reflect the origin of T cells from a lymphoid precursor common to B and T cells. However, in this context it is surprising that B cells show only one hypersensitive site in this region (U.S. and B.A., unpublished results) compared with at least three in T cells. Second, these sites may have become activated in the course of the activation of some other T-cell-specific gene in the vicinity. This would be analogous to the finding of aberrant rearrangements of immunoglobulin genes in B lymphocytes which express some other functionally rearranged immunoglobulin gene[24–27]. The part which B-cell immunoglobulin genes play in T-cell function remains to be elucidated.

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1. Kemp, D. J., Harris, A. W., Cory, S. & Adams, J. M. *Proc. natn. Acad. Aci. U.S.A.* **77**, 2876–2880 (1980).
2. Kemp, D. J., Wilson, A., Harris, A. W. & Shortman, K. *Nature* **286**, 168–170 (1980).
3. Kronenberg, M., Davis, M., Early, P., Hood, L. & Watson, J. *J. exp. Med.* **152**, 1745 (1980).
4. Near, R. & Storb, U. *Biochemistry* **20**, 2386–2394 (1981).
5. Kurosawa, Y. *et al. Nature* **290**, 565–569 (1981).
6. Wu, C., Bingham, P. M., Livak, K. J., Holmgren, R. & Elgin, S. C. R. *Cell* **16**, 797–806 (1979).
7. Sundin, O. & Varshavsky, A. *J. molec. Biol.* **132**, 535–446 (1979).
8. Wu, C. *Nature* **286**, 854–860 (1980).
9. Stalder, J. *et al. Cell* **20**, 451–460 (1980).
10. Keene, M., Corces, V., Lowenhaupt, K. & Elgin, S. *Proc. natn. Acad. Sci. U.S.A.* **78**, 143–146 (1979).
11. Storb, U. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2467–2471 (1976).
12. Storb, U., Wilson, R., Selsing, E. & Walfield, A. *Biochemistry* **20**, 990–996 (1981).
13. Wilson, R., Miller, J. & Storb, U. *Biochemistry* **18**, 5013–5021 (1979).
14. Ralph, P. *J. Immun.* **110**, 1470–1475 (1973).
15. Marcu, K. B., Banerji, J., Penncavage, N. A., Lang, R. & Arnheim, N. *Cell* **22**, 187–196 (1980).
16. Liu, C.-P., Tucker, P. W., Mushinski, J. F. & Blattner, F. R. *Science* **209**, 1348–1353 (1980).
17. Cory, S., Adams, J. M. & Kemp, D. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 4943–4947 (1980).
18. Findly, R. C. & Pederson, T. *J. Cell Biol.* **88**, 323–328 (1981).
19. Davis, M. M., Kim, S. K. & Hood, L. E. *Science* **209**, 1360–1365 (1980).
20. Ravetch, J. V., Kirsch, I. R. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6734–6738 (1980).
21. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676–683 (1980).
22. Takahashi, N., Nakai, S. & Honjo, T. *Nucleic Acids Res.* **8**, 5983–5991 (1980).
23. Cory, S., Webb, E., Gough, J. & Adams, J. *Biochemistry* **20**, 2662–2671 (1981).
24. Storb, U., Arp, B. & Wilson, R. *Nucleic Acids Res.* **8**, 4681–4687 (1980).
25. Walfield, A., Selsing, E., Arp, B. & Storb, U. *Nucleic Acids Res.* **9**, 1101–1109 (1981).
26. Schnell, H., Steinmetz, M. & Zachau, H. *Nature* **286**, 170–173 (1980).
27. Max, E. E., Seidman, J. G., Miller, H. & Leder, P. *Cell* **21**, 793–799 (1980).
28. Southern, E. M. *J. molec. Biol.* **98**, 503–517 (1975).

Introduction of a rabbit β-globin gene into the mouse germ line

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The introduction of cloned foreign genes into cultured mammalian cells[1–7] has been used to identify DNA sequences required for correct transcription *in vivo*[8–10]. It is not clear, however, to what extent these systems will be useful for an analysis of the sequences necessary for tissue-specific gene expression. A more appropriate approach for such an analysis might be the production of mice that contain a cloned foreign gene in all their cells, throughout development. This could be accomplished by the transfer of a cloned gene into germ-line cells, and the subsequent transmission of that gene to offspring. Previously, SV40 DNA sequences[11] and a cloned HSV-1 thymidine kinase gene[12] have been introduced into somatic tissues of mice by microinjection of the DNAs into blastocysts[11] or eggs[12], but germ-line transmission of these sequences has not been demonstrated. The only foreign DNA sequences which have been transferred into and transmitted by the mouse germ-line have been exogenous Moloney leukaemia virus genomes introduced by viral infection of early embryos[13]. We now report the introduction of a cloned rabbit DNA fragment containing the adult β-globin gene into the germ-line of mice. We have analysed 24 mice derived from eggs microinjected with this DNA. Nine mice contain the rabbit β-globin gene in liver DNA, and at least four males from this group transmit the gene to a fraction of their progeny.

