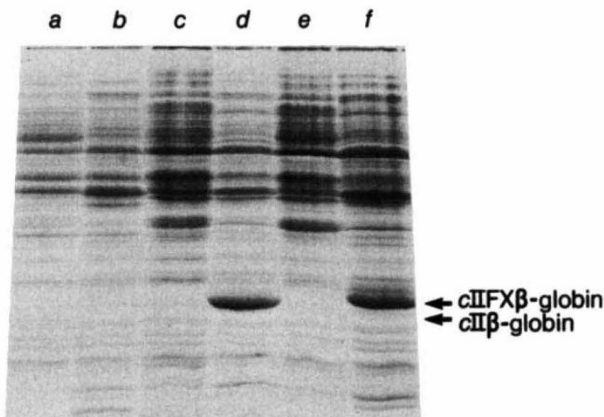

Fig. 2 Expression of cIIβ-globin hybrid protein in *E. coli*. Total cellular proteins from MZ-1 carrying pLcII, pLcIIβ and pLcIIFXβ, with and without 2 h of heat induction, were analysed on an 18% polyacrylamide-SDS gel and visualized with Coomassie blue. *a*, pLcII at 30 °C; *b*, pLcII at 42 °C; *c*, pLcIIβ at 30 °C; *d*, pLcIIβ at 42 °C; *e*, pLcIIFXβ at 30 °C; *f*, pLcIIFXβ at 42 °C. **Methods:** MZ-1 cells harbouring the expression plasmids pLcII, pLcIIβ and pLcIIFXβ were grown to $A_{600} = 0.7$ in 2×TY medium (16 g Tryptone, 10 g yeast extract, 5 g l^{-1} NaCl) at 30 °C and for each of the cultures, one half was mixed with an equal volume of 2×TY preheated to 65 °C and grown at 42 °C. The other half was grown further at 30 °C as a control. The total cellular protein was extracted with an equal volume of phenol and spun down after precipitation with 5 vol. ethanol[8]. The pellet was dissolved in SDS sample buffer and analysed on an 18% polyacrylamide-SDS gel[28].

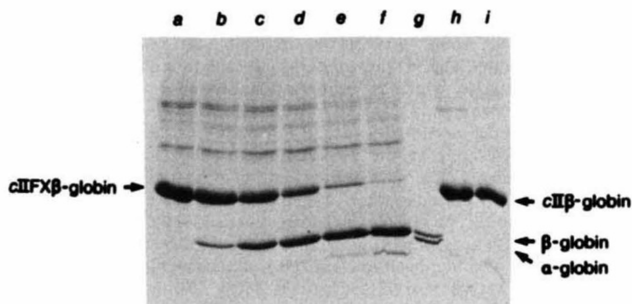

Fig. 3 Digestion of cIIFXβ-globin protein with bovine blood coagulation factor X_a. The cIIFXβ-globin hybrid protein was digested with factor X_a at an enzyme to substrate molar ratio of 1:100 at 25 °C and analysed on an 18% polyacrylamide-SDS gel[28]. *a*, Undigested cIIFXβ-globin; *b*, 5 min; *c*, 15 min; *d*, 30 min; *e*, 60 min; *f*, 120 min after addition of factor X_a; *g*, human adult haemoglobin comprising the α (faster band) and β (slower band) chains. The cIIβ-globin hybrid protein which lacks the cleavage site for factor X_a was treated with factor X_a in the same conditions: *h*, after 120 min of treatment; *i*, untreated cIIβ-globin. **Methods:** MZ-1 cells harbouring pLcIIFXβ were grown in 500 ml of 2×TY medium. At $A_{600} = 0.7$, the culture was mixed with 500 ml of 2×TY preheated to 65 °C, then grown at 42 °C. After 2 h, cells were collected, and the high salt precipitate was prepared[29]. The pellet was dissolved in 30 ml of 10 mM Na-phosphate *p*H 6.4, 1% SDS (BDH), 1% β-mercaptoethanol and incubated in a boiling water bath for 5 min. The sample was dialysed against 10 mM Na-phosphate *p*H 6.0, 1 mM dithiothreitol, 0.1% SDS and purified on a hydroxylapatite (Bio-Rad, DNA grade) column[30]. The cIIFXβ-globin hybrid protein was concentrated by ultrafiltration (Amicon, PM-10 membrane) then precipitated with 6 vol. acetone/0.1 M HCl to remove SDS. The precipitate was air-dried, dissolved in a minimal amount of 8 M urea, 50 mM Tris-HCl *p*H 8.0 and dialysed against 100 mM NaCl, 50 mM Tris-HCl *p*H 8.0 and 1 mM $CaCl_2$. Bovine blood coagulation factor X (given by Dr M. P. Esnouf) was activated to factor X_a with Russell's viper venom (Sigma)[11].

