letters to nature

Together, these data show that the large family of activating homeodomain transcription factors, such as Pit-1, exert their functions through a balance between the N-CoR co-repressor complexes and the CBP co-activator complexes. Pit-1 activity is regulated by distinct signal-transduction pathways, through mechanisms that do not appear to involve modification of Pit-1 itself, but at least in part through regulation of the recruited co-activator complex. A surprising consequence of these events is that a single transcription factor, Pit-1, actually uses the HAT functions of different proteins, and requires the function of different domains of CBP when activated in response to cAMP or growth-factors (Fig. 5d). These results have implications for the mechanism of integration of signalling events that control complex patterns of gene expression by many classes of transcription factors.

Methods

Protein-protein interaction assays. All the GST-fusion proteins were expressed and purified as described previously^{4,15}. *In vitro* protein–protein interaction assay, immunoprecipitation, GST pull-down and DNA-dependent protein–protein interaction (ABCD) assays were done as described^{4,6,18}.

Nuclear microinjection, staining and fluorescence microscopy. Microinjection analysis was done as described previously using affinity-purified IgGs^{6,15,26,28}.

Transient transfection. HeLa and 293 cells were maintained in DMEM supplemented with 10% fetal bovine serum. Calcium precipitation mediated transient transfection was done according to standard protocol.

Received 13 March; accepted 29 June 1998

- Gray, S. & Levine, M. Transcriptional repression in development. Curr. Opin. Cell Biol. 8, 358–364 (1996).
- Hermesz, E., Mackem, S. & Mahon, K. A. Rpx: a novel anterior-restricted homeobox gene progressively activated in the prechorda plate, anterior neural plate and Rathke's pouch of the mouse embryo. *Development* 122, 41–52 (1996).
- Ingraham, H. A. et al. A tissue-specific transcription factor containing a homeodomain specifies a pituitary phenotype. Cell 55, 519–529 (1988).
- Horlein, A. J. et al. Ligand-independent repression by the thyroid hormone receptor mediated by a nuclear receptor co-repressor. Nature 377, 397–404 (1995).
- Chen, J. D. & Evans, R. M. A transcriptional co-repressor that interactions with nuclear hormone receptors. *Nature* 377, 454–457 (1995).
- Heinzel, T. et al. A complex containing N-CoR, mSin3 and histone deacetylase mediates transcriptional repression. Nature 387, 43–48 (1997).
- Alland, L. et al. Role for N-CoR and histone deacetylase in Sin3-mediated transcriptional repression. Nature 387, 49–55 (1997).
- Laherty, C. D. et al. Histone deacetylases associated with the mSin3 corepressor mediate Mad transcriptional repression. Cell 89, 349–356 (1997).
- Chrivia, J. C. et al. Phosphorylated CREB binds specifically to the nuclear protein CBP. Nature 365, 855–859 (1993).
- Yang, X.-Y., Ogryzko, V. V., Nishikawa, J., Howard, B. H. & Nakatani, Y. A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature* 382, 319–324 (1996).
- 11. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H. & Nakatani, Y. The transcriptional coactivator p300 and CBP are histone acetyltransferases. *Cell* 87, 953–960 (1996).
- Bannister, A. J. & Kouzarides, T. The CBP coactivator is a histone acetyltransferase. *Nature* 384, 641-643 (1996).
- Yang, W.-M., Inouye, C., Zeng, Y., Bearss, D. & Seto, E. Transcriptional repression by YY1 is mediated by interaction with a mammalian homolog of the yeast global regulator HDAC2/mRPD3. *Proc. Natl Acad. Sci. USA* 93, 12845–12850 (1996).
- Kurokawa, R. et al. Polarity-specific activities of retinoic acid receptors determined by a co-repressor. Nature 377, 451–454 (1995).
- Kamei, Y. et al. A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. Cell 85, 403–414 (1996).
- Lavinsky, R. M. et al. Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. Proc. Natl Acad. Sci. USA 95, 2920–2925 (1998).
- Lundblad, J. R., Kwok, R. P., Laurance, M. E., Harter, M. L. & Goodman, R. H. Adenoviral E1Aassociated protein p300 as a functional homologue of the transcriptional co-activator CBP. *Nature* 374, 85–88 (1995).
- Glass, C. K., Lipkin, S. M., Devary, O. V. & Rosenfeld, M. G. Positive and negative regulation of gene transcription by a retinoic acid-thyroid hormone receptor heterodimer. *Cell* 59, 697–708 (1989).
- Jacobson, E. M., Li, P., Leon-del-Rio, A., Rosenfeld, M. G. & Aggarwal, A. K. Structure of Pit-1 POU domain bound to DNA as a dimer: unexpected arrangement and flexibility. *Genes Dev.* 11, 198–212 (1997).
- Elsholtz, H. P., Lew, A. M., Albert, P. R. & Sundmark, V. C. Inhibitory control of prolactin and Pit-1 gene promoters by dopamine. J. Biol. Chem. 266, 22919–22925 (1991).
- Murdoch, G. H., Waterman, M., Evans, R. M. & Rosenfeld, M. G. Molecular mechanism of phorbol ester, thyrotropin-releasing hormone, and growth factor stimulation of prolactin gene transcription. *J. Biol. Chem.* 260, 11852–11858 (1985).
- Howard, P. W. & Maurer, R. A. A composite Ets/Pit-1 binding site in the prolactin gene can mediate transcriptional responses to multiple signal transduction pathways. J. Biol. Chem. 270, 20930–20936 (1995).
- 23. Okimura, Y., Howard, P. W. & Maurer, R. A. Pit-1 binding sites mediate transcriptional responses to cyclic adenosine 3',5'-monophosphate through a mechanism that does not require inducible

