SECRETORY GLYCOPROTEINS OF THE ROOF AND FLOOR PLATES AND THEIR ROSTRAL DERIVATIVES, THE SUBCOMMISSURAL AND FLEXURAL ORGANS, IN THE DEVELOPING CENTRAL NERVOUS SYSTEM OF VERTEBRATES. AN IMMUNOCYTOCHEMICAL STUDY

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The central nervous system of vertebrates differentiates from a neural tube lined by a germinative neuroepithelium that originate all nerve cells. Neuroepithelial cells finally differentiate into ependymal cells. In the mid-sagittal plane, the roof and floor plates secrete informative molecules that control growing and wiring of neuronal axonal processes and neuronal differentiation[1]. They also synthesize and release into the lumen of the neural tube high molecular weight glycoproteins capable to condense and form fibrous structures called Reissner´s fiber (RF). In the urochordate *Oikopleura dioica*, a single ependymal cell located in the rostral end of the neural tube releases materials that form a RF. In the cephalochordate *Branchiostoma lanceolatum* the infundibular organ, in the floor of the brain, synthesizes a RF immunologically similar to bovine RF. In the developing nervous system of vertebrates, cells of the roof and floor plates secrete RF-like glycoproteins. Most of these cells are located in the rostral floor plate constituting the flexural organ (FO) and in the rostral roof plate forming the subcommissural organ (SCO). The FO ceases to secrete around birth. At variance, the SCO remains active in adults in most species[2]. Being so early in ontogeny and so extended in phylogeny suggest that RF-substances should play a role in the developing and adult central nervous system. Lesion studies in Xenopus tadpoles suggested that RF were involved in the development of the axial skeleton and the epidermis of the tail tip[3, 4]. More recently, it has been shown that RF improves survival of cultured neurons[5].

We have studied the embryonic development of FO and SCO in all vertebrate classes by using several monoclonal and polyclonal antibodies against the SCO secretory glycoproteins of bovine and dogfish[6, 7]. We used: fishes (dogfish, *Scyliorhinus canicula*; sea bream, *Sparus aurata*), amphibians (*Rana perezi*, *Xenopus laevis*), reptiles (snake, *Natrix maura*), birds (chicken, *Gallus domesticus*), and mammals (bovine, *Bos taurus*). We have identified secretory glycoproteins of SCO and FO on thin, semithin and ultrathin sections. In order to analyse the glycosidic component we also used lectin histochemistry with concanavalin A, wheat germ agglutinin and *Limax flavus* agglutinin.

In figures 1-8 an overview of the structures stained by anti bovine-RF in embryonic vertebrate central nervous system is shown. In fishes and amphibians embryos, the FO differentiated soon after the closure of the neural tube and before the SCO. Then OF and SCO coexist during the remaining embryonic development and, after hatching, the FO regresses (Figs. 1-4). In *Xenopus laevis*, in addition to SCO and FO (Fig. 4), secretory cells were seen along the whole floor plate (Fig. 5). In all vertebrates, the SCO differentiated soon and remained active for all embryonic period and thereafter during adult life (Figs 1, 2, 4, 6, 7, 8); RF was evident both in embryonic and in adult life. The use of different antibodies have led to interesting comparative results. Anti-bovine SCO secretory glycoproteins identified materials in the SCO and the FO of several vertebrate classes thus indicating that conservative universal epitopes may be present in all vertebrates. Conversely, anti-dogfish SCO sera recognized selectively selachian SCOs and monoclonal antibodies against bovine RF identified materials only in bovine or, at most mammalian species, thus indicating that class specific, and species specific, epitopes should also exist in RF-like glycoproteins. Since, in lower vertebrates, both SCO and FO are stained by the same antisera against SCO secretory proteins, it is likely that secretions from both organs are similar.

In conclusion specialized ependymal glandular cells of chordates derived from the rostral floor and roof plates, secrete large glycoproteinst that could play important roles. Elucidation of these roles appears as an important future task. Molecular biology studies and the development of simple functional models would aid to the understanding of these enigmatic ancient molecules.

