Tunicate Neurogenesis: The Case of the *SoxB2* Missing CNE

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Abstract The discovery of the *SoxB2*/*Sox21* regulatory element, conserved from basal metazoa to human, opened novel perspectives to study the conservation among distant related genomes. This discovery represents exceptional maintenance of an almost identical enhancer structure controlling a gene that is fundamental for nervous system development. The activity of metazoan *SoxB2* enhancers was previously demonstrated in zebrafish embryos by cross-species experiments.

Here we tested the activity of human and amphioxus orthologue *cis*-regulatory sequences in embryos of the tunicate *Ciona intestinalis* through a transgenic approach, and found out that *SoxB2* enhancers retained their activity in neuronal differentiation even in a non-vertebrate chordate.

This result was unexpected since the conserved *SoxB2* enhancer was not found in *Ciona* in previous studies. Nevertheless, we adopted a different comparative approach and performed a phylogenetic footprinting analysis using two congeneric tunicate species, *C. intestinalis* and *Ciona savignyi*, that, in fact, evidenced a conserved *SoxB2* 3′ element. The discovered element could potentially be the missing orthologous *SoxB2* enhancer previously identified in human, zebrafish, and amphioxus.

A detailed search for possible transcription factors revealed the massive presence of Sox, Pou and Fox binding sites as found in other deuterostomes. Nevertheless, whether the conserved *SoxB2* element of *Ciona* possesses a functional ability as gene transcriptional enhancer remains to be demonstrated experimentally.

Keywords Evolution • Transgenesis • Nervous system • *Ciona* • *Cis*-regulatory enhancers

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