phosphorylation of Pit-1. Mol. Endocrinol. 8, 1559-1565 (1994).

- Kapiloff, M. S., Farkash, Y., Wegner, M. & Rosenfeld, M. G. Variable effects of phosphorylation of Pit-1 dictated by the DNA response elements. *Science* 253, 786–789 (1991).
- Õnate, S. A., Tsai, S. Y., Tsai, M.-J. & O'Malley, B. W. Sequence and characterization of a coactivator for the steroid hormone receptor superfamily. *Science* 270, 1354–1357 (1995).
- Torchia, J. et al. The transcriptional co-activator p/CIP binds CBP and mediates nuclear-receptor function. Nature 387, 677-684 (1997).
- Korzus, E. et al. Transcriptional factor-specific requirements for coactivators and their acetyltransferase functions. Science 279, 703–707 (1998).
- Rose, D. W., McCabe, G., Feramisco, J. R. & Adler, M. Expression of c-fos and AP-1 activity in senescent human fibroblasts is not sufficient for DNA synthesis. J. Cell. Biol. 119, 1405–1411 (1992).

Supplementary information is available on *Nature's* World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of Nature.

Acknowledgements. We thank C. Laherty, R. Eisenman, W.-M. Yang and E. Seto for antibodies, C. Nelson for cell culture, L.-M. Philips for assistance, and P. Meyer for her expertise in the preparation of the illustrations. This work was supported by an American Heart Association Predoctoral fellowship (L.X.), the NSF (R.M.L.), the Lucille Markey Fund (J.S.D.), and an NIH predoctoral fellowship (D.S.), a US Army Medical Research Program Award (E.K.), by grants from HHMI and NIH (to M.G.R.), and by an American Diabetes Association Career Development Award (to D.W.R.). C.K.G. is an Established Investigator of the American Heart Association and M.G.R. is an investigator of the Howard Hughes Medical Institute.

Correspondence and requests for materials should be addressed to M.G.R. (e-mail: mrosenfeld@ucsd.edu).

errata

Evidence for the shikimate pathway in apicomplexan parasites

Fiona Roberts, Craig W. Roberts, Jennifer J. Johnson, Dennis E. Kyle, Tino Krell, John R. Coggins, Graham H. Coombs, Wilbur K. Milhous, Saul Tzipori, David J. P. Ferguson, Debopam Chakrabarti & Rima McLeod

Nature 393, 801-805 (1998)

The correct designation of symbols in Figs 1b, d and 3a is as follows. In Fig. 1b, open squares: PABA alone; filled squares: sulphadiazine and PABA; open circles: glyphosate and PABA; filled circles: pyrimethamine and PABA. Figure 1d shows *in vivo* activity against *T. gondii* of glyphosate (100 mg kg⁻¹ day⁻¹) and pyrimethamine (12.5 mg kg⁻¹ day⁻¹), alone or in combination. Open squares, with 50% survival at 30 days: combination of glyphosate and pyrimethamine; filled circles: pyrimethamine alone; open circles: glyphosate alone. Open squares, with 0% survival at 9 days: untreated controls. In Fig. 3a, diamonds: PABA alone; squares: folic acid alone; upright triangles: glyphosate and PABA; inverted triangles: glyphosate and folic acid.

Phase-mapping of periodically domain-inverted LiNbO₃ with coherent X-rays

Z. W. Hu, P. A. Thomas, A. Snigirev, I. Snigireva, A. Souvorov, P. G. R. Smith, G. W. Ross & S. Teat

Nature **392**, 690–693 (1998)

The labels for Fig. 2b and c should be interchanged.