PAX6 and MSX1, two homeobox genes involved in the development of the subcommissural organ

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ABSTRACT

During mouse central nervous system (CNS) development, the homeobox-containing genes Pax6 and Msx1, have a spatial and temporal restricted expression in the CNS and craniofacial skeleton. Both genes are highly expressed in the glial secretory cells that forms the subcommissural organ (SCO), a circumventricular organ located at the forebrain-midbrain boundary, in the pretectal dorsal midline neuroepithelium beneath the posterior commissure. Pax6 (Small eye, Sey/Sey) and Msx1 (-/-) null mutants homozygous fail to develop the SCO and a normal posterior commissure. Pineal gland is also absent in Small eye heterozygotes demonstrated specific Pax6 defects in the developing SCO, with an important reduction in the secretory basal cell processes, in accordance with the dosage effect of Pax6. Also for Msx1, a gene-dosage effect was found since heterozygous showed a reduced (about one half) SCO. In both mutants, homozygotes and sometimes heterozygotes develop hydrocephalus. This suggests a causal relationship between the development of the SCO and of the posterior commissure, and between the absence of a normal SCO and the development of hydrocephalus.

The development of vertebrate CNS follows a metameric pattern characterized by different segments (neuromeres) separated by boundaries. The borderline between prosomere 1 (prosencephalic neuromere 1) and the mesencephalon (the forebrain-midbrain limit) is the pretectal region, characterized by a landmark, the posterior commissure, a conspicuous decussation of fibers originating in the pretectal nuclei serving auxiliary visual functions. Even before the appearance of this commissure, the pretectal dorsal neuroepithelium begins to secrete into the tubular lumen high amounts of characteristic spondin-like glycoproteins (Gobron et al., 1996) that polymerize in a fiber called Reissner’s fiber (RF) extending along the cerebral ventricles and the spinal central canal. This secretory region grows concomitantly to the posterior commissure penetrating it by basal processes of the secretory neuroepithelial (ependymal) cells. This way, the roof of the differentiated caudal diencephalon virtually contacts the posterior commissure and the underlying secretory neuroepithelium that forms a brain gland called the subcommissural organ (SCO) (Schöebitz et al., 1986; Oksche et al., 1993). There is also evidence indicating a basal release of RF-glycoproteins into the leptomeningeal spaces through the SCO cell processes (Schöebitz et al., 1986) that could influence structures beneath the organ such as the posterior commissure. The recent works involving SCO-spondin in mechanisms of neural aggregation and differentiation (Rodríguez and Yulis, 2001) and the SCO location, have led us to hypothesize an interrelationship between the SCO and the posterior commissure formation. The development and establishment of segments and boundaries patterning the CNS, depend on spatial and temporal restricted expression of regulatory genes encoding numerous proliferation and differentiation factors. SCO development depends on the expression of such genes. To present, some of them have been reported to be expressed in the pretectal region or its neighbourhood, being good candidates to control SCO and/or the posterior commissure development. We show here evidences that two of them, Pax6 and Msx1, are highly expressed in the SCO.

Pax6, a transcription factor encoded by a homeobox-containing gene, is expressed in the developing CNS with a spatially and temporally restricted pattern (Estivill-Torrues et al., 2001). Small eye homozygous mutant mice (Sey/Sey) lacking Pax6, have severe defects affecting the eyes, nose and the proliferation and differentiation of the forebrain neural precursors (Walther and Gruss, 1991; Stoykova et al., 1996). Sey/Sey homozygotes also are characterized by the loss of structures derived from the dorsal region of prosomere 1, this is, pretectal structures such as the posterior commissure. The Msx1 homozygous mutant gene, is expressed in the developing CNS with a spatially and temporally restricted pattern concerning, in this case, the craniofacial and axial skeleton (Orested-Cardoso et al., 2001). In embryonic CNS, Msx1 is expressed in the dorsal midline of the neural tube and of the brain. Msx1 (-/-) null mutant mice display numerous abnormalities in craniofacial and neural tube development including hydrocephalus. In this report, we state previous (Gobron et al., 2000) and present evidences suggesting developmental mechanisms for Pax6 and Msx1 in the SCO. Remarkably, a link between Msx1 and Pax6 expression has been suggested in other structures. The analysis from homozygous and heterozygous Small eye and Msx1 null mutants, has provided evidences of specific functions of these genes in the SCO cells.

The mouse mutation Small eye obtained at the University of Edinburgh results in a non-functional protein lacking the homeodomain. The availability in our group of antibodies that selectively recognize the SCO secretion and the Pax6 protein led us to demonstrate that Pax6 is expressed in the SCO secretory cells (Fig. 1 b). Expression, starting from E14 (14 days post coitum) and coincident with SCO development, reaches a maximum at E18, when SCO is fully developed (Fig. 1 a,c). Few days after birth Pax6 expression ceases. When Small eye mutants were examined we found no SCO and no secretion in homozygotes (Sey/Sey) (Fig. 1d)
and a decreased activity in heterozygotes (Sey/+). Additionally, the posterior commissure was defective and partially absent in homozygotes. In addition to a diencephalic indirect environmental effect, the expression in the SCO cells itself appears to have a specific effect. By comparison with normal mice (Fig. 1 c,g) in Small eye mice heterozygous (Fig. 1e), the SCO developed a wild type pattern but the secretory material was absent from its cell basal processes (Fig. 1f). Considering that the diencephalon seemed to develop correctly in heterozygotes, this defect only could be attributed to the Pax6 expression in the SCO cells and, in homozygous where defects are not partial but absolute, the SCO absence should result from the aggravation of those heterozygous milder defects. Pax6 seems to influence morphogenetic mechanisms in the absence should result from the aggravation of those heterozygous milder defects. Pax6 may influence morphogenetic mechanisms in the absence of Pax6 expression.

**References**


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