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Short Report

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* mice. Analysis of *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 consists of 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 leaded us to hypothesize a 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-Torrús *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 homeobox gene has a spatial-temporal expression 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 demonstrated 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)

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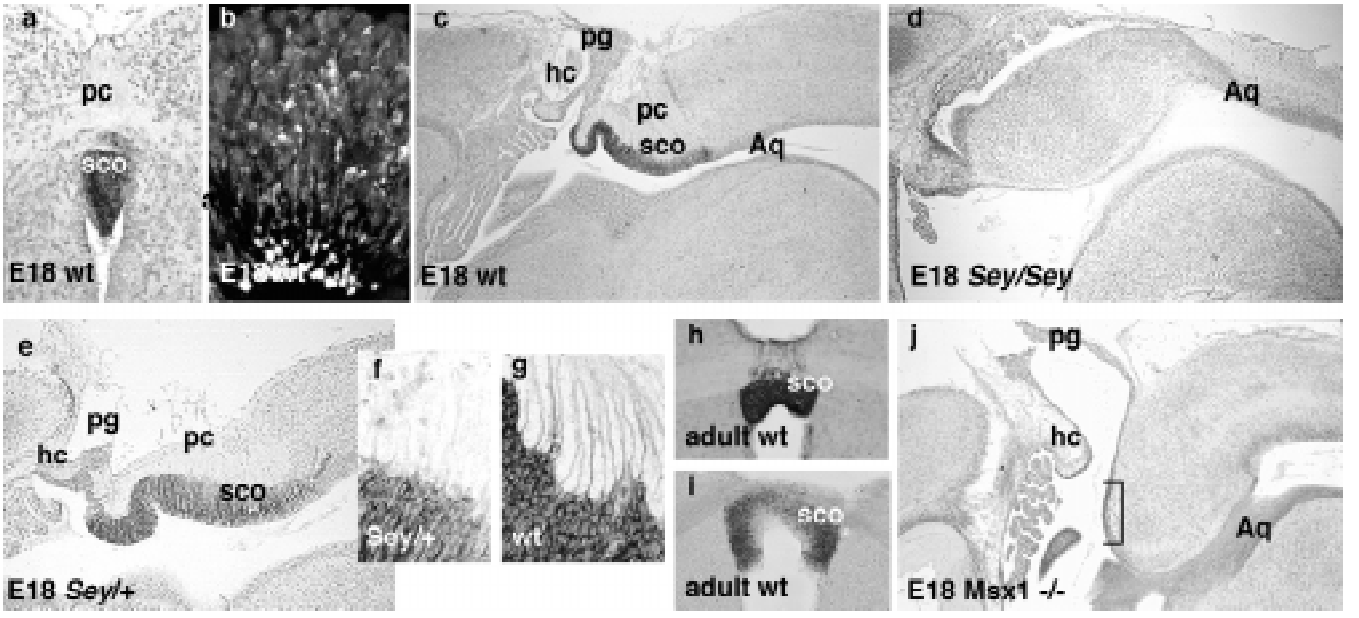

Fig. 1. Coronal (a,b,h,i) and sagittal (c-g, j) sections from E18 (a-g, j) and adult (h,i) mice analyzed for SCO secretion and/or gene expression. *(a-c, g-i) Wild type mice (wt). (d) Small eye homozygous (Sey/Sey). (e,f) Small eye heterozygous (Sey/+). (j) Msx1 null mutant (Msx1 -/-).* **(a, c-g, h, j)** *Immunostaining for antibodies specific for SCO secretion products (AFRU).* **(b)** *Double staining for Pax6 (nuclear) and SCO secretion products (bright, cytoplasmic).* **(i)** *In situ hybridization for Msx1.* **(f,g)** *Detail of SCO basal processes. sco, subcommissural organ; pc, posterior commissure; hc, habenular commissure; pg, pineal gland; aq, cerebral aqueduct. Open key in j labels the remain of the SCO region in mutant.a, x40; b, x275; c,d,j, x15; e, x22; f,g, x225: h,I, x75.*

and a decreased activity in heterozygotes (*Sey/+*) (Fig. 1e) (Gobron *et al.,* 2000). 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 SCO and their secretory routes. Interestingly, R- and OB-cadherin expression, observed in the SCO cells, followed a similar defective pattern in the mutants, suggesting a link between these cell adhesion molecules and the Pax6 function (Gobron *et al.,* 2000).

Msx1 null mutants, obtained in the Prof. Robert laboratory, in Paris by a targeted gene disruption, were similarly analyzed. In wild type mice, the ependymal cells of the embryonic and adult SCO immunoreactive for the specific antibodies against their secretion (Fig. 1h) showed Msx1 expression by *in situ* hybridization (Fig. 1i). In null homozygous mutant (-/-) SCO was absent (Fig. 1j) and the posterior commissure was also missing. Heterozygotes for Msx1 mutation showed a reduced (about 50%) SCO suggesting, as for Pax6, a gene dosage effect. Additionally, both Pax6 and Msx1 mutants (homozygous and some heterozygous) developed hydrocephalus. In Msx1 mutants stenosis of the cerebral aqueduct was evident. This suggests a causal relationship between the miss-development of the SCO and hydrocephalus. In addition to the Pax6 and Msx1 requirement for the SCO development reported here, the existing link between these genes and their effect in the SCO and posterior commissure development bring us, again, the hypothesis of an interrelationship between both structures. The significance of heterozygotes where the SCO basal secretory route is defective may bring new insights for the role of the SCO in CNS development.

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