Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Marcello Cantini, Massimiliano Aloisi

In vitro differentiation of striated muscle from chick embryo iris epithelium

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8, Vol. **60** (1976), n.6, p. 859–863.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1976_8_60_6_859_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1976.

SEZIONE III

(Botanica, zoologia, fisiologia e patologia)

Biologia. — In vitro *differentiation of striated muscle from chick embryo iris epithelium* (*). Nota di MARCELLO CANTINI e MASSIMILIANO ALOISI, presentata (**) dal Socio M. ALOISI.

RIASSUNTO. — I muscoli intrinseci dell'iride negli Uccelli (come nei Rettili e in alcuni Anfibi) sono costituiti da fibre striate ed hanno una origine neuroepiteliale, che è comune a tutti i muscoli iridei delle altre classi di animali, ove esistono. Nel pollo, il primo abbozzo che si differenzia dalla lamina esterna del rivestimento epiteliale pigmentato dell'abbozzo irideo, si reperisce all'8° giorno di incubazione in forma di piccola cresta sporgente, ma ancora racchiusa dietro la membrana basale, in una zona dell'abbozzo immediatamente adiacente al margine pupillare. È sembrato interessante studiare la possibilità di coltivare materiale cellulare proveniente da questa zona per assistere alla eventuale differenziazione delle cellule epiteliali in cellule muscolari. Si è potuto infatti accertare che tale differenziazione è possibile *in vitro*, ma solo a partire dall'8° giorno di incubazione; essa peraltro non richiede che fossero già presenti mioblasti nel materiale espiantato, potendo assistere alla loro continua formazione dalla popolazione di cellule neuroepiteliali in crescita. I mioblasti e miotubi formati denunciano del resto la loro origine epiteliale in quanto contengono pigmento e per un certo tempo continuano a formarlo.

It is known that in Birds, as well as in Reptiles and in some Amphibia, the iris intrinsic muscles (sphincter and dilatator) are constituted by striated fibres very similar to those of skeletal muscles [5, 6, 7, 8, 9, 13]. The occasional appearance of single striated fibres among the majority of smooth muscle cells has been observed also in Mammals [3]. In all cases these iris intrinsic muscles are of epithelial origin in all species, and they differentiate from the outer layer of the iris pigmented epithelial anlage, starting from a region immediately adjacent to the pupillar border.

The first modification of the outer epithelial layer of the embryonic chick iris is seen at the 8th day of incubation [11] in the form of a small circular ridge of epithelial cells which protrudes externally into the mesenchymal part of the iris anlage. Its cells are still pigmented, though to a lesser extent than their mother cells. Soon after its formation the ridge grows through proliferation of the cells, apposition of new cells from the epithelial layer, and increase in volume of single cells. These cells do not fuse until the 12th–13th day, which is the stage in which they form myotubes and then young striated muscle fibres.

(*) This investigation was carried out in the National Research Council Unit for Muscle Biology and Physiopathology, Institute of General Pathology, University of Padua, and was supported in part by a grant from the Muscular Dystrophy Association, Inc. to prof. M. Aloisi.

(**) Nella seduta del 10 giugno 1976.

In vitro experiments on the growth of embryonic iris, retina and whole eyes are not numerous [2, 4, 10, 12] and it is still not known how far back in the process of development we can obtain muscle from the iris epithelial anlage. It appeared worthwhile to us to check this point and to follow the process of differentiation of muscle from epithelia in tissue cultures, in order to provide supplementary information and supplementary material for the ultrastructural observations on normal ontogenesis of the iris muscles which are carried out in our laboratory.

Materials and methods

We used chick embryos from 5 to 12 days of incubation. Under a dissecting microscope we separated aseptically the distal portion of their optic cup including the iris and the ciliary body anlages. From these annular portions we cut several small sectors corresponding to the very margin of the pupilla, so far excluding the ciliary body region. Ultrathin sections of fixed tissue for electron microscopy were used to assess the mean extent of the iris portion which had to be used for cultivation and to check the stage of development of the iris. Obviously, the uncertainty about the morphogenetic potency of our experimental material was directly proportional to the precociousness of the stage used: at the 6–7 day stage it appeared more difficult to avoid the ciliary body region, but in general fragments containing this region were discarded and in any case they were easily recognised under the microscope even after the explants were grown.

