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Protein synthesis in different regions of Xenopus laevis embryo

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Embriologia e morfogenesi. — *Protein synthesis in different regions of* Xenopus laevis *embryo* (*). Nota di FIORENZA DE BERNARDI, presentata (**) dal Socio S. RANZI.

RIASSUNTO. — Embrioni di *X. laevis* sono stati tagliati separando la regione dorsale da quella ventrale dallo stadio di gastrula fino allo stadio di neurula. Gli RNA estratti dalle regioni isolate sono stati separati su oligo (dT) cellulosa in poliadenilati e nonpoliadenilati e tradotti *in vitro*. Il rapporto tra radioattività incorporata nei prodotti di traduzione ottenuti rispettivamente con mRNA poliadenilati e nonpoliadenilati suggerisce che nella regione dorsale la maggior parte delle proteine viene sintetizzata su mRNA poliadenilati in tutti gli stadi considerati, mentre, nella regione ventrale, sembra che la quota maggiore di sintesi proteica avvenga sullo stampo di messaggeri non poliadenilati fino allo stadio di neurula precoce; nella neurula tardiva sembra aumentare l'attività degli mRNA poliadenilati. La presenza e la traducibilità degli mRNA poliadenilati sembrano quindi essere in rapporto con l'incremento della sintesi proteica e con l'inizio del differenziamento morfologico, che iniziano rispettivamente allo stadio di gastrula nella regione dorsale e allo stadio di neurula nella regione ventrale.

During the early development of the amphibian embryo, the cells initiate distinct programmes of gene expression as they follow a distinct spatial pattern of differentiation. Among the informational macromolecules operating during the early development to bring about the terminal differentiation, particular interest has been given to messenger RNA, as the first product of the gene activation (Mohun *et al.*, 1984).

One approach to the analysis of regionally localized RNA molecules is to dissect early embryos and to look for qualitative and quantitative differences in their distribution within the different portions of the embryos.

The stages between gastrulation and neural groove were chosen and dissected in dorsal and ventral region because during these few hours of development the most important organs are determined. The objective was to reveal the different translation efficiencies of polyadenylated mRNAs in the dorsal and the ventral regions.

(*) Ricerche eseguite nel Dipartimento di Biologia dell'Università Statale di Milano con un contributo del C.N.R.

(**) Nella seduta dell'8 marzo 1986.

Materials and methods

Xenopus laevis eggs were obtained, reared, and the dorsal and ventral regions were separated as described (De Bernardi, 1984). RNAs from the separated regions were extracted in 0.1 M Tris-HCl pH 9,1 mM EDTA, 0.1% SDS buffer (Brawerman, 1974), deproteinized by phenol-chloroform and loaded onto an oligo (dT) cellulose column. Polyadenylated and nonpolyadenylated RNAs were eluted and translated in vitro by nuclease-treated rabbit reticulocyte lysate with ^{35}S-methionine as label. The translation products were processed for TCA-precipitable cpm count and for electrophoresis on 0.1% SDS-10% polyacrylamide gel.

Results and discussion

The counts per minute listed in Table I showed a stepwise increase of the in vitro translation products directed by the RNAs extracted from the gastrula to neural groove stage. The overall increase was about 2 in the dorsal and about 1.6 in the ventral region.

Table I.

Cpm of TCA-*precipitable radioactivity from 1* µl *of the* in vitro *translation products directed by polyadenylated or nonpolyadenilated* RNAs *extracted from the dorsal and ventral region of* X. laevis *embryos* (*stages from Nieuwkoop and Faber, 1956*)

Stage	Dorsal region		Ventral region	
	poly (A) $^+$RNA directed cpm	poly (A) $^-$RNA directed cpm	poly (A) $^+$RNA directed cpm	poly (A) $^-$RNA directed cpm
Early gastrula	28,206	27,671	23,828	24,640
Neural plate	39,956	39,910	29,116	34,643
Neural folds	46,224	35,904	32,729	46,468
Neural groove . . .	51,079	41,149	38,912	44,669
Endogenous (no added RNA)	7,860	—	—	—

Moreover in the dorsal region more counts per minute were generally incorporated by translation of the polyadenylated RNAs than by translation of the nonpolyadenylated RNAs.

