Origin of the irideal striated muscle in birds

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SUMMARY
The aim of the present study was to elucidate the origin of the striated muscle cells in the avian iris. For this purpose we adopted interspecific transplantation between quail and chick embryos because quail cells can be used as biological markers in this system. We transplanted isotopically and isochronically (6- to 7-somite stage) a fragment of a dorsal part of the quail neural anlage into a chick embryo at the level corresponding to the posterior prosencephalon and the mesencephalon on the right-hand side.

In the chimaeric embryo, the iris epithelium comprised host chick cells, while most of the stromal cells of the iris on the operated side possessed the quail nuclear marker. At 19 days after the operation, the striated muscle cells had differentiated in the chimaeric embryo. These cells, as well as connective tissue cells and the Schwann cells of the iris of the chimaera, were shown to possess typical quail nuclei by light and transmission electron microscopy. From these findings, we conclude that the striated muscle cells originate from the neural crest.

INTRODUCTION
It has been known for a hundred years that the intrinsic ocular musculature in birds is striated (Geberg, 1884). Smooth muscle cells have also been found in the chicken iris (Gabella & Clarke, 1983), although their number is very small.

The origin of the irideal muscle has been a matter of dispute. There have been many descriptive studies asserting that the sphincter and the dilator muscles derive from the pigment epithelium (Lewis, 1903; Brini, Porte & Stockel, 1964; Ferrari & Koch, 1984a, b). Duke-Elder (1958) described in his textbook that the striated fibres of the iris derived from the pigment epithelium. Recently, Ferrari & Koch (1984a, b) described growth and differentiation of the chicken iris in vivo and in vitro. They interpreted their findings to support the view that the sphincter and the dilator muscles originated from the pigment epithelium. In contrast, Gabella & Clarke (1983), by observing the iris of normally developing chick embryos and of adult chickens, suggested that the striated muscle cells originated from the mesenchymal cells and that the smooth muscle cells originated from the pigment epithelium.

After Le Douarin (1969, 1971, 1973) found that chick and quail nuclei are distinguishable by light and transmission electron microscopy, it became possible to follow the fate of quail tissue transplanted into a chick host by using the quail

Key words: iris, striated muscle, neural crest, quail-chick chimaera.
cell as a biological marker. By this method, the contribution of the cephalic neural crest and the mesoderm to the ocular and periocular tissues of birds was investigated (Johnston et al. 1979; Noden, 1975, 1978a, b). Johnston et al. (1979) showed that the connective tissue cells in the iris are of crest origin. It was also shown by them that ciliary striated muscle cells are derived from the neural crest. Their findings raised the possibility that the striated muscle cells in the avian iris might also arise from the neural crest.

In the present study, we transplanted the quail neural crest into the chick host isotopically and isochronically in order to identify the origin of the striated muscle cells in the avian iris.

MATERIALS AND METHODS

Quail (Coturnix coturnix japonica) and chick (Gallus gallus domesticus) embryos obtained from local farms were incubated in a humidified atmosphere at 37 °C until they had reached the 6- to 7-somite stage (stage 9 of Hamburger & Hamilton, 1951). Isotopic and isochronic grafting of a quail neural primordium into a chick embryo was carried out unilaterally on the right-hand side according to the following technique (Fig. 1). A fragment of the dorsal part of the neural tube

Fig. 1. Schematic drawing of the transplantation. The neural crest of the chick host embryo with 6–7 somites (A) is removed at the level from the posterior prosencephalon to the mesencephalon. The equivalent fragment from the quail embryo (C) at the same level and the same stage is transplanted into the groove of the host (B). P, prosencephalon; M, mesencephalon; R, rhombencephalon; nc, neural crest; nt, neural tube.
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Together with neural crest and overlying superficial ectoderm was excised with a sharpened steel needle from a chick host at the level of the posterior prosencephalon to the mesencephalon. The corresponding length of neural primordium was taken from a quail embryo at the same developmental stage and implanted into the groove of the host left by the previous excision (Fig. 1). Rostrocaudal and lateromedial orientations of the implant were adjusted to the host by marking it with carbon particles.

Chimaeras were reincubated, and were sacrificed 15 h, 5 days, 7 days, and 19 days after the operation. After fixing in Zenker's fluid for light microscopy, specimens were dehydrated through a graded series of ethanol and embedded in paraffin. The 8 μm horizontal sections were cut serially and stained by the Feulgen–Rossenbeck procedure (1924), which makes it possible to distinguish between chick and quail cells (Le Douarin, 1969, 1973), and counterstained with picro-indigocarmine.

