

### ROLE OF SULFATED PROTEOGLYCAN IN EARLY LENS DEVELOPMENT

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The extracellular matrix has been seen to play a key role in many of the morphogenetic processes which take place during early ocular anlage development. For some time now it has been known that sulfated proteoglycans are present in initial lens development, as these molecules have been implied in morphogenetic processes such as lens invagination and induction (Webster et al., 1983, Peterson et al., 1995). The implication of sulfated proteoglycans in these phenomena is attributed to their distribution pattern and their coinciding with a particular process, or to the changes found in this distribution pattern in certain mutant strains of aphakic mice (Zwaan and Webster, 1984). Nevertheless, there is no direct evidence to support the implication of proteoglycans in the formation processes of the lens vesicle and its subsequent development. In this paper, we offer the preliminary results of an experimental study concerning selective disruption of sulfated proteoglycans synthesis by means of a  $\beta$ -D Xyloside, and its effect on lens growth.

For this study, stage 13 (Hamburger and Hamilton, 1951) chick embryos were injected in ovo, subgerminally, with a 24  $\mu$ l. dose of a 4mM solution of P-Nitrophenyl  $\beta$ -D Xylopyranoside or sterile Ringer's solution, after which they were reincubated until they reached stage 24 H.H. The embryos were fixed in Bouin for optic microscopy or in Carnoy for immunohistochemistry with a CS 56 anti-Chondroitin Sulfate antibody, which recognizes the glycidic fraction of this proteoglycan.

23 H.H.-stage control embryos display a normal degree of development and morphology both in the lens and in the other ocular structures (Fig. 1-A). Immunostaining with anti-Chondroitin Sulfate (CS) antibody reveals the abundance of this molecule in the lens capsule, especially on the posterior wall, and discrete marking on the apex and the intercellular spaces of the anterior epithelium cells (Fig. 2-A).

The  $\beta$ -D Xyloside-treated embryos display dramatic changes in ocular growth, and above all in that of the lens, which is smaller and flattened (Fig 1-B). The lens cavity is still present and inside it is a fibrillary material together with cells which are rounded in appearance. Most apparent is the lack of growth of the primary, and especially the anterior, lens fibres, and occasionally the whole of the posterior epithelium shows signs of degeneration. The lens capsule is still complete, yet appears frayed and less compact than in the posterior section controls. The retina neural layer appears abnormally folded and with areas of hypertrophy. Anti-CS antibody immunomarking on these embryos (Fig 2-B) reveals the existence of dramatic changes in CS distribution; the presence of this in the lens anterior epithelium is considerably greater than in control embryos, and appears in the intercellular spaces and lens cavity.

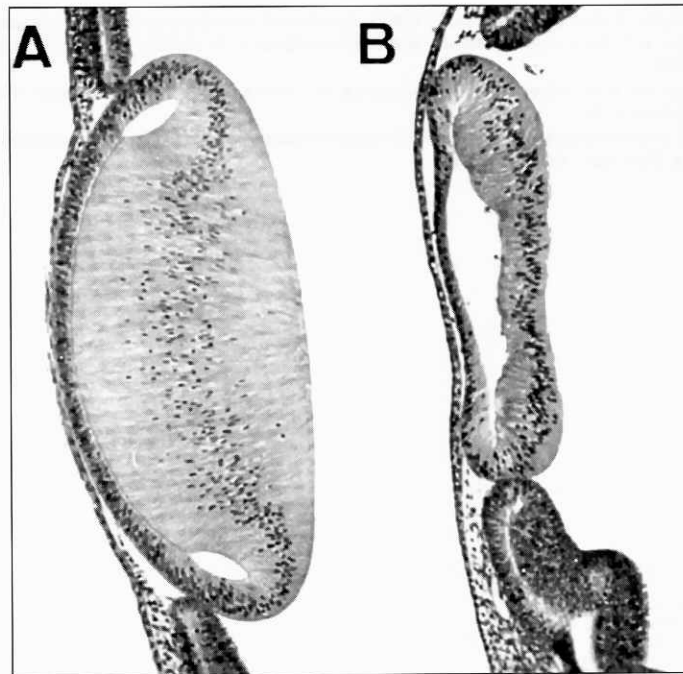


Figure 1: Comparison at similar magnification of lens anlage development of a 23 H.H. control embryo (A) and a 23 H.H.  $\beta$ -D Xyloside treated embryo (B).

