Rapid radiolabel-sparing thin-layer chromatography method for the visual assessment of chloramphenicol acetyltransferase gene expression

R. Aubin, R. Fourney, M. Weinfeld and M. C. Paterson

Molecular Genetics and Carcinogenesis Laboratory, The W.W. Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta T6G 1Z2, Canada
Submitted September 2, 1987

Recombinant vectors bearing the gene cartridge encoding *E. coli* chloramphenicol acetyltransferase (CAT; acetyl-CoA:chloramphenicol 3-O-acetyltransferase, EC 2.3.1.28) are widely used as convenient "reporters" to study various features of eucaryotic gene promoters and associated regulatory elements (1,2). Sequences to be assayed for their effects on gene expression are fused to the CAT gene and the hybrid constructs introduced by gene transfer into suitable recipient cells in culture. Two to three days following transfection, the levels of transiently expressed CAT enzyme are assayed *in vitro* by preparing cell extracts and measuring the extent of acetylation of ^{14} C-labeled chloramphenicol using acetyl coenzyme A as the acetyl group donor (3). Finally, labeled products are separated from the substrate by thin-layer chromatography (TLC) and the corresponding spots are localized by autoradiography and quantitated by liquid scintillation counting (3). We have significantly shortened the overall procedure through the use of plastic-backed fluorescent silica gel TLC sheets (E. Merck #60F-254), thus eliminating the time-consuming autoradiography step. Using this modification, chloramphenicol and its acetylated derivatives are resolved within 10-15 minutes when samples are run on TLC strips (12 cm in length) with chloroform:methanol (95:5) as the solvent. The reaction products are then visualized under UV light (254-304 nm) and the spots are simply excised and radioactivity is quantitated by liquid scintillation spectrometry. A permanent photographic record of the chromatogram can also be obtained using a Polaroid MP4 Industrial Land camera system equipped with a Kodak Wratten No. 9 (yellow) gelatin filter and type 655 posi-negative film. The accompanying figure provides an example of such a chromatogram in which CAT activity was determined for NIH 3T3 cells transfected with the plasmid pSV2cat (3). Not only does this modification provide a rapid visual assessment of CAT activity but it is also more cost effective, since a quantitative determination of chloramphenicol acetylation can be routinely performed in the presence of 7.5 nmol unlabeled chloramphenicol (3) supplemented with as little as 0.01 μ Ci ^{14} C-labeled substrate per reaction.

Separation of chloramphenicol (Cm), 1-acetyl chloramphenicol (1-AcCm) and 3-acetyl chloramphenicol (3-AcCm) for 20 μ l of cell extract incubated for 7.5 minutes (lane 1) and 30 minutes (lane 2) at 37°C. The arrow denotes the origin of migration.

ACKNOWLEDGEMENTS

We are grateful to the Alberta Heritage Foundation for Medical Research and the Medical Research Council of Canada for supporting this work.

REFERENCES: 1. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. and Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA*, 79: 6777-6781. 2. Yang, J.-Q., Remmers, E. F. and Marcu, K. B. (1986) *EMBO J.* 5: 3553-3562. 3. Gorman, C. M., Moffat, L. F. and Howard, B. H. (1982) *Mol. Cell. Biol.* 2: 1044-1051.