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Rostrocaudal polarity formation of chick optic tectum

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ABSTRACT
The optic tectum receives retinal fibers in a topographically ordered manner. For the formation of the precise connections, the tectum is believed to be positionally specified by gradients of molecules along axes. Rostrocaudal polarity of the tectum is first detectable at embryonic day 2 (E2) in the chick, by the caudorostral gradient of en expression, then by the rostrocaudal gradient of cytoarchitectonic development. Tectum rotation experiments showed that tectum rostrocaudal polarity is not determined at around 10-somite stage, but is fixed on E3. Ectopic tectum was produced in the diencephalon by transplanting the mesencephalic alar plate heterotopically. In the ectopic tectum, en expression was weakest at the caudal (nearest to the host diencephalo-mesencephalon junction) and strongest at the rostral end. Consequently, the pattern of en expression in the host and ectopic tecta was nearly a mirror image. Retinal fibers projected to the ectopic tectum in a topographic order in accordance with the inverted gradient of the en expression pattern. Ectopic tecta was also produced by heterochronal transplants between E3 host and E2 donor, where the en pattern was preserved. Retinotectal projection pattern was also preserved, suggesting that en expression patterns are followed by retinotopic order with regard to rostrocaudal polarity.

KEY WORDS: optic tectum, rostrocaudal polarity, engrailed retinotectal projection

Introduction
Optic tectum is a visual center of lower vertebrates, and receives retinal fibers in an exact retinotopic manner. Fibers from nasal part of the retina project to the caudal tectum, and those from temporal part of the retina project to the rostral part of the tectum (Crossland and Uchwat, 1979). For the precise neural circuit formation, Sperry (1963) proposed a chemoaffinity theory. For the retinotectal circuit formation, it is now accepted that the positional specification of both tectal and retinal cells are represented by gradients along axes rather than by different labeling of each point. Thus retina and tectum should be positionally specified by the time retinal fibers reach the tectum. In this review, we will focus on the development of the rostrocaudal polarity of the avian optic tectum.

Developmental events concerning rostrocaudal polarity formation of the tectum
The main role of the optic tectum is to receive retinal fibers in a retinotopic manner. Hence the final positional specification of the tectum may be represented by one that retinal fibers read to find their target. There are some events concerning rostrocaudal polarity, and the final positional specification may be established through a cascade of these developmental events.

The first developmental event along the rostrocaudal axis of the tectum known so far is expression of engrailed (en). en is a homolog of Drosophila segment polarity gene engrailed, and contains homeobox. In chick, 2 engrailed genes are identified, en-1 and en-2 (Logan et al., 1992). en-2 is detected from E2 (embryonic day 2) by a monoclonal antibody, 4D9. We used 4D9 antibody to detect en expression in a series of our experiments. en is expressed strongly at the mesencephalo-metencephalon junction, and weakened toward the diencephalo-mesencephalon junction (Fig. 1). In other words, en is expressed in a caudorostral gradient (Gardner et al., 1988; Patel et al., 1989). From about E5, there is a rostrocaudal gradient of cytoarchitecture. Rostral part of the tectum differentiates faster and has more tectal laminas than the caudal (LaVail and Cowan, 1971). Finally, the tectal surface is positionally specified along a rostrocaudal axis by E8 (Walter et al., 1987a,b), when the retinal fibers invade the tectum. The retinal fibers may read the difference to find their target.

Plasticity in rostrocaudal polarity of the tectum
It is an interesting question whether the polarity of the tectum is determined from an early stage of development. To answer this question, rotation of tectal anlagen was performed. A quail tectum anlage was transplanted into a chick mesencephalon by rotating its rostrocaudal axis 180° at about 10-somite stage.

Development of the rotated tectum proceeded similarly to the contralateral host tectum. Twenty-four hours after the rotation, the en expression pattern was already regulated to the host pattern (Martínez and Alvarado-Mallart, 1990). en was expressed strongly at the caudal part of the rotated tectum (though it was originally
rostral), and weakly at the rostral part. Rostral part of the rotated tectum (original caudal) differentiated faster than the caudal and received temporal retinal fibers (Ichijo et al., 1990; Matsuno et al., 1991).