Fertilized eggs were recovered 3–7 h before first cleavage from (C57BL/6×CBA/H)F_1 female mice that had been mated to (C57BL/6×CBA/H)F_1 males, and one pronucleus was injected with DNA, using a glass micropipette[12]. The DNA was prepared from a recombinant bacteriophage, λCH4A.RβG2 (λRβG2), which contains the rabbit adult β-globin gene, β1, and a β-like pseudogene, Ψβ2, in a 19-kilobase pair (kbp) chromosomal DNA fragment[14–16] (see Fig. 1). Most of the λRβG2 DNA was in the form of linear monomers but a small fraction (10–20%) consisted of multimers, noncovalently joined by the cohesive ends of λ DNA. Approximately 1 pl of DNA solution, at concentrations ranging from 5 to 50 μg ml^{-1}, corresponding to 100–1,000 λRβG2 molecules, was injected into a pronucleus. Eggs that survived injection (50%) were transferred to the oviducts of pseudo-pregnant (C57BL/6×CBA/H)F_1 females[17], and 15% of them developed to term, yielding a total of 51 live-born mice. Partial hepatectomies were performed on 24 of the mice at 6–8 weeks of age. Total liver DNA was extracted, digested with the restriction endonuclease *Kpn*I and screened for rabbit β-globin sequences by Southern blot hybridization[18].

Figure 2 shows the results for the first 18 mice. Eight of these mice contain sequences in their liver DNA which hybridize to probes for the rabbit β-globin genes, β1 and Ψβ2. The most intensely hybridizing bands correspond in size to three λRβG2 *Kpn*I fragments homologous to the probes (18.5, 4.7 and 2.0 kbp; see Fig. 1). Of six more mice screened (data not shown), one also contains the same λRβG2 *Kpn*I fragments. The average number of copies of the rabbit β-globin gene per liver cell in each of the positive mice may be estimated from the intensities of hybridization relative to known amounts of *Kpn*I-digested λRβG2 DNA in parallel lanes. Mice 40 (not shown) and 7 contain 1 or 2 copies, mice 3, 4, 13, 37 and 38, 5–10 copies, and mice 9 and 23, more than 20 copies. Although the sample size is small, there is no obvious correlation between the concentration of the injected DNA and the presence or copy number of λRβG2 sequences in these mice.

To determine whether the λRβG2 sequences are transmitted through the male germ line, we mated four male mice containing the rabbit sequences with normal CBA/H females. Total DNA was prepared from several whole newborn progeny, restricted with *Eco*RI and screened for λRβG2 sequences using ^{32}P-labelled λRβG2 DNA as a probe. As shown in Fig. 3, all four mice transmitted λRβG2 sequences to a fraction of their progeny. These fractions are: one out of four progeny for mice 4 and 23, two out of four for mouse 7, and two out of six for mouse 13. Progeny from the other five positive mice have not yet been analysed. For at least two of the mice transmitting rabbit β-globin sequences (mice 4 and 7), the intensity of hybridization to λRβG2 is significantly greater for the progeny DNAs than for the parent liver DNAs. One likely explanation is that these mice are chimaeric, and only a fraction of their liver cells contain the λRβG2 sequences, whereas all the cells of their progeny contain them. If this is so, the apparent number of copies in liver DNA may underestimate the true copy number in those cells that do contain λRβG2 sequences. A further analysis of the tissue distributions and transmission patterns of the rabbit DNA sequences should distinguish between this and alternative explanations.

