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On the induction of somites in chick embryo

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SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Zoologia. — *On the induction of somites in chick embryo* (*). Nota di ANNAMARIA BOLZERN, MARISA CIGADA LEONARDI, FIORENZA DE BERNARDI, ROSALBA MACI e SILVIO RANZI, presentata (**) dal Socio S. RANZI.

RIASSUNTO. — I somiti non si differenziano nell'espianto postnodale del blastoderma di pollo coltivato *in vitro* col metodo di New. Somiti si differenziano invece se al liquido di allevamento è aggiunto mRNA di miosina 26S estratto da muscoli di embrioni di pollo di 14 giorni. Lo stesso risultato si ottiene se il pezzo postnodale è coltivato in un pool di mRNA di muscolatura. Somiti sono anche indotti se al liquido di allevamento si aggiunge miosina. L'aggiunta di puromicina impedisce l'induzione di somiti per azione di mRNA, ma non quella per azione di miosina. Si deduce che per l'induzione è necessaria la traduzione mRNA–miosina. Il pool di messaggeri di fegato ovvero di rene di pollo è senza azione. Attivo invece è il mRNA di miosina di coniglio.

L'actina in forma F ovvero G non induce. Nel blastoderma, allo stadio di linea primitiva, è presente, secondo Ebert (1955), miosina. Possiamo confermare questo dato e di conseguenza pensiamo che sia proprio questa miosina il normale induttore dei somiti. I somiti così formati necessitano di un secondo induttore per il normale sviluppo, induttore che Packard e Jacobson hanno visto venire dagli organi assili.

The action of RNA—particularly of the messengers, mRNAs—in developmental processes is a current argument of research work. The study of the importance of mRNA during the development of primitive organs is very interesting. Recent researches identified the messenger ribonucleic acids which are able to direct the synthesis of typical proteins in differentiated tissues (Brawerman, 1974). Heywood and Nwagwu (1969) and Morris *et al.* (1972) were able to extract a 26S RNA from muscle cells coding for myosin synthesis in cell-free systems.

Niu (1964) summarizes some papers by himself and other research workers. In these researches the ribonucleic acids turned out to be able to direct embryonic differentiation; researches by Ranzi *et al.* (1963) are quoted; in these researches elements of striated muscle are induced by muscular ribonucleoprotein preparations and cells able to synthesize glycogen are induced by liver ribonucleoprotein in the chorio-allantois of chick embryo.

The purpose of this paper is to examine the importance of messenger RNA in inducing the rudiments of the embryonic organs.

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(**) Nella seduta del 15 giugno 1978.

Fertilized chick eggs (Ross–Italia) were incubated at 38.5 °C for 24 hours up to the primitive streak stage. Older embryos were discarded. The embryos at primitive streak stage were cultured *in vitro* following New's method (1955). Some embryos were cultured as a control. In the rest of the embryos the anterior part was excised to 0.6 mm from the node and the remaining part (postnodal piece) was cultured in Pannet-Compton solution and albumen (Fig. 1). In the Table I and in the figs. these explants are referred to as controls.

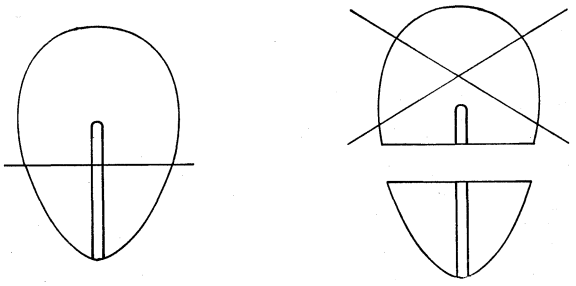

Fig. 1. – Explant of the postnodal piece.

Postnodal pieces, called treated, were cultivated in presence of mRNA. This mRNA (40–60 μg/ml in Pannet–Compton solution) was diluted 4–6 times in culture medium: albumen or Pannet–Compton solution both under and over the explant; consequently each explant received 10 μg of RNA.

