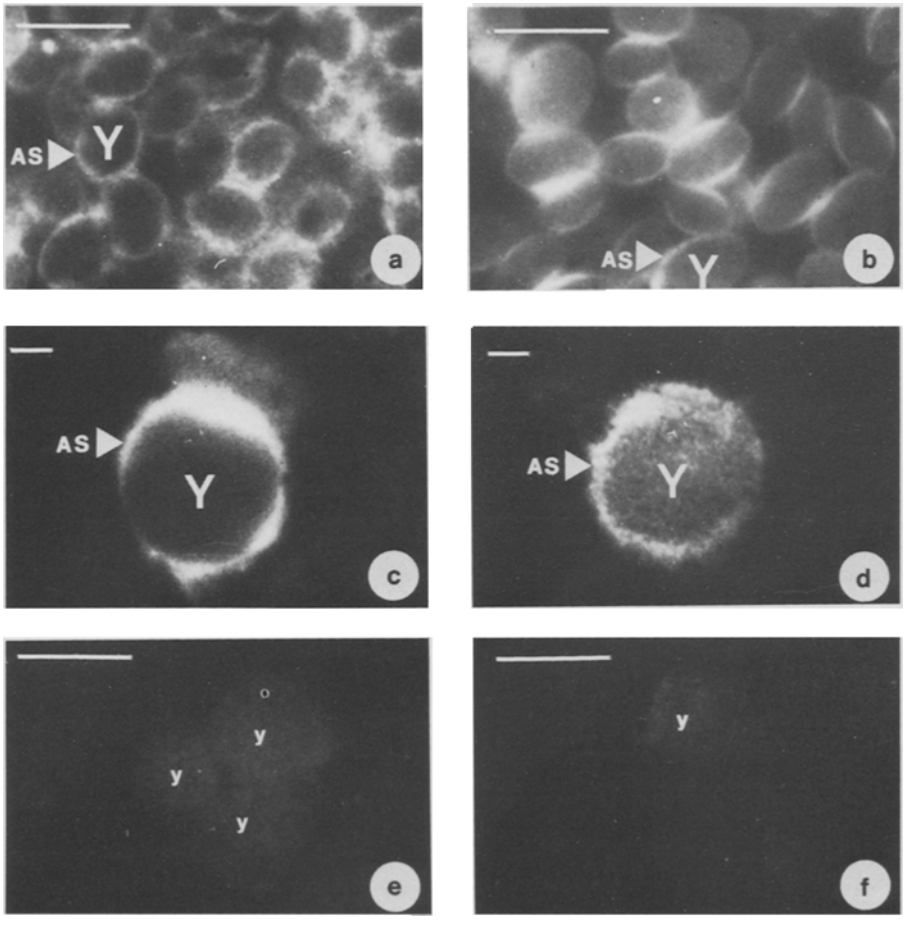

Figure 1. UV-light microphotographs: (Y) yolk platelet, (AS) actin-shell. *a* Detail of unfertilized Xenopus egg deutoplasm. Histological section obtained by the Sainte-Marie method; sample treated with anti-Xenopus muscle actin rabbit serum (AXMA) and fluorescent labeled anti-Rabbit γ-globulins serum (ARG). Scale bar 5 µm. *b* Detail of unfertilized Xenopus egg deutoplasm. Histological section obtained by Sainte-Marie method. Sample treated with DNase I-FITC complex (DFC). Scale bar 5 µm. *c* Isolated Xenopus yolk platelet. Sample treated with AXMA and ARG. Scale bar 1 µm. *d* Isolated Xenopus yolk platelet. Sample treated with DFG. Scale bar 1 µm. *e* Isolated Xenopus yolk platelets. Sample treated with unconjugated DNase I and then with DFC. Scale bar 5 µm. *f* Isolated Xenopus yolk platelet. Sample treated with DFC and then washed with guanidine-HCl 3 M; scale bar 5 µm.

About 10 g of Sephadex G 25 was suspended in water and washed by repeated sedimentation and decanting. The slurry was poured into a column, allowed to pack by gravity, and equilibrated with phosphate buffer 0.1 M pH 7.2.

The fluorochrome-protein reaction mixture was then poured into the column; the conjugated protein was quickly separated from unreacted dye and then easily collected.

