

doi: 10.1387/ijdb.140197LV

**SUPPLEMENTARY MATERIAL**

**corresponding to:**

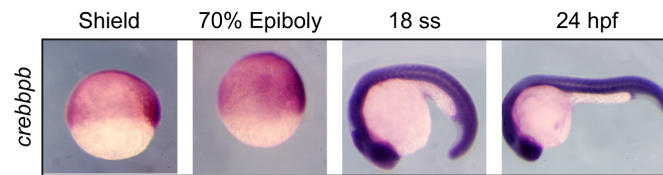
**Expression patterns of CREB binding protein (CREBBP) and  
its methylated species during zebrafish development**

JULIE BATUT, CARINE DUBOÉ and LAURENCE VANDEL

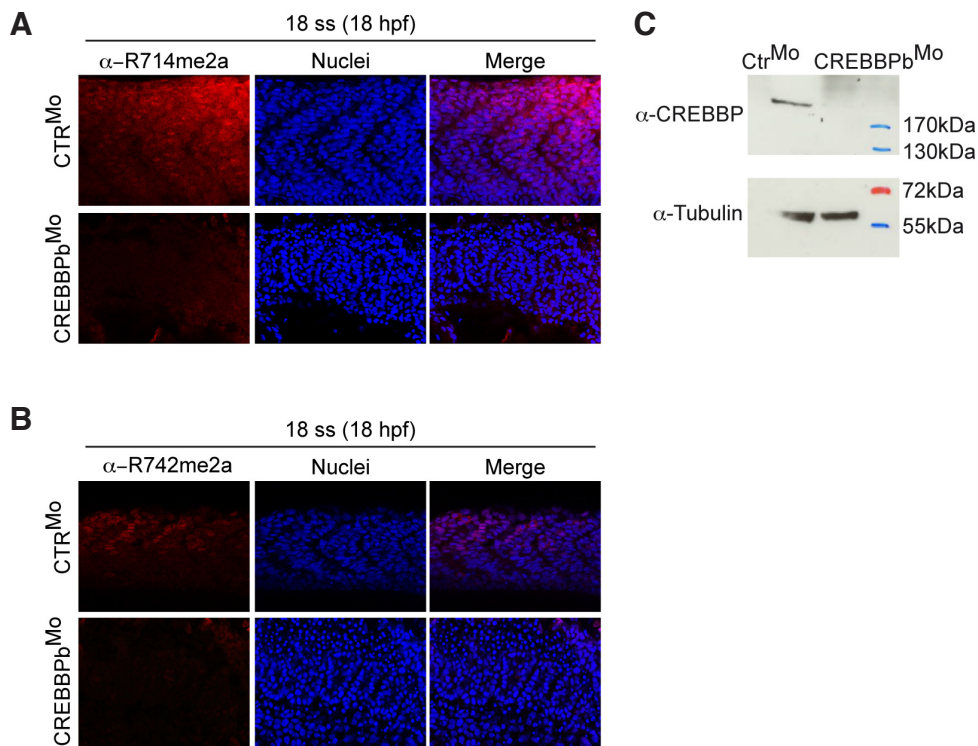
**\*Address correspondence to:** Laurence Vandel, CBD UMR5547 CNRS, 118 route de Narbonne, 31062 Toulouse cedex, France. Tel: (33)-561-558383.  
Fax: (33)-561-556507. E-mail: laurence.vandel@univ-tlse3.fr

**Full text** for this paper is available at: <http://dx.doi.org/10.1387/ijdb.ijdb.140197LV>

*Accepted:* 15 January 2015.



**Supplementary Figure S1.** *In situ hybridization against crebbpb at the indicated stages of development. The crebbpb transcript is ubiquitously expressed from gastrulation to 24 hpf.*



**Supplementary Figure S2.** *Validation of CREBBP-methylated specific antibodies on zebrafish embryos. (A,B) Immunohistochemistry with CREBBP-methylated specific antibody at 18 ss using (A) R714me2a or (B) R742me2a antibody on embryos injected with Control morpholino (CTR<sup>Mo</sup>) and CREBBPb morpholino (CREBBPb<sup>Mo</sup>). Nuclei are stained in blue with To-Pro3 and a merged picture is shown. Expression of CREBBP-R714me2a and CREBBP-R742me2a is strongly reduced in CREBBPb<sup>Mo</sup>- as compared to control<sup>Mo</sup>-injected embryos. Injections were performed at the 1-cell stage with 6 ng of CTR<sup>Mo</sup> or CREBBPb<sup>Mo</sup> (Gene-tools). Morpholino sequences were: CREBBPb 5'-GACTGTTGCCACCTGCCATGCCCAT-3' and Control 5'-CCTCTTACCTCAGTTACAATTATA-3'. (C) Knock-down of CREBBPb reduces CREBBPb protein level in 14 ss old zebrafish embryos. Embryos injected with either CTR<sup>Mo</sup> or CREBBPb<sup>Mo</sup> were collected at 14 ss and processed for western blot analysis to detect CREBBP expression. Tubulin was used as a loading control and a protein ladder is shown. Whole cell extracts from 20 embryos were classically prepared in 40 ml Laemmli sample buffer. 5 ml of each sample (4 embryos) were loaded per lane and subjected to SDS-PAGE analysis. The following antibodies were used: anti-CREBBP (1/200, A22, santa Cruz), anti  $\alpha$ -Tubulin (1:5000, Sigma, T9026), anti-Rabbit IgG, HRP conjugate (1:50000, Promega, W4011) and anti-Mouse IgG, HRP conjugate (1:10000, Promega, W4021). Protein Ladder, PageRuler prestained Protein Ladder (Fermentas, SM0671).*