TABLE I

	Explanted embryos	Surviving explants	Inductions	% Induction
Controls	83	56	0	0
myosin mRNA	126	86	32	37,2
mRNA pool from muscles	204	144	76	52,8
mRNA pool from liver	12	8	0	0
mRNA pool from kidney	9	6	0	0
rabbit myosin mRNA	8	6	4	66,7
soluble myosin	23	20	8	40,0
myosin mRNA + puromycin	30	9	0	0
myosin + puromycin	9	6	3	50,0
F actin	5	4	0	0
G actin	18	14	0	0

The first series of explants was treated with myosin 26S mRNA extracted from a leg muscle of 14-day-old chick embryo following the method of Heywood and Rourke (1974). The explants were kept at 38.5 °C. After twenty hours 37.2 % of treated surviving explants showed roundish mesodermic masses (Plate I, Figs 2, 3, 4). These masses were usually found to be arranged in a single series, one near the other; the number of masses differs in the different cases and varies from two or three to over ten, once up to twenty-one. These masses can be double-paired, never more than five pairs if the explants are not stretched crosswise but this seldom happens. These masses are larger than the normal somites of the control embryos.

The area of the sixth somite in the control embryo is $3281.3 \pm 331.0\ \mu^2$, and the area of induced mesodermic masses is $5625.0 \pm 885.1\ \mu^2$; the somite thickness of the control embryo is less than the thickness of the induced masses; consequently the volume of the induced masses is greater than that of the somites. In the crosswise stretched explants the induced mesodermic masses are laid out on the anterior edge of the explant.

In section (Plate I, Figs 5, 6) these masses appear to be a large somite. The cells appear stretched, converging toward the center, where mitosis is more frequent. This arrangement corresponds to the arrangement which can be observed in the normal somites. At the electron microscope the mesodermic stretched cells appear to be young myoblasts (Plate I, Fig. 7) like the normal myoblasts described by Shimada (1971) with numerous mitochondria and ribosomes in their basal region, distal in respect of the somite centre.

In a second series of experiments a pool of messengers from embryonic muscle was used. This pool was separated on a Millipore filter following the method of Brawerman (1974) or on Poly(U) Sepharose column. In this messengers of myosin and other muscular protein are present (Heywood and Rich, 1968). The result was the same as that obtained in the first experiment. The frequency of somite induction (52.8 %) seems to be greater.

Controls were set up in order to test the specificity of the reaction. To this purpose postnodal explants were treated with mRNA pool extracted from chick liver or kidney. No mesodermic masses appeared.

On the contrary, mRNA extracted from rabbit muscles was able to induce somites in postnodal explants of blastoderm of chick embryo.

Induced somites are stable for about 24 hours, after which time they degenerate and disappear; in some cases they remain like indifferentiated vesicles (Plate I, Fig. 8).

We can conclude that myosin mRNA induces the formation of myoblasts. These myoblasts aggregate to form somites. The processes of embryonic cell organisation are well-know (Moscona and Moscona, 1952; Trinkaus, 1969). Notochord and the ventral part of the neural tube are necessary for the further development of somites (Packard and Jacobson, 1976). The factor which comes from the rudiments is not present in our explants, consequently the somites induced in this way become small vesicles built by dedifferentiated cells.

This conclusion suggested testing the action of soluble myosin. Chick myosin was extracted following the method of Mommaerts and Parrish (1951) and placed in culture medium of postnodal explants (15–10 μg/ml); in these explants somites were induced.

We also wanted to test if the translation myosin mRNA → myosin is necessary to induce the somites. The translation can be blocked by puromycin. Postnodal explants were consequently treated with myosin mRNA (10 μg/ml) and puromycin (5 μg/ml). No mesodermic masses appeared.

In order to exclude the possibility that the puromycin blocks the induction of the somites we placed the explants in culture with myosin (15–10 μg/ml) and puromycin (5 μg/ml). We obtained 33 % induction. We can conclude that the myosin induces somites without a detectable new protein synthesis.

The induction obtained with the pool of muscle messengers raises the question of actin. The explants were cultivated in the presence of actin (monomeric or polymerized) (30 μg/ml). No induction was observed.

