

Supplementary method 2: Chromatin immunoprecipitation (ChIP).

Nuclear pellet was resuspended in cross-linking buffer containing 15 mM HEPES pH 7.9, 0.34 M sucrose, 60 mM KCl, 2 mM MgCl₂, 15 mM NaCl, 0.15 mM β-Mercaptoethanol, 0.5 mM PMSF and complete protease inhibitor cocktail. Formaldehyde was then added to a final concentration of 1% and nuclei were incubated for 13 min at 30°C. Addition of 0.125 M glycine (final) stopped the cross-linking reaction. Nuclei were centrifuged for 10 min at 10000g, 4°C, resuspended in sonication buffer containing 50 mM HEPES pH 7.9, 140 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% Na-deoxycholate, 0.1% SDS, 0.5 mM PMSF and complete protease inhibitor cocktail, and split into seven 400 µl aliquots. Chromatin aliquots were extensively sonicated in ice water (7 times 5 min at power output 7 with a duty cycle of 50% using the indirect sonication setup) with a W-375 sonicator (Ultrasonics Inc.) and centrifuged for 20 min at 21000g, 4°C. At this point one aliquot was saved as input sample. After centrifugation the soluble chromatin was precleared and subjected to immunoprecipitation as previously described (Soutoglou et al., 2001). In this study we used the polyclonal rabbit α-HNF1α-NC-AR and α-HNF1β-NC-AR antibodies depicted above. Immune complexes were collected by adsorption to protein A-Sepharose (Sigma).

References

- Soutoglou, E., Viollet, B., Vaxillaire, M., Yaniv, M., Pontoglio, M. and Talianidis, I. (2001) Transcription factor-dependent regulation of CBP and P/CAF histone acetyltransferase activity. *Embo J*, **20**, 1984-1992.