From the data in Table I the translation ratio for the two types of mRNAs seems to be similar in the dorsal and ventral regions at the gastrula stage, and

it seems to be very near to 1. During the neurula stage, however, the ratios are clearly more than 1 for the dorsal and less than 1 for the ventral region (fig. 1).

This means that in the dorsal region of the embryo during the postgastrula stages the bulk of protein synthesis is supported by polyadenylated mRNAs.

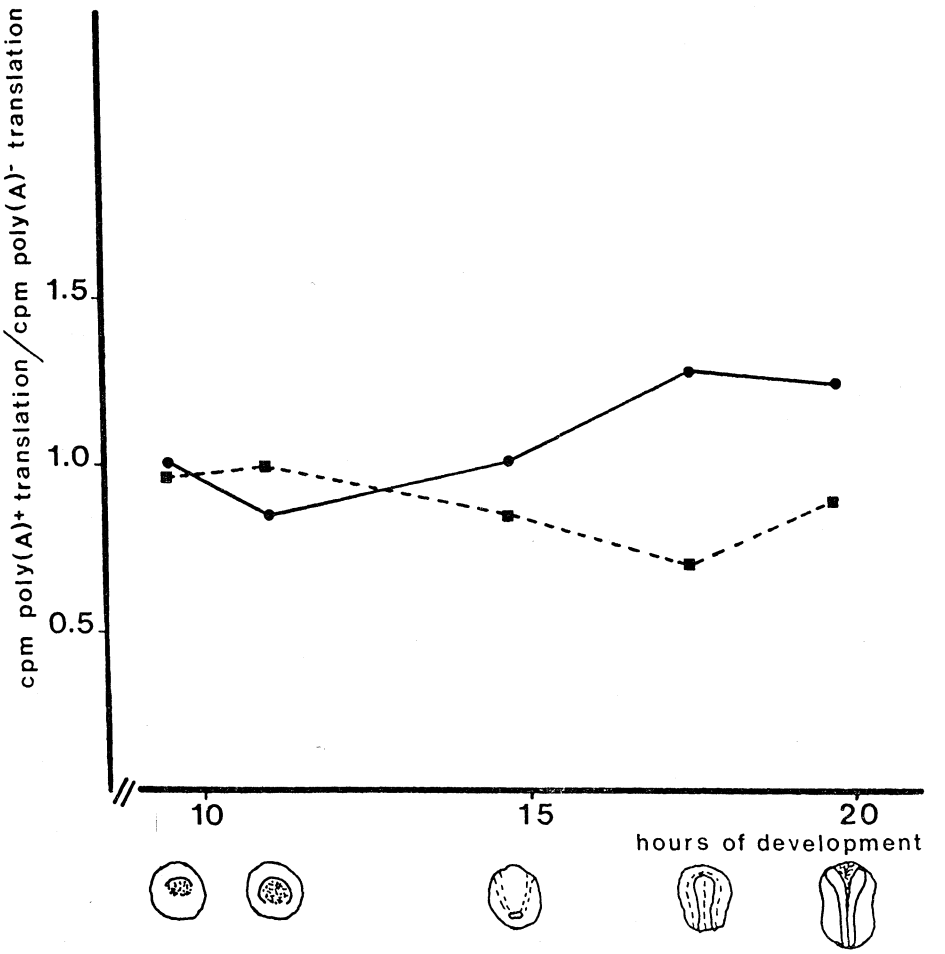

Fig. 1. – Ratios of the in vitro translation of polyadenylated and nonpolyadenylated RNAs. Continuous line = dorsal region; dotted line = ventral region.

On the contrary in the ventral region more radioactivity was incorporated by translation of nonpolyadenylated mRNAs extracted from the neurula stages, thus suggesting a higher translational efficiency of the nonpolyadenylated mRNAs.

In previous studies we compared the rates of protein synthesis in the dorsal and ventral regions of *Xenopus laevis* embryos in postgastrula stages and we reported that the mRNA codifying for myosin heavy chain is detectable in

the dorsal region, just at the end of the gastrulation and it seems to be under transcriptional control (De Bernardi, 1982).

The data here discussed suggest that the presence and the translability of polyadenylated mRNAs have to be correlated with the time of increase of protein synthesis and with the morphological differentiation, both detectable in the dorsal region earlier than in the ventral one.

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