The presence of myosin already found by Ebert (1955) in chick blastoderm at primitive streak stage is confirmed by immunodiffusion research. We can consequently conclude that myosin is the inductor of the somites in normal development.

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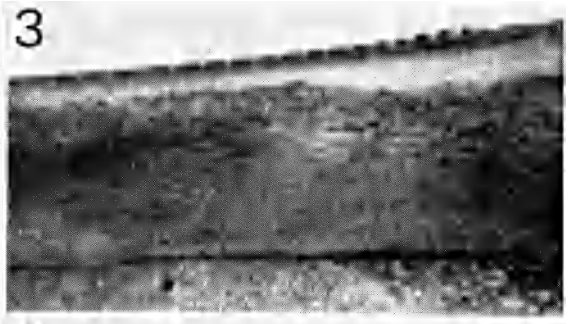
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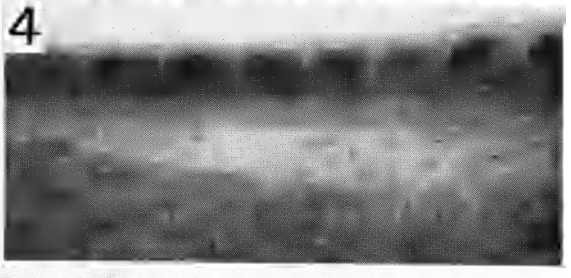
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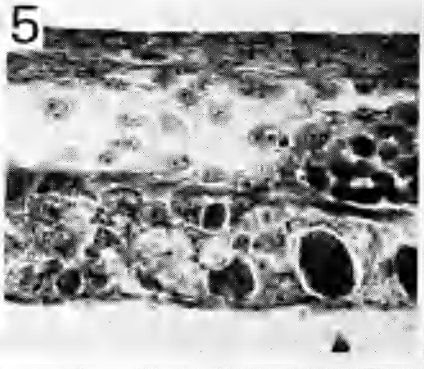
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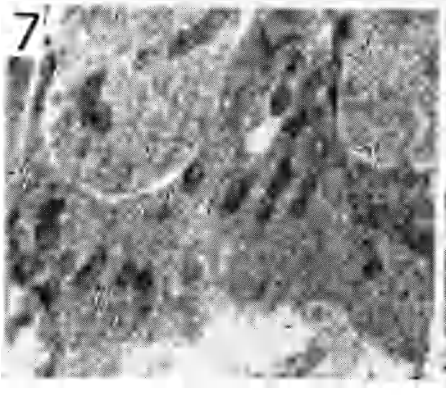
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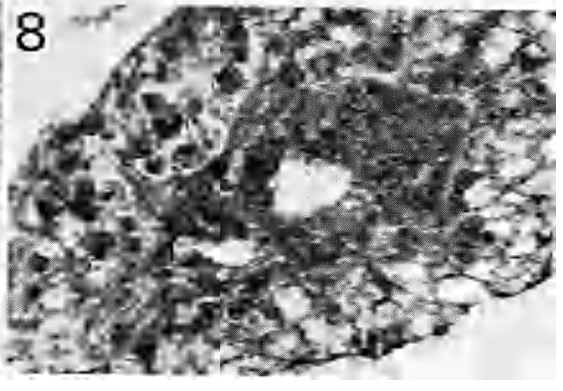
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EXPLANATION OF PLATE I

Fig. 2. – Control explant (after 18 hours' culture) (×8.5).

Fig. 3. – Postnodal explant cultured 18 hours in the presence of myosin mRNA (×8.5).

Fig. 4. – The same explants as in Fig. 3 (×40).

Fig. 5. – Transverse section of the control explant (after 20 hours' culture) (×370).

Fig. 6. – Transverse section of the induced somite (after 20 hours' culture in the presence of myosin mRNA) (×370).

Fig. 7. – Young myoblast of induced somite (after 17 hours in the presence of myosin mRNA (×9300).

Fig. 8. – Transverse section of degeneration of a somite induced by myosin mRNA (48 hours' culture in the presence of mRNA) (×370).