A small part of the iris of the chimaera taken 19 days after the operation was fixed with 2.5 % glutaraldehyde in Hanks' balanced salt solution, pH 7.2, for transmission electron microscopy. After postfixing in 1 % osmium tetroxide in Hanks' balanced salt solution, pH 7.2, and dehydration through a graded series of ethanol, the specimen was embedded in Epok 812 (Oken Shoji, Tokyo). Ultrathin sections were cut radially with a Sorvall MT-1 ultramicrotome, stained with uranyl acetate and lead citrate, and observed with a Hitachi HU-11D or a Hitachi H-300 transmission electron microscope.

RESULTS

External observations

No abnormalities were found in the chimaeras when the operation was successful. The boundaries of the graft were faintly observable in the chimaeras 15 h after the operation (stage 15). In the chimaeras taken 19 days after the operation (stage 45), the upper beak was a little shorter than that of a normally developing chick embryo. Pigmentation of the quail pattern was seen on the head and on the eyelids on the right-hand side of the chimaera (Fig. 2). A small amount of quail-type pigmentation was also observed on the left-hand side of the chimaera.

Light microscopic observations

In the chimaeras at 15 h after the operation, the sectioned zones of the neural tube were almost perfectly repaired at the dorsal part of the host. In our chimaeric embryos, only the dorsal part of the diencephalon was replaced by the quail tissue. The optic vesicle, a protrusion of the lateral wall of the prosencephalon (Balinsky, 1981), was composed of chick cells. The quail cells were migrating near the optic vesicle (Fig. 3). Although quail cells were found on both sides, they were much more numerous on the right-hand side, i.e., the side of the operation.

In the chimaeras at 5 days after the operation (stage 31), all the cells in the irideal stroma except for the vascular endothelium were of quail type. On the other hand, the two layers of the irideal epithelium consisted of the host chick cells. The striated structures could not be seen in the iris. The lens and the epithelium of the cornea were composed of chick cells, whereas the stroma and the endothelium of the cornea consisted of quail cells (Fig. 4).

In the chimaeras at 7 days after the operation (stage 35), an epithelial bud was projecting into the stroma at the pupillary margin. The epithelial bud together
Fig. 2. Head of the chimaeric embryo 19 days after the operation. Note the pigmentation of quail pattern (arrows) on the head and the eyelids. The upper beak is slightly short.

with the irideal epithelium consisted of chick type cells, while surrounding stromal cells were of quail type (Fig. 5).

In the chimaera at 19 days after the operation, the irideal epithelium was densely pigmented. The pigment cells were also found just over the pigment epithelium at the ridge of the pupil and at the anterior surface of the iris in the pupillary portion (Fig. 6). Vessels were abundant in the iris and their endothelium was composed of chick cells. Most of the stroma of the iris was occupied by cells containing the quail nuclei and the cytoplasm was stained yellow with picro–indigocarmine. The striated structure of the dilator muscle could be seen immediately anterior to the pigment epithelium on the radial section. The striation is not conspicuous because the specimen was stained according to Feulgen–Rossenbeck procedure to facilitate the identification of the nuclear type. Nuclei of quail type were seen over the striated myofibrils (Fig. 7). Near the pupillary margin, a cluster of chick cells existed in the stroma (Fig. 8). These cells may be the descendants of the epithelial bud observed at 7 days after the operation.

Transmission electron microscopic observations

In the striated muscle cells of the iris of the quail embryo, a large mass of heterochromatic DNA was closely associated with the nucleolar RNA (Fig. 9). On the other hand, the nucleolar RNA and heterochromatic DNA were intermingled
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Fig. 3. Transverse section through the diencephalon of the chimaeric embryo 15 h after the operation. The dorsal part of the neural tube (asterisks) is made up of quail cells, while the optic vesicle (ov) is made up of chick cells. The migrating neural crest cells (arrows) can be seen dorsally and near the optic vesicle. nt, neural tube; se, surface ectoderm. (x330).

Fig. 4. Cornea, iris and lens of the chimaeric embryo 5 days after the operation. Iris stroma (is), corneal endothelium (cen) and corneal stroma (cs) are made up of quail cells. Irideal pigment epithelium (pe), vascular endothelium (ve), corneal epithelium (cep) and lens (L) are made up of chick cells. (x600).
Fig. 5. Iris of the chimaeric embryo 7 days after the operation. At the pupillary portion, the chick type epithelium is budding (arrow) into the stroma which is composed of quail cells. *cen*, corneal endothelium; *v*, a vessel; *L*, lens; *pe*, irideal pigment epithelium. (×800).

in the striated muscle cells of the iris of the chick embryo, and the large mass of DNA was not seen (Fig. 10).