The caudal part of the rotated tectum received the nasal retinal fibers, and the rostral part of the rotated tectum received temporal retinal fibers. These results indicate that the rostrocaudal polarity of the optic tectum is not fixed at around the 10-somite stage, and that it is established through interaction with surrounding tissues.

Then we checked when the polarity of the tectum anlage is fixed. For this purpose, we performed tectum rotation experiment at E3. Since it is very difficult to rotate whole tectum, we took caudal half of quail left tectum and transplanted into the rostral part of the chick right tectum (double caudal tectum) (Fig. 2A). In a similar way, double rostral tectum was produced at E3.

When the operation was performed after the 20-somite stage, the transplant was not regulated by the host, but kept its original characteristics of en expression. Original caudal tectum anlagen kept expressing en strongly at the rostral part of the tectum (Itasaki et al., 1991). On the other hand, original rostral tectum anlagen did not express en strongly at the caudal part of the tectum. On the double caudal tectum in which en had been expressed strongly at the rostral part, nasal retinal fibers were attracted by the transplant (Itasaki et al., 1991). On the double rostral tectum, in which en had not been expressed strongly at the rostral part, the nasal retinal fibers extended to the caudal part, but could not find their target (Fig. 3). These results indicate correlation between the en expression pattern and the final polarity of the tectum.

Ectopic tectum differentiated by heterotopic transplantation of the tectum anlage

We have mentioned that the tectum rostrocaudal polarity is not determined at E2, and it is regulated by the surrounding tissue. On the other hand, the fate of the tectum anlage is already determined by the 10-somite stage, and it differentiates as an optic tectum even when transplanted ectopically into the diencephalon or into the mesencephalon (Nakamura, 1990). Ectopic tectum which differentiated at the diencephalon can receive retinal fibers (Alvarado-Mallart and Sotelo, 1984).

We studied the rostrocaudal polarity formation in the ectopic tectum produced in the diencephalon (Fig. 2B). First, E2 tectal anlage was transplanted into the E2 diencephalon. In the host tectum, en was expressed strongly at the caudal part and weakly at the rostral part. On the other hand, in the ectopic tectum, en expression was weak at the caudal part and strong at the rostral part. The further from the diencephalo-mesencephalon junction, the stronger the en expression (Fig. 4). Consequently, the pattern of en expression in the host and in the ectopic tecta was nearly a mirror image, suggesting the existence of a repressive influence on en expression around the diencephalo-mesencephalon junction (Itasaki et al., 1991).
Fig. 3. Trajectories of nasal retinal fibers on the double rostral tectum. Transplantation was performed between 24- and 26-somite stage quail and chick embryos. Nasal retinal fibers extended to the caudal pole of the double rostral tectum [A, C], but they could not make terminal arborsizations. Instead they are wandering (arrows in A) as if to find the target near the expected target zone (TZ). (B) shows the boundary between the host (h) and the transplant (t). The transplant is easily identified because quail cells have condensed heterochromatin (arrows). (C) represents camera lucida drawing of the tectum (upper) and retina (lower). Shadowed area represents the transplant. C: caudal, R: rostral, M: medial, L: lateral, D: dorsal, T: temporal, N: nasal, V: ventral, Dil: place where Dil was put.

E7 ectopic tecta had the laminar structure characteristics of the developing tectum. Cytoarchitectonic development of the ectopic tecta proceeded in a mirror image to the host tectum (Fig. 5). The caudal part of the ectopic tecta had thicker wall and more laminae than the rostral (Itasaki et al., 1991). This result suggests correlation between the en expression pattern and the pattern of cytoarchitectonic development. In both ectopic and host tecta, the place where the en is expressed most weakly differentiated faster than the place where en is most strongly expressed.

