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Andrea Delpino, Ornella Senatori, Raffaele Scopelliti, Harry Maneli

Dissociation behaviour of monomers from Bufo bufo unfertilized eggs and tailbud-stage embryos

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Embriologia e morfogenesi. — *Dissociation behaviour of monomers from* Bufo bufo *unfertilized eggs and tailbud-stage embryos* (*). Nota di ANDREA DELPINO (**), ORNELLA SENATORI (***), RAFFAELE SCOPELLITI (***) e HARRY MANELLI (***), presentata (****) dai Soci S. RANZI e A. STEFANELLI.

RIASSUNTO. — I monomeri 80 S isolati da uova vergini e da embrioni allo stadio di bottone caudale di *Bufo bufo* sono stati solubilizzati in tamponi contenenti una quantità fissa di $MgCl_2$ (2,5 mM) e quantità crescenti di KCl. Il grado di dissociazione indotta dal sempre più elevato rapporto K^+/Mg^{++} è stato valutato mediante analisi dei profili di sedimentazione in gradienti di saccarosio.

È stato osservato che i monomeri ottenuti dalle uova vergini sono pressocchè interamente composti da ribosomi « fuori ciclo », mentre i monomeri ottenuti dagli embrioni sono costituiti in parte da ribosomi « fuori ciclo » ed in parte da ribosomi impegnati nella traduzione del messaggero (monosomi).

Si è osservato, inoltre, che sia nelle preparazioni di monomeri ottenute dalle uova vergini sia nelle preparazioni di monomeri ottenute dagli embrioni, la totalità dei ribosomi « fuori ciclo » risulta dissociata nelle due subunità costitutive quando il rapporto K^+/Mg^{++} del mezzo viene elevato ad 80.

Per quanto riguarda la resistenza alla dissociazione, pertanto, non si apprezza alcuna differenza tra i monomeri « fuori ciclo » ottenuti dalle uova vergini ed i corrispondenti monomeri « fuori ciclo » ottenuti dagli embrioni.

In order to characterize the functional state of ribosomes from unfertilized eggs in respect to that of ribosomes from developing embryos, much attention has been paid to the comparative evaluation of their synthetic activity in cell-free systems (Vittorelli *et al.*, 1969; Clegg and Denny, 1974; Van Der Saag *et al.*, 1976). However, the data obtained from these experiments are conflicting, and the problem is still open.

An alternative approach has been followed by Maggio *et al.*, 1968, who have studied the dissociation behaviour of monomers from unfertilized eggs and from embryos of sea urchin when dialyzed against low-Mg^{++} solutions. These authors have reported that monomers from unfertilized eggs resist salt-promoted dissociation better than monomers from embryos; they have also speculated that this stronger " stickiness " between the two constitutive subunits of unfertilized egg ribosomes may be the cause of their functional restriction.

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(**) Biophysical Laboratory Regina Elena Institute for Cancer Research, Rome, Italy.
(***) Institute of Zoology, University of Rome, Italy.
(****) Nella seduta del 9 febbraio 1980.

In our researches concerning the characterization of *Bufo bufo* ribosomes during embryonic development we have determined that monomers—as well as their constituent subunits—from unfertilized eggs, from developing embryos and from adult tissues display the same CsCl buoyant density, indicative of an equal relative protein content (Senatori *et al.*, 1979). Furthermore, we have determined that monomers from *Bufo bufo* unfertilized eggs are not impaired in their synthetic activity, when tested in poly-U programmed cell-free systems (Delpino *et al.*, 1980).

In this context, it has appeared to be of interest to study whether in *Bufo bufo* the monomers from unfertilized eggs and from developing embryos display also the same resistence towards the salt-promoted dissociation, or whether some difference exists between them in this respect, and likewise the situation described in sea urchin.

Monomers from unfertilized eggs and from tailbud–stage embryos of *Bufo bufo* were used. It is to be pointed out that, as we have previously reported (Scopelliti *et al.*, 1978), in *Bufo bufo* the protein synthesis rate is severely depressed in unfertilized eggs, while in the tailbud–stage embryos an active recycling of the ribosomes on messenger RNA is taking place.

Particles have been suspended in buffers containing a fixed amount of $MgCl_2$ (2.5 mM) and variable amounts of KCl (from 100 to 250 mM, as indicated at the top of each panel in Fig. 1). After a brief incubation at 4 °C, samples were centrifuged onto sucrose gradients, made under the same ionic conditions as the material they were layered onto, and sedimentation profiles were recorded (Fig. 1).

At the lower KCl concentration tested (100 mM), both monomers from unfertilized eggs (panel A) and monomers from tailbud–stage embryos (panel E) sediment almost exclusively as a single peak at the 80 S position. When the KCl concentration is increased to 150 mM some of the particles dissociate into 40 S and 60 S subunits: the 80 S peak almost disappears in the case of unfertilized egg monomers (panel B), and is reduced to about half in the case of monomers from tailbud-stage embryos (panel F). It is remarkable that, under these ionic conditions, in both samples the profile of the peak corresponding to the small ribosome subunit is markedly irregular, with a double peak sometimes evident. When monomers are exposed to 200 mM KCl the amount of particles dissociated into subunits still increases for both egg (panel C) and embryo (panel G) monomers. At this KCl value the dissociation attains its maximum for both kinds of monomers; in fact no further dissociation occurs if the KCl concentration is raised to 250 mM (panels D and H, respectively) or even further (not shown).