Transmission electron microscopic observation was performed on a chimaera sacrificed 19 days after the operation. The cytoplasm, which was stained yellow with picro-indigocarmine on light microscopic observation, was identified as sarcoplasm of the sphincter muscle. Most of the dilator and the sphincter muscle fibres were of striated type. Myelinated nerve fibres were abundant all over the

Fig. 6. Iris of the chimaeric embryo 19 days after the operation. Irideal pigment epithelium (*pe*) is densely pigmented. Note the pigment cells (*pc*) at the pupillary portion and at the anterior surface of the iris. The vessels (*v*) are abundant. Sphincter muscle (*spm*) occupies a large area of the iris and the dilator muscle (*dim*) a small area. (×460).

Fig. 7. High magnification of the middle portion of the iris from the chimaeric embryo 19 days after the operation. Irideal stroma is almost entirely made up of quail cells. The striation (arrows) of the dilator muscle can be seen faintly, just over the pigment epithelium and the quail cells (arrowheads) on it. On the other hand, the endothelium (*en*) of the vessel is of chick type. (×1300).

Fig. 8. High magnification of the pupillary portion of the iris from the chimaeric embryo 19 days after the operation. A clump of chick cells can be seen near the pupillary margin. Quail cells (arrowheads) are also seen though the number of them is small in this region. (×1300).
irideal stroma and the Schwann cells possessed quail nuclei. Fig. 11 shows one of the irideal striated muscle cells. This cell has a large mass of DNA granules which are associated with two clumps of nucleolar material. This feature is characteristic to the quail cell. The sphincter and dilator muscle cells both possessed the quail nucleolar structure.

DISCUSSION

Our results demonstrate that implanted quail cells differentiate into irideal striated muscle cells. To verify the neural crest origin of the quail cells and to rule out the possibility of their arising from the optic cup, it was necessary first to select the appropriate site and stage for transplantation. It has been shown that the neural crest cells at the level of the posterior prosencephalon to the mesencephalon migrate into the ocular region (Le Lièvre & Le Douarin, 1975; Le Lièvre, 1978; Noden, 1978a), and that individualization of the cephalic neural crest cells takes place between the 8- and 11-somite stages in the posterior prosencephalon and between the 7- and 12-somite stages in the mesencephalon (Duband & Thiery, 1982). Based on these observations, we operated at the level from the posterior prosencephalon to the mesencephalon and between the 6- and 7-somite stages. As the irideal epithelium is an extension of the lateral part of the diencephalon (Fig. 3), only the dorsal part of the quail neural tube was transplanted into the chick embryo. In our chimaeric embryos, the neural retina, retinal pigment epithelium, ciliary pigmented and non-pigmented epithelium and two layers of the irideal epithelium were composed of host chick cells, though the roof of the diencephalon and the mesencephalon was replaced by quail tissue. In the chimaeric eye, the mesenchyme of the iris was made up of quail cells that had migrated from the transplanted neural crest (Fig. 4). Figs 3, 4 and 5 of the present study indicate that our system provides a valid method for pursuing the origin of the striated muscle cells in the avian iris.

In our chimaeric embryos, the eye development was externally normal and the migration of the neural crest cells into the eye was enough for the study of muscle differentiation (Figs 2, 3, 4). The upper beak of the chimaeric embryo 19 days after the operation was slightly shorter than that of a normally developing embryo. The neural crest cells from the level where we operated migrate into the upper beak (Le Lièvre, 1978; Noden, 1978a; Johnston et al. 1979). It might be not because the migration is disturbed but because the length of the beak depends on the mesenchyme; the beak is shorter in the quail than in the chick.

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Fig. 9. A part of a quail striated muscle cell of the iris (just before hatching). Note a large mass (asterisk) of heterochromatic DNA which associates with nucleolar RNA (arrow). (×35 000).

Fig. 10. A part of chick striated muscle cell of the iris (just before hatching). The nucleolar RNA and heterochromatic DNA are intermingled. There is no large mass of heterochromatic DNA. (×35 000).
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Fig. 11. A part of a striated muscle cell in the iris of the chimaeric embryo, 19 days after the operation. Note that a large mass (asterisk) of DNA is associated with two masses of nucleolar RNAs (arrows), which is characteristic of a quail cell. (×21 500).