As mentioned earlier, the plasticity in the rostrocaudal polarity of the tectum is lost in the E3 embryo. It has also been suggested that the rostrocaudal polarity of the tectum is determined by the interaction of tectum and surrounding tissues. We pursued the mechanisms of polarity formation by heterochronic transplantation.

Fig. 4. Chimeras with ectopic tecta (arrow heads) at various positions in the diencephalon. The further from the mes-diencephalon junction (arrows), the stronger the en expression was. This rule is also applicable to the host tectum. Bar, 500 μm.
Fig. 5. Histology of chimeric brain with ectopic tectum. In the host tectum, the rostral part (D) where en was more weakly expressed, differentiates faster than the caudal part (E). On the other hand, in the ectopic tectum, caudal part (C), where en expression was weaker, differentiates faster than the rostral (B). Bar, A: 200 μm, B: 20 μm.

Fig. 6. Chimeras after heterochronic transplantations, stained with anti-en antibody. (A) A chimera after transplantation of E3 mesencephalon into the E2 diencephalon. (B) A chimera after transplantation of E2 mesencephalon into the E3 diencephalon. Ectopic tectum in A has had its en expression pattern reversed, while the ectopic tecta in B kept its original pattern of en expression. This finding indicates that the E3 tectum anlage is competent to respond to regulating factors for rostrocaudal polarity, whereas the E3 embryo may have lost the regulating factors. Bar, 500 μm.

(i)naaki et al., 1991), i.e. we wanted to know whether E3 embryos lost plasticity in tectal rostrocaudal polarity formation because tectal anlage itself lost the competence or because the regulative activity was lost.

When the E3 tectal anlage was transplanted into the E2 diencephalon, the en expression pattern was reversed (Fig. 6A), i.e. en expression was strong at the rostral and weak at the caudal (near the diencephalo-mesencephalon junction), just as in the chimeric embryos produced by the transplantation between E2 donor and E2 host embryos. In other words, E3 tectal anlage changed the en expression pattern by regulative influence from the host embryo. On the other hand, when the E2 tectal anlage was
made terminal arborsations at the caudal part of the ectopic tecta (Fig. 7) (Itasaki and Nakamura, 1992).

Our experimental results on rostrocaudal polarity all show the consistent relation between the en expression pattern and subsequent development including the retinotectal projection pattern. When the en expression pattern is reversed in the ectopic environment, subsequent cytoarchitectonic development and retinotectal projection patterns were in reverse to the host.

Concluding remarks

Our study series showed close correlation between en expression pattern and subsequent tectal polarity including the polarity to receive retinal fibers. It may be interesting to look at retinal projection patterns after inducing en expression ectopically by expression vectors such as retrovirus. Such experiments are being carried out in our lab.

en is a homeobox containing gene originally identified as a segment polarity gene in Drosophila (Kornberg, 1981). In Drosophila, many genes which are involved in morphogenesis have been identified. Recent studies have shown that the homolog of such genes are active in vertebrate development. en is one such gene. wnt-1, which is a homolog of wingless (wg) in Drosophila, has been shown to be essential for mesencephalon and cerebellum development in mice by the gene targeting method (McMahon and Bradley, 1990; Thomas and Capocchi, 1990). In Drosophila, it has been shown that en and wnt-1 mutually interact for their expression (Martinez-Arias et al., 1988). Thus it may be interesting to elucidate genes which regulate en expression in chicken mesencephalon. It may be also interesting to study what gene en regulates because en is a homeobox containing gene.

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References


Fig. 7. Summary in the ectopic tectum. When E2 mesencephalon is transplanted into the E2 diencephalon (upper), en expression is a mirror image to the host tectum. Subsequent developmental events proceed in a mirror image to the host. The caudal part of the ectopic tectum may have acquired the 'rostral' property and receives nasal retinal fibers, and rostral part of the ectopic tectum receives temporal retinal fibers. When E2 mesencephalon is transplanted into the E3 diencephalon (lower), the transplant keeps its original fate of en expression pattern. Caudal part of the ectopic tectum, in this case, receives nasal retinal fibers. C: caudal, R: rostral, t: temporal, n: nasal.


