CLONING AND CHARACTERISATION OF TWO CHICK HOMEOBOX GENES, MEMBERS OF THE SIX/SINE OCULIS FAMILY, EXPRESSED DURING EYE DEVELOPMENT.

Paola BOVOLENTA[1], Antonello MALLAMACI [2] and Edoardo BONCINELLI [2,3]

[1] *Dept. Neurobiologia del Desarollo Instituto Cajal , CSIC, Dr Arce 37, 28002Madrid, Spain;* [2] *DIBIT, Istituto Scientifico H.S. Raffaele, via Olgettina 60, 20132 Milano, Italia.* [3]*. Centro Infrastrutture Cellulari, CNR, via Vanvitelli 32, 20129 Milano, Italia*

The vertebrate eye formation is a very complex phenomenon that relays on a sequence of inductive interactions. The eye development begins with the formation of the optic vesicle, a protrusion of the lateral forebrain. The optic vesicles upon contact with the overlying ectoderm will determine the formation of the lens placode. At this stage the optic cup is formed from which the optic stalk, pigmented epithelium and neural retina will arise. The chick embryo is an ideal model to study these early steps of eye formation, since it is accessible to surgical manipulations and it allows rapid attempts of interference with the expression of specific genes. Transcription factors in general and homeobox genes in particular, are fundamental for the genetic control of different basic developmental processes (Gehring et al., 1994). Therefore, the study of these genes might be crucial to understand the steps leading to the formation of a well organised and functional eye. Recently, several homeobox genes have been detected at early and later stages of vertebrate eye and retinal development (Saha et al, 1992; Halder et al, 1995). Many of them are homologues of genes known to be crucial in the development of the Drosophila compound eyes. Thus, in the search for new genes that might control the early steps of vertebrate eye formation, we have looked for the chick homologue of the Drosophila gene *sine oculis*. *Sine oculis* is a homeobox containing gene that, in the fly, appears to be fundamental for the development of the entire visual system (Cheyette et al., 1994).

Using two different pairs of degenerate primers, we have amplified by nested PCR, the homeobox region of the homologue of *sine oculis* starting from a E2 chick cDNA library. Sequence analysis of the amplified fragments revealed the existence of at least four different genes related to the Drosophila *sine oculis*. The aa sequence of two of them were identical to the homeobox of the recently isolated murine six-1 and six-2 (Oliver et al., 1995a). The remaining two were used to screen the cDNA library and isolate two cDNA inserts of 1.5 and 2.1 kb, respectively. Sequence analysis revealed that the first cDNA contained the entire coding sequence of a gene related to the Drosophila *sine oculis,* and highly homologue to that of the recently described mouse *six-3* (Oliver et al., 1995b). The second contained part of the coding sequence and the 3' end region of a gene related to the Drosophila *sine oculis*, but different from those so far described. We named them *csix-3* and *csix-4,* respectively. The csix-3 gene presented a homeodomain identical to that of the mouse six-3 (70% homology with that of *sine oculis*).