Our results demonstrate that selective disruption in the synthesis of sulfated proteoglycans such as CS, causes a radical change in early lens development; basically, the anlage cavity remains and the growth of primary fibres is defective, which suggests that this proteoglycan is a fundamental component of the extracellular matrix implicated in early lens development (Webster et al., 1983). Coincidence in  $\beta$ -D Xyloside-induced changes in chick lens growth, both as regards morphological

appearance and the distribution pattern of sulfated proteoglycans, with the changes described by Zwaan and Webster (1984) in a mutant strain of aphakic mouse, suggests that there is a direct relation between changes in lens morphogenesis and those of the ocular extracellular matrix described by these authors.

Moreover, similar changes, such as cavity persistence, the lack of lens fibre growth and the presence of apoptosis in the posterior epithelium, have been dealt with by Chow et al. (201995) in transgenic mouse embryos with an altered receptor-1 FGF. Given that this growth factor has been implicated in apoptosis suppression and lens fibre differentiation, our findings support the hypothesis that this factor might be stored in chick embryo ocular tissues and bound to sulfated proteoglycans, as one of the extracellular matrix morphogenetic roles.

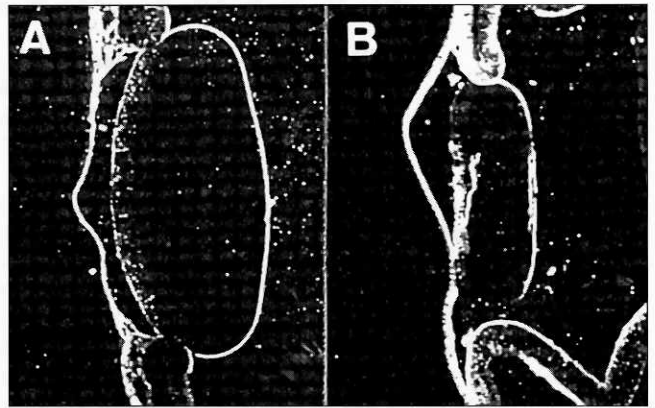


Figure 2: Immunolocalization of chondroitin sulphate in lens anlage of a 23 H.H. control (A) and  $\beta$ -D Xiloside treated chick embryo (B). Magnification was similar in both images.

#### References

- Beebe, D.C., Silver, M.H., Belcher, K.S., Van Wyk, J.J., Svoboda, M.E. and Zelenka, P.S. (1987). Lentropin, a protein that controls lens fiber formation, is related functionally and immunologically to the insulin-like growth factors. *Proc. Natl. Acad. Sci.* 84:2327-2330.
- Chow, R.L., Diez Roux, G., Roghani, M., Palmer, M.A., Rifkin, D.B., Moscatelli, D.A. and Lang, R.A. (1995). FGF suppresses apoptosis and induces differentiation of fibre cells in the mouse lens. *Development* 121:4383-4393.
- de longh, R. and McAvoy, J.W. (1993). Spatio-Temporal distribution of acidic and basic FGF indicates a role for FGF in rat lens morphogenesis. *Dev. Dyn.* 198:190-202.
- Hamburger, V. and Hamilton, H.L. (1951). A series of normal stages in the development of the chick embryo. *J. Morphol.* 88:49-92.
- Peterson, P.E., Pow, C.S.T., Wilson, D.B. and Hendrickx, A.G. (1995). Localisation of glycosaminoglycans during early eye development in the macaque. *J. Anat.* 86:31-42.
- Webster, Jr., E.H., Silver, A.F. and Gonsalves, N.I. (1983). Histochemical analysis of extracellular matrix material in embryonic mouse lens morphogenesis. *Dev. Biol.* 100:147-157.
- Zwaan, J. and Webster, Jr., E.H. (1984). Histochemical analysis of extracellular matrix material during embryonic mouse lens morphogenesis in an aphakic strain of mice. *Dev. Biol.* 104:380-389.