In the chimaeras, most of the irideal stroma was occupied by the quail cells except for the vascular endothelium as was shown by Johnston et al. (1979). The sphincter muscle occupied a large part of the irideal stroma, but the dilator muscle occupied a narrow area between the sphincter muscle and the pigment epithelium. In the chimaera 19 days after the operation, the striation was observed in the dilator muscle just over the pigment epithelium on the radial section. By light microscopic observations, both the dilator and the sphincter muscles seemed to contain quail nuclei. But further confirmation was needed because it was very difficult to ascertain by light microscopy whether the quail type nuclei belonged to the striated structure or to the adjacent cells. Transmission electron microscopy revealed that both in the dilator and the sphincter striated muscles of the chimaera, a large mass of DNA was closely associated with one or two masses of the nucleolar RNA (Fig. 11). Le Douarin (1982) classified the patterns of nucleolar organization into four types; i.e., Type 1, 2 and 3 are specific for quail nucleoli and the remaining one is usually encountered in vertebrate cells. The striated muscle cells in the chimaeric iris may correspond to Type 1 of the quail nucleolus according to her classification. These light and transmission electron micrographs
clearly show that the neural crest cells migrated into the iris and differentiated into striated muscle cells.

Ferrari & Koch (1984a) reported that clusters of elongated epithelial cells were present within the irideal stroma in the 11-day chick embryo, and thought that some of these elongated cells formed myotubes of the sphincter muscle. The same authors (Ferrari & Koch, 1984b) cultured organotypically the intact iris, the isolated irideal mesenchyme and the isolated irideal epithelium. They found striated muscle in each kind of culture, although the number of cultures in which myotubes developed from the isolated epithelium was small. From these findings they concluded that the striated muscle cells in the avian iris come from the irideal epithelium.

Gabella & Clarke (1983) made chronological observations on the avian iris similar to those of Ferrari & Koch (1984a), but, in contrast to the latter authors, surmised that the pigment epithelium differentiated, not into striated muscle, but into smooth muscle cells because only the smooth muscle cells contained pigment granules. They suggested that striated muscle cells might differentiate from the mesenchyme. Our result that the epithelial bud of chick type is surrounded by the quail type mesenchymal cells (Fig. 5) may support the idea of Gabella & Clarke.

One cannot fully pursue the ancestors of some tissues by a chronological study, hence we adopted the cell marking technique to reveal the origin of the striated muscle cells in the avian iris, and our experiments clearly show that the striated muscle cells migrate from the neural crest. The study in vitro by Ferrari & Koch (1984b) showed that the isolated irideal mesenchyme developed into striated muscle cells, and that in a few cases, isolated irideal epithelium also developed into striated muscle cells. They took their results to mean that the stromal epithelial cells had already dispersed into the mesenchyme at the time of isolation of this tissue and, second, that influence from the mesenchyme was necessary for the epithelium to differentiate into the striated muscle cells.

Our results are contrary to those of Ferrari & Koch (1984a,b). As the neural crest cells migrate into the irideal stroma and, in fact, constitute the major part of the stromal mesenchyme (Johnston et al. 1979; our results), it might be natural to interpret the results of Ferrari & Koch (1984b) as that the irideal mesenchyme developed into striated muscle cells. One cannot exclude the possibility of contamination with a small number of mesenchymal cells in the culture of isolated irideal epithelium, as the number of cultures which developed the striated muscle in the isolated irideal epithelium was not great in their experiments. On the other hand, the results of our experiments do not rule out the possibility that a few striated muscle cells originate from the irideal epithelium.

In conclusion, striated muscle cells and the Schwann cells of the iris originate from the neural crest. The connective tissue cells in the iris also come from the neural crest, as Johnston et al. (1979) showed. On the other hand, vascular endothelium originates from the mesoderm.
We wish to thank Professors M. Yasuda and K. Choshi, Hiroshima University School of Medicine, and Professor N. M. Le Douarin, Institut d'Embryologie du CNRS et du Collège de France, for their critical reading of the manuscript. We are indebted to Dr J. Smith, Institut d'Embryologie du CNRS et du Collège de France, for reviewing the English manuscript.

This work was supported in part by grants No 83-01-32 from National Center for Nervous, Mental and Muscular Disorders of the Ministry of Health and Welfare, and No. 59213011 for Special Project Research from the Ministry of Education, Science and Culture.

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(Accepted 25 February 1985)