Tissue cultures from muscle explants of fragments of this material were obtained according to the following variant procedures: *a*) the explants were deposited on a Millipore (0.22 μ) dish floating on a layer of agar dissolved in Simms fluid; *b*) the explants were deposited on the bottom of a Falcon plastic dish where they were anchored by a plasma clot, 50 % embryo extract (EE) and citrated chicken plasma or by a layer of 1 % gelatin (g. Difco) in phosphate buffer solution (PBS).

The culture media were respectively the following: *a*) 109 NTC Difco Medium, calf foetal serum, and chick EE [1] (7 : 2 : 1) 1 part; Agar-Simms (2 %) 1 part; *b*) Eagle Medium modified by Dulbecco, horse serum, and chick embryo extract (7.5 : 1.5 : 1). In both media 100 I.U. of penicillin, 40–100 μg streptomycin, 2.5 μg fungizone per ml medium were added. The cultures were incubated at 37 °C for intervals of time, varying from 2 to 10 days; the atmosphere was air with 5 % CO_2. The cultures were observed while still living by phase contrast microscopy and polarized light microscopy; then they were fixed and stained (Haematox-Eosin).

(1) The chick embryo extract was the supernatant of homogenates of chick embryo between 9–11 days, in equal parts of PBS.

RESULTS

Iris explants taken from chick embryos up to the 8th day of incubation (Pl. I, fig. 1) give mostly growths of epithelial cells which form a monocellular layer of pigmented elements (Pl. I, fig. 2). The pigment is decreasing in the outer part of the growth where the cells are products of several divisions. Besides these cells also fibroblast-like elements are occasionally seen, but not in great number and not frequently. There is no evidence of a transformation of the epithelial cells into myoblasts, even in the older cultures (5–10 days). Only in one case, which concerned an explant taken from a 6-day embryo, did we obtain myoblasts and well developed myotubes; but this was a case in which the explant was most probably larger than usual and included the ciliary region; proof of this supposition is offered by the fact that there was no pigment in the myoblasts and myotubes and that in addition to the muscle growth there was also a chondroblastic growth with spots of mature cartilage, as can be expected from cultures of the more distal part of the anlage, in respect to the pupillary margin.

The epithelial cells are provided with a dense peripheral layer of oriented material which is able to produce a strong birefringence with an appearance which is very similar to that of the so-called tono-filaments. The electron microscopy of these cultures shows the factual existence of a dense peripheral aggregation of microfilaments, mostly oriented parallel to the cell surface. In this way the birefringence appears to be a coherent property of the epithelial layer as a whole and it is stronger in those regions of the culture where tensile forces are in operation.

When the explants originated from chick embryos older than 8 days of incubation, the cell population of the culture was clearly different: in addition to epithelial cells another type of cell is recognisable, namely several multifariously shaped elements showing prolongations of their cytoplasm. They are very similar to the cells of glial nature in culture [1], and they show a strong birefringence particularly in their cell processes. But at this stage the explants produce also elongated and relatively thicker elements which have the appearance of myoblasts and which actually fuse together in multinucleated myotubes. Noteworthy is the observation that, even when the explants were taken after the stage of 8 1/2 days of incubation, that is when presumptive myoblasts have already differentiated from the outer epithelial layer, it was possible to see the formation of myoblasts and myotubes directly from the epithelial part of the growth in culture (Pl. I, fig. 3). The cytoplasm of these myotubes is strongly positively birefringent along the longitudinal axis and has a clear longitudinal striation, whereas a cross striation was difficult to assess. It can be frequently observed (particularly in cultures obtained with procedure *b*) that new myotubes or some of their branches fuse together to form several arches at the periphery (Pl. I, fig. 4).

When the small portion of the pupillar edge of the iris was properly deposited on the culture support, the growth of myotubes was observed only on one side of the fragment, the other side of the culture being occupied by epithelia and fibroblast-like cells only.