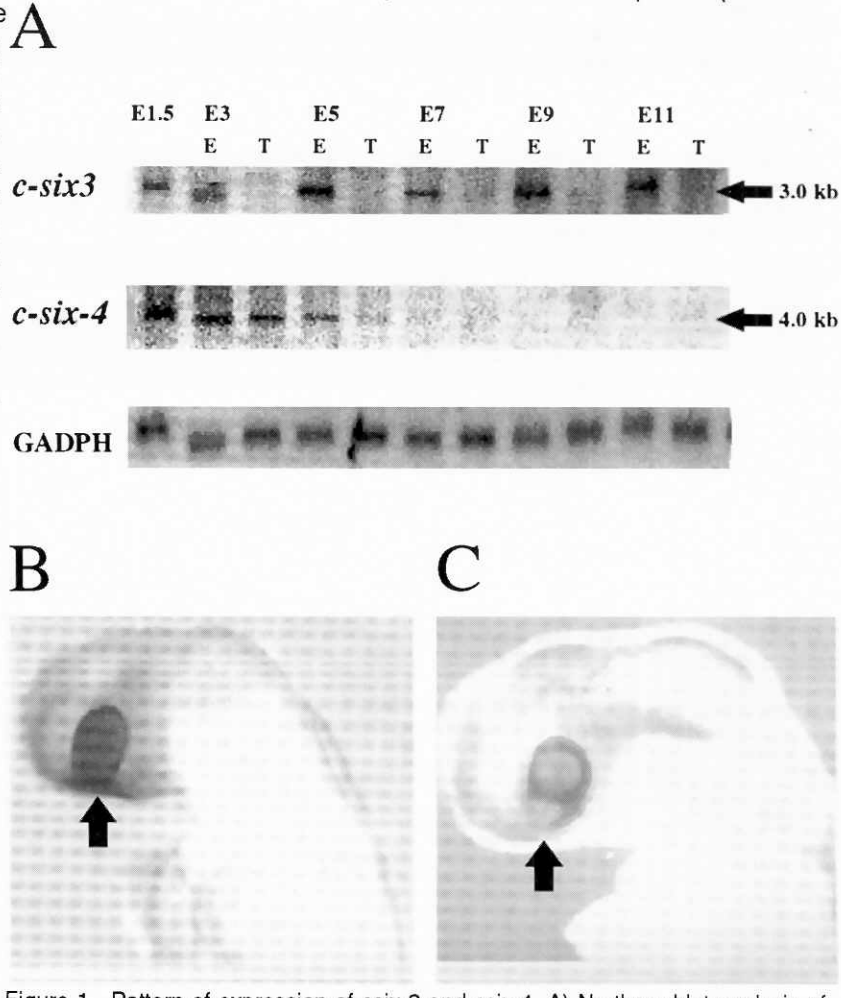

Figure 1. Pattern of expression of csix-3 and csix-4. A) Northern blot analysis of *csix-3* and *csix-4*, determined using total RNA extracted from E1.5 embryos and from isolated eye (E) and tectum (T) of chick embryos from E3 to E11. Whole mount in situ hybridisation of E2.5 embryos, using DIG-labelled probes for *c-six3* (B) and *csix-4* (C).

Further, csix-3 presented 100% identity with the mouse gene in the *Six* domain (a region comprising 110 aa in the 5' flanking region of the homeodomain), but diverged from it in the remaining of the sequence. The csix4 presented 75% and 81% homology with the Drosophila *sine oculis* in the homeo- and *Six* domain, respectively. Northern blot analysis of eye and tectum mRNA, isolated from E1, E3, E5, E7, E9 and E11 embryos showed one single transcript of 3.0 and 4.0 kb for *csix-3* and *csix-4*, respectively. Further, *csix-3* was expressed from very early stages, with a peak of expression in the E5 eye, while its expression was much lower in the tectum. The *csix-4* was expressed at early embryonic stages as well, but its maximal expression was at E3 both in the eye and in the tectal region (Fig 1A).

Using DIG-labelled probes we further studied the pattern of expression of *csix-3* and *csix-4* in chick embryos ranging from HH 5 up to E14, using in situ hybridisation in tissue sections and in whole mounts. Early on, *csix-3* is restricted to the anterior neural plate including areas that later on will give rise to ectodermal and neural derivatives. At later stages *csix-3* is expressed mainly in the eye (Fig. 1B) and at the base of the diencephalon. As development proceeds *csix-3* is expressed only in the neural retina but not in the pigmented epithelium or in the lens. The expression pattern of *csix-4* is confined the eye region (Fig.1C) especially to the neural retina.

The role of the six/sine oculis family during eye formation needs to be determined. However, based on the proposed function for the Drosophila gene and pattern of expression of these genes, it appears that they might be among the mayor players of eye formation.

Acknowledgements

This work was initiated while P.B. was holding an EC contract (BIO2-CT-94-6293) in E.B. lab. This study was supported by grants from EC Biotech, the Telethon-Italia Programs and the Italian Association for Cancer Research (AIRC) to E.B. and PB94-0102, DGICYT from Spanish Ministry of Education to P.B.

References

Cheyette, BNR, Green, PJ, Martin, K., Garren, H., Hartenstein, V., Zipursky, SL (1994) The *Drosophila sine oculis* locus encodes a homeodomain-containing protein required for the development of the entire visual system., Neuron, 12, 977-996.

Gehring, W.J., Quian, YQ, Billeter, M., Furokubo-Tokonaga, K., Schier, A.F., Resendez-Perez, D., Affolter, M., Otting, G. and Würthrich (1994) Homeodomain-DNA recognition. Cell, 78: 211-223.

Halder, G., Callaerts, P. and Gehring, W.J. (1995) New perspective on eye development. Curr. Opin. Genet. Dev. 5, 602-609.

Oliver, G., Wehr, R., Jenkins, N.A. Copeland, N.G., Cheyette, B.N., Hartenstein, V., Zipursky, SL and Gruss, P. (1995a) Homeobox genes and connective tissue patterning. Development, 121, 693-705.

Oliver, G., Mailhos, A., Wehr, R., Copeland, N.G., Jenkins, N.A. and Gruss, P. (1995b) *Six3*, a murine homologue of the sine oculis gene, demarcates the most anterior border of the developing neural plate and is expressed during eye development. Development, 121, 4045-4055.

Saha., M., Servetnick, M. and Grainger, R.M. (1992) Vertebrate eye development. Curr. Opin. Genet. Dev.. 2: 582-588.