De-jelled unfertilized eggs of *Xenopus laevis* were prepared for fluorescence technique exposure by Sainte-Marie's method[5]. Likewise, Xenopus yolk platelets were isolated as previously described[2].

When these experimental samples were exposed to DNase I-FITC treatment (30 min at 37 °C), the histological pictures obtained were very similar to those revealed using anti-Xenopus muscle actin antibodies (fig. 1, a–d).

Isolated yolk platelets, previously treated with unconjugated DNase I, remained unstained after DNase I-FITC supply (fig. 1, e). This fact can be explained by the complete saturation of actin by means of FITC-free DNase I.

Furthermore when the yolk platelets were exposed to DNase I-FITC treatment and then washed with guanidine-HCl 3 M solution, the same dark result was obtained (fig. 1, f). It is a well known fact that guanidine-HCl concentrations higher than 2 M are deleterious to the inhibiting activity of actin[6].

The DNase I-Fluorochrome technique was first used by Wang and Goldberg[7] with unsatisfactory results. That their experiments failed to produce definite results was due, in our opinion, to the following factors:

1. In their experiments, Wang and Goldberg used tetramethylrhodamine isothiocyanate (MRITC). This fluorochrome has a lower titer of staining than FITC[8].
2. When an enzyme (the DNase I in our case) is conjugated with a fluorochrome, it loses a part of its activity[9,10]. If we immobilize an excess of muscle actin on a CNBr activated Sepharose column and then pour the DNase I-FITC complex into the column, only 25% of the coupled enzyme stays bound to the resin (Colombo, unpublished data). Hence, if there is a decrease in DNase affinity for the actin, it is very important to use a fluorochrome with a high titer of staining.
3. MRITC dissociates easily from the conjugate, resulting in nonspecifically localized fluorescence.

Our experiments demonstrated that a DNase I-FITC conjugate is sufficient to detect cytoplasmic actin localization.

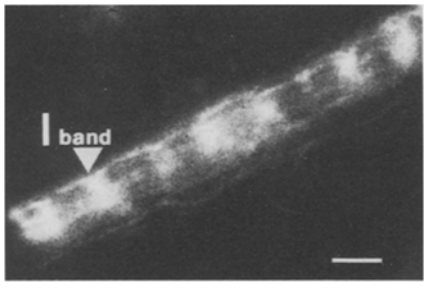

Figure 2. UV-light microphotograph. Rabbit myofibril isolated from stretched and glycerinated limb muscle by the method of Sobieszeck and Bremel[11]. Sample treated with DNase I-FITC complex. Scale bar 4 µm.

In order to emphasize the specificity of DNase I-FITC in actin location, we tested the conjugate on an actin-containing structure whose actin filament arrangement is well known, i.e. a skeletal muscle myofibril. When a myofibril from a stretched and glycerinated skeletal muscle fiber is treated with DNase I-FITC, a characteristic fluorescent series of bands appears (fig. 2). The size of each fluorescent band roughly corresponds to that normally found in an I muscle band.

The recognition of cytoplasmic actin by means of the DNase I-FITC method is thus a rapid, specific and inexpensive technique, by which we have demonstrated the presence of an 'actin-shell' around the yolk platelets of *Xenopus laevis* eggs.

Since the oocyte uses a receptor-mediated endocytosis as a mechanism for cellular yolk uptake[12], we have previously suggested[2] that the actin-shell could be an indication of how the oocyte yolk uptake process took place. In particular we have suggested that the actin microfilaments of the cell plasmalemma might facilitate the formation of coated pits and coated vesicles and/or the clustering of receptors in the cell's external membrane.

Then, when the yolk uptake process is complete, actin microfilaments should remain on the external surface of the yolk platelet membrane, where they can be detected.

The recent publication of Salisbury et al.[13], showing the close relationship between the clathrin-coated vesicles and the actin microfilament system of the cell cortex, in a human B lymphoblastoid cell line, heightens the probability of our 1st hypothesis.

Indirect immunofluorescence experiments made on *Ciona* eggs (Colombo, unpublished data), autosynthetic in yolk formation, according to Anderson[14], showed that this animal's yolk platelets have no actin-shell. This gives us an indication of the possible biological function of the actin around the yolk platelets; eggs which get their yolk from an external environment would have an actin-shell; autosynthetic eggs, on the other hand, would not. This suggests a means to distinguish between the 2 different egg types.

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