We thank Dr M. F. Perutz for suggesting this project; Drs K. McKenney, S. Anderson and S. Brenner for many helpful suggestions; Dr D. M. Brown for help with the oligonucleotide synthesis; Dr A. Klug for criticism; Drs M. P. Esnouf, E. Remaut, K. McKenney, M. Rosenberg, K. D. Smith, J. Clegg, J. V. Kilmartin and B. S. Sproat for essential materials; and Dr Y. Enoki for encouragement. K.N. was supported by a Thomas Usher Fellowship and recently by NIH grant RO1 HL 31461-01. H.C.T. was supported by an EMBO long-term fellowship. This paper is dedicated to Max Perutz on the occasion of his 70th birthday.

Received 17 April; accepted 1 May 1984.

1. Remaut, E., Tsao, H. & Friers, W. *Gene* **22**, 103–113 (1983).
2. Queen, C. *J. molec. appl. Genet.* **2**, 1–10 (1983).
3. Shatzman, A., Ho. Y.-S. & Rosenberg, M. in *Experimental Manipulation of Gene Expression* (ed. Inouye, M.) 1–14 (Academic, New York, 1983).
4. Shine, J. & Dalgarno, L. *Nature* **254**, 34–38 (1975).
5. Steitz, J. A. in *Biological Regulation and Development, 1: Gene Expression* (ed. Goldberger, R. F.) 349–399 (Plenum, New York, 1979).
6. Harris, T. J. R. in *Genetic Engineering* Vol. 4 (ed. Williamson, R.) 127–185 (Academic, London, 1983).
7. Magnusson, S., Petersen, T. E., Sottrup-Jensen, L. & Claeys, H. in *Proteases and Biological Control* (eds Reich, E., Rifkin, D. B. & Shaw, E.) 123–149 (Cold Spring Harbor Laboratory, New York, 1975).
8. Remaut, E., Stanssens, P. & Fiers, W. *Gene* **15**, 81–93 (1981).
9. Rosenberg, M., Court, D., Shimatake, H., Brady, C. & Wulff, D. L. *Nature* **272**, 414–423 (1978).
10. Towbin, H., Sraehelin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).
11. Fujikawa, K., Legaz, M. E. & Davie, E. W. *Biochemistry* **11**, 4892–4898 (1972).
12. Itakura, K. *et al. Science* **198**, 1056–1063 (1977).
13. Shine, J., Fettes, I., Lan, N. C. Y., Roberts, J. L. & Baxter, J. D. *Nature* **285**, 456–461 (1980).
14. Fujikawa, K., Legaz, M. E. & Davie, E. W. *Biochemistry* **11**, 4882–4891 (1972).
15. Walz, D. A., Hewett-Emmett, D. & Seegers, W. H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1969–1972 (1977).
16. Butkowski, R. J., Elion, J., Downing, M. R. & Mann, K. G. *J. biol. Chem.*, **252**, 4942–4957 (1977).
17. Magnusson, S., Sottrup-Jensen, L., Petersen, T. E., Dudek-Wojciechowska, G. & Claeys, H. in *Proteolysis and Physiological Regulation* (eds Ribbons, D. W. & Brew, K.) 203–238 (Academic, New York, 1976).
18. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning* (Cold Spring Harbor Laboratory, New York, 1982).
19. Murray, N. E., Bruce, S. A. & Murray, K. *J. molec. Biol.* **132**, 493–505 (1979).
20. Tait, R. C., Rodriguez, R. L. & West, R. W. Jr *J. biol. Chem.* **255**, 813–815 (1980).
21. Sproat, B. S. & Bannmarth, W. *Tetrahedron Lett.* **24**, 5771–5774 (1983).
22. Gait, M. J., Matthes, H. W. D., Singh, M., Sproat, B. S. & Titmas, R. C. *Nucleic Acids Res.* **10**, 6243–6254 (1982).
23. Vieira, J. & Messing, J. *Gene* **19**, 259–268 (1982).
24. McKenney, K. thesis, Johns Hopkins Univ. (1982).
25. Wilson, J. T. *et al. Nucleic Acids Res.* **5**, 563–581 (1978).
26. Lawn, R. M., Efstratiadis, A., O'Connell, C. & Maniatis, T. *Cell* **21**, 647–651 (1980).
27. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
28. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
29. Gilmer, T. M., Parsons, J. T. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2152–2156 (1982).
30. Moss, B. & Rosenblum, E. N. *J. biol. Chem.* **247**, 5194–5198 (1972).

Erratum

Expression and amplification of the N-*myc* gene in primary retinoblastoma

W.-H. Lee, A. L. Murphree & W. F. Benedict
Nature **309**, 458–460 (1984).

THE first paragraph on page 460 was printed incorrectly. It should read: 'amplification in neuroblastoma and N-*myc* expression in the retinoblastoma strongly suggests that the N-*myc* gene may belong to the proto-oncogene family. The fact that the N-*myc* gene is amplified and expressed in both retinoblastoma and neuroblastoma is of obvious interest. This is especially so because both tumours originate from neural crest ectodermal cells, although retinoblastoma may develop from a more primitive cell than neuroblastoma[22]. However, we have no evidence to exclude the possibility that genes other than N-*myc* are involved in these two types of tumour formation.'

© 1984 Nature Publishing Group