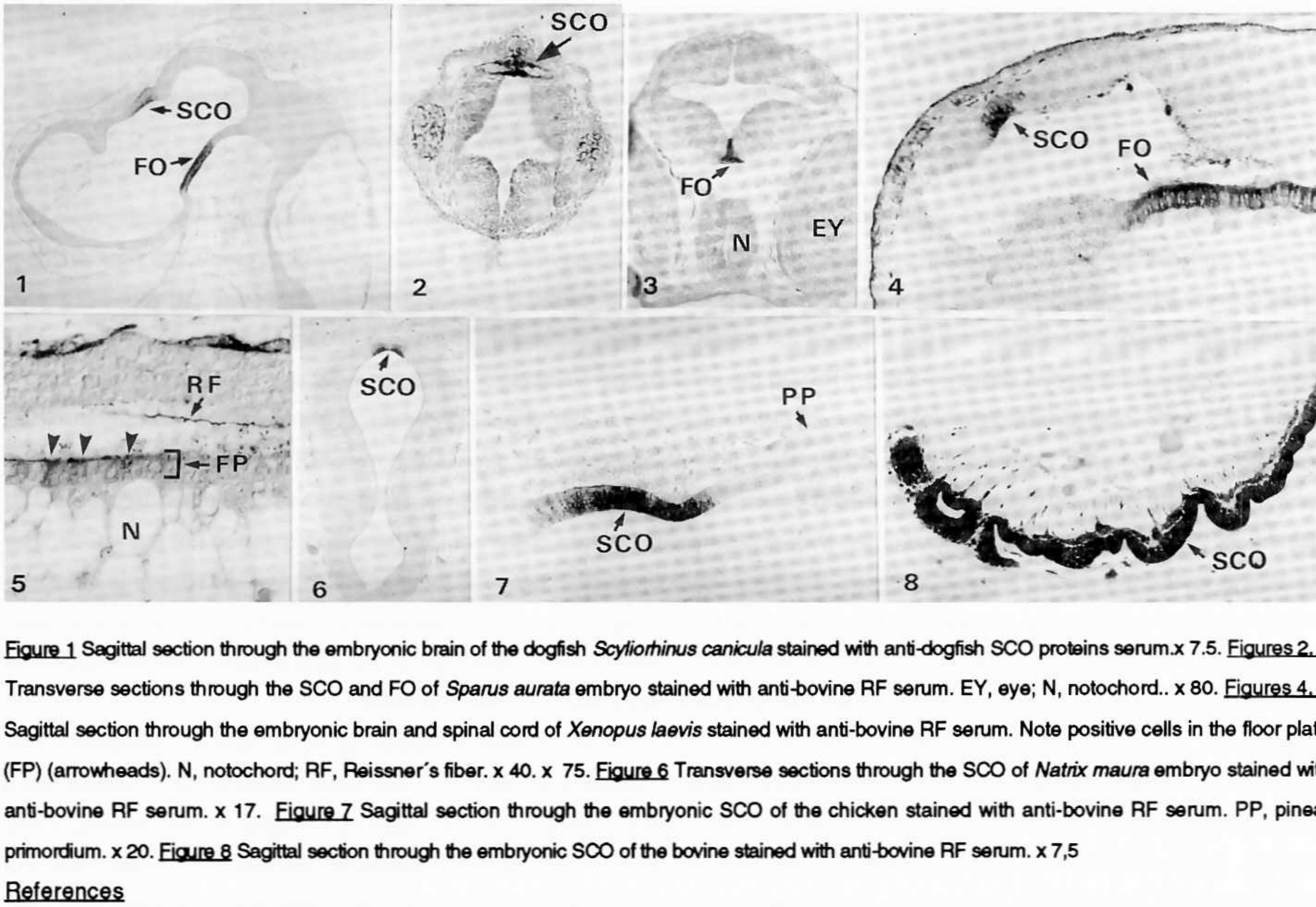

Figure 1 Sagittal section through the embryonic brain of the dogfish *Scyliorhinus canicula* stained with anti-dogfish SCO proteins serum.x 7.5. Figures 2, 3 Transverse sections through the SCO and FO of *Sparus aurata* embryo stained with anti-bovine RF serum. EY, eye; N, notochord.. x 80. Figures 4, 5 Sagittal section through the embryonic brain and spinal cord of *Xenopus laevis* stained with anti-bovine RF serum. Note positive cells in the floor plate (FP) (arrowheads). N, notochord; RF, Reissner's fiber. x 40. x 75. Figure 6 Transverse sections through the SCO of *Natrix maura* embryo stained with anti-bovine RF serum. x 17. Figure 7 Sagittal section through the embryonic SCO of the chicken stained with anti-bovine RF serum. PP, pineal primordium. x 20. Figure 8 Sagittal section through the embryonic SCO of the bovine stained with anti-bovine RF serum. x 7,5

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