CDYL : a new transcriptional repressor

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Supplementary Materials

Figure 1. Selection of Xenopus cDNAs based on their homology to mouse sequences.

Xenopus (tadpoles) double stranded cDNAs fragments homologous to cDNA fragments from differentiated mouse B16 cells were isolated as described in materials and methods. **A)** An ethidium bromide-stained gel containing Xenopus cDNAs before selection (lane 1), and cDNAs obtained after the first and second rounds of selection (lanes 2 and 3 respectively) is shown. The gel was then transferred onto a membrane and probed with a fraction of "selection 2" cDNAs (lower panel). **B)** Homology between the mouse CDYL cDNA and one of the Xenopus cDNA fragments from "selection 2". Numbers indicate the position of nucleotides in the Xenopus and mouse cDNAs respectively.

Figure 2. Colocalisation of CDYL with HDAC1 and HDAC2.

HeLa cells were transfected with Flag-HDAC1(left) or Flag-HDAC2 (right) expression vectors, without (upper) or with (lower) a Ha-CDYL expression vector. Twenty four hours post-transfection, the cells were fixed and the intracellular localisations of the Flag-HDACs and Ha-CDYL were monitored by immunofluorescence using anti-flag and anti Ha-antibodies.

Supplementary Materials and Methods

Isolation of Xenopus cDNAs with sequences highly homologous to mouse sequences

One μg polyA+ RNA isolated from Xenopus tadpoles (taken at stage 45) or from mouse B16 cells induced to differentiate for twenty four hours with 10 mM butyrate was used to synthesize double stranded cDNAs using an appropriate kit (Amersham) according to the vendor's instruction. Each cDNA was digested either with RsaI or with AluI in separate tubes. Digested

cDNAs were pooled and ligated to either Xenopus or mouse specific linkers. Linkers were as follows : Xenopus1, tagtccgaattcaagcaagagcaca; Xenopus 2, ctcttgcttgaattcggacta ; mouse 1, gcggtgacccgggagatctgaattc ; mouse 2, gaattcagatc. Non-ligated linkers were then removed and Xenopus and mouse cDNAs were PCR amplified using Xenopus 2 and mouse 1 primers respectively. Ten μg of amplified mouse cDNA were incubated with 3' terminal transferase and dATP (Boehringer) according to the supplier's recommendations. After phenol-chloroform extraction, the tailed cDNAs were mixed with 1 μg of amplified Xenopus cDNA in 10 μl hybridization buffer (750 mM NaCl, 25 mM hepes pH, 7.6, 5 mM EDTA and 0.1% SDS), denatured at 100°C for 10 min, and incubated at 68°C for 20 hours. These materials were then diluted and loaded onto an oligo dT column according to the standard protocol for the selection of poly A+ RNAs. Retained cDNAs were PCR amplified using the Xenopus 1 primer. One μg of this cDNA was used to hybridize with 10 μg of mouse poly A-tailed cDNAs as above. Another round of selection was performed and the final Xenopus cDNAs were cloned in the bluescript vector.

Immunolocalisation

Immunolocalisation of HA-tagged CDYL and Flag-tagged HDACs were carried out as previously described (Lemercier *et al*., 2002).

Reference

Lemercier, C., Brocard, M.P., Puvion-Dutilleul, F., Kao, H.Y., Albagli, O. and Khochbin, S. (2002). Class II histone deacetylases are directly recruited by BCL6 transcriptional repressor. *J Biol Chem*, **277**, 22045-22052.

Supplementary Figure 1

Caron *et al.* (top)

Supplementary Figure 2