Two considerations can be made concerning the above data.

The first is that monomers from unfertilized eggs and from tailbud-stage embryos include different percentages of salt-stable and salt-dissociable particles. As stated by Falvey and Staehelin, 1970, the unprogrammed ribosomes dissociate into their constituent subunits when exposed to high concentrations

of monovalent ions, while translating ribosomes, engaged with template and carrying a peptidyl-tRNA, are stable under the same conditions. Consequently, it can be observed that the monomers from unfertilized eggs are almost entirely constituted by unprogrammed, salt-dissociable, particles (see panel D); by contrast only about 50 % of the monomers from embryos are in this situation, the remainder being constituted by true, salt indissociable, monosomes (see panel H).

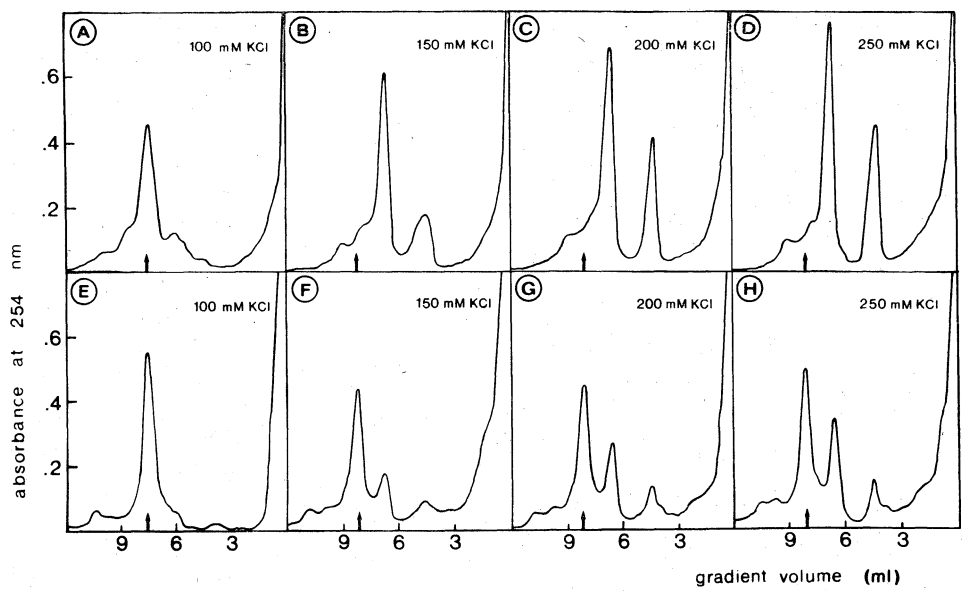

Fig. 1.

Monomers from unfertilized eggs and from tailbud-stage embryos of *Bufo bufo* were obtained directly by centrifuging the detergent-treated postmitochondrial supernatant onto linear 15 to 40 % sucrose gradients, (containing also 50 mM, Tris-HCl pH 7.6 25 mM KCl, 5 mM $MgCl_2$) as previously described (5,6). Monomers were resuspended in buffers of increasing ionic strength as described in the text, and centrifuged onto linear 15 to 40% sucrose gradients containing the same ionic conditions as the material layered onto. Centrifugation was carried out in the Spinco rotor SW 41, at 40.000 rpm, for 4 hours, at 4 °C. Direction of sedimentation is from right to left. The gradients were displaced from the bottom of the tube and continuously monitored at 254 nm in the flow cell of an ISCO model 640 apparatus. The arrows indicate the position of the 80 S peak.

PANELS A to D: monomers from unfertilized eggs.

PANELS E to H: monomers from tailbud-stage embryos.

The second consideration is that the unprogrammed ribosomes from eggs and the corresponding unprogrammed ribosomes from embryos are equally sensitive to salt dissociation. In both samples the greatest part of salt-labile monomers already dissociates at 150 mM KCl, but the subunits arising from dissociation, particularly the small ones, sediment as broad peaks, indicating

that some intermediate forms are present. When the K^+/Mg^{++} ratio is elevated to 80, both in eggs and in embryos, all labile particles are dissociated, and the ribosomal subunits generated by the dissociation sediment as symmetrical peaks, at the expected 40 S and 60 S positions.

The fact that a difference in the strength of subunit association in unprogrammed ribosomes could not be detected in our experiments confirms that ribosomes from unfertilized eggs of *Bufo bufo* are fully comparable with the functionally correspondent ribosomes obtained from developing embryos. Therefore, no special inhibitory mechanisms seem to be operative in *Bufo bufo* ribosomes during embryonic development.

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