Discussion

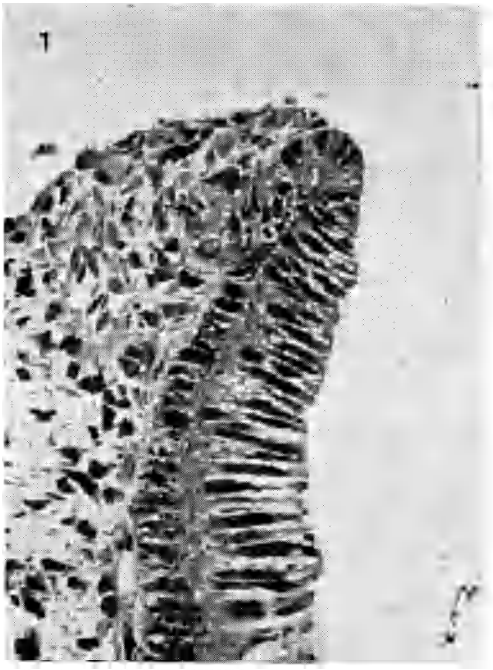
From our observations it is possible to conclude that the margin of the iris anlage of chick embryo is able to produce striated muscle even in culture, provided that the explant is taken around the 8th day of incubation, when in the embryo there are not yet any muscle cells and myoblast, because at this stage the outer epithelial layer of the anterior part of the optic cup is still unaffected by differentiation. Moreover, it is possible to observe, even at a later stage, the direct derivation of myoblasts and myotubes from the peripheral parts of a growing culture which can be defined epithelial in character.

It is very probable that only one of the two layers of the embryonic iridial epithelia is capable of differentiating into muscle in culture, after the 8th day; this is deducible from the observation of the different composition of the cell population growing on the two sides of the explant and it is in agreement with the ontogenetic events *in vivo*.

The structure of the myotubes derived from myoblasts of neuroepithelial origin is not grossly different from that of developing skeletal muscle; however, they go on containing pigment at least for a certain time after fusion, and this particular is a further demonstration of their unusual origin. The stage of a clear cross-striation of the myoplasm of these myotubes has not been frequently reached in our cultures, as observed with the light microscope. This point will be better checked with electron microscopy of the same material.

References

[1] A. Bairati (1949) – « Biologica Latina », *2*, 601.
[2] F. Dorris (1938) – « J. Exp. Zool. », *78*, 385.
[3] G. Gabella (1974) – « Proc. R. Soc. Lond., B », *186*, 369.
[4] J. R. Harrisom (1951) – « J. Exp. Zool », *118*, 209.
[5] A. Hess (1966) – « Anat. Record », *154*, 357.
[6] M. L. Lucchi, R. Bortolami and E. Callegari (1974) – « J. Submicr. Cytol. », *6*, 205.
[7] H. Oehme (1969) – « Zool. Jb. Anat. », *86*, 96.
[8] G. Pilar and P. C. Vaugham (1971) – « J. Physiol. », *219*, 253.
[9] J. F. Reger (1966) – « Anat. Record », *155*, 197.
[10] R. Reimbold (1951) – « C.R. Soc. Biol. », *148*, 1493.
[11] A. L. Romanoff (1960) – In: *The Avian Embryo*, p. 405. MacMillan, New York.
[12] T. S. P. Strangeways and H. B. Fell (1926) – « Proc. Roy. Soc., B », *100*, 273.
[13] W. Zenker and E. Krammer (1967) – « Z. Zellforsch. », *83*, 147.


1


2


3

EXPLANATION OF PLATE I

Fig. 1. – Semi-thin section of the margin of the iris anlage of a chick embryo of 8 days' incubation. The arrow indicates the small bud of the outer epithelial layer from which the iris sphincter muscle will take origin (×400).

Fig. 2. – Growth of epithelia from an iris margin of a 7.5 days chick embryo. The culture was 4.5 days old. Polarized light. Birefringence of the peripheral part of the cells (×250).

Fig. 3. – Culture of the iris anlage of a chick embryo of 12 days' incubation. The culture was 4 days old. From the epithelial layer of the culture originates a symplast which has the characteristic of a myotube (×250).

Fig. 4. – Culture of the iris anlage of a chick embryo of 8.5 days' incubation. The culture was 5 days old. Formation of several arches of myotubes (×250).