The results shown in Fig. 3 also provide information about the structure of the λRβG2 sequences present in the cells of the

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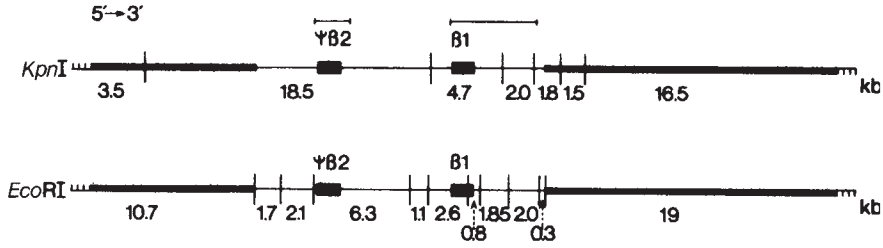

Fig. 1 Restriction maps of λRβG2 DNA. λRβG2 consists of a 19-kbp rabbit chromosomal DNA fragment (thin line) cloned in a bacteriophage λ-vector (thick lines)[14,24]. β1 and Ψβ2 (solid boxes) are, respectively, the adult β-globin gene and a β-like pseudogene[15,16]. The symbols ⊔⊔ and ⊓⊓ represent the single-stranded cohesive ends of the λ vector DNA. DNA was isolated from purified phage particles[24] and dissolved in 10 mM Tris *p*H 7.4, 0.1 mM EDTA for injection into mouse eggs. The bars drawn above the *Kpn*I restriction map depict the regions of λRβG2 that are contained in the probes used in Fig. 2.

positive mice and their progeny. Whereas *Eco*RI digestion of linear λRβG2 DNA generates fragments of 19 and 10.7 kbp, corresponding to the two arms of the λCH4A vector (see Fig. 1), the mouse DNAs do not contain the 19- and 10.7-kbp fragments; but in every case, a 30-kb band equal in length to the sum of the two λ vector arms is seen. In addition, all the bands expected from *Eco*RI digestion of the rabbit DNA insert in λRβG2 are detected in the DNAs of the positive mice. Together with the results of Fig. 2, these data suggest that the parent liver and progeny DNAs contain intact copies of λRβG2. The 30-kbp *Eco*RI band in the mouse DNAs is not sensitive to denaturation in conditions that melt the cohesive ends of λ DNA[19] (65 °C, 0.01 M Tris, *p*H 8), which indicates that the λRβG2 copies exist as either circular or multimeric molecules whose cohesive ends have been covalently joined in the mouse cells.

One possible interpretation of these data is that the λRβG2 sequences are integrated into mouse chromosomes in tandem arrays. In this case, the 30-kbp *Eco*RI fragment in the mouse DNAs would be derived from the joining of the left and right ends of adjacent λRβG2 molecules. Linear DNA molecules are rapidly ligated into high molecular weight concatemers following injection into fertilized frog eggs[20], and DNA-mediated transformation of mammalian cells by the calcium phosphate precipitation method is believed to involve the formation of large DNA concatemers before chromosome integration[21,22]. Possibly, microinjected λRβG2 DNA integrates by a similar mechanism in mouse eggs or early embryos. This interpretation predicts that new *Eco*RI fragments which hybridize to λRβG2 should be generated at the ends of each integrated array of λRβG2 molecules. Most of the positive mouse DNAs do, in fact, show one or more new, fainter *Eco*RI bands in addition to the strong bands that are due to multiple copies of λRβG2. These are visible in several of the lanes of Fig. 3. Mouse 13, for example, shows a new *Eco*RI fragment about 12 kbp in length (panel *e*, lane *B*) which is inherited by both of its positive progeny (panel *d*, lanes *B* and *F*). Mice 7 and 23 (panels *b* and *c*) also show new *Eco*RI fragments which are transmitted to their progeny. The observation that these new bands are inherited is consistent with the prediction that the bands represent junction fragments generated at sites of chromosomal integration. As no more than two or three new bands are seen in either *Kpn*I or *Eco*RI digests of the positive DNAs, it is unlikely that the mice could contain many individually integrated copies of λRβG2. Although our data support a model of chromosomal integration, they do not formally eliminate other interpretations, such as the autonomous replication and germ-line transmission of free λRβG2 circles. We are now attempting to detect integration of the rabbit DNA sequences by *in situ* hybridization to metaphase chromosomes[22].

In conclusion, we have demonstrated that a large eukaryotic DNA fragment can be introduced at a high frequency into mouse tissues, in the absence of selection, and transmitted through the germ line. Therefore, many strains of mice can now be produced that carry the adult rabbit β-globin gene and its flanking sequences in all their cells. With such strains of mice it will be possible to examine the expression of the rabbit β-globin gene and to investigate whether expression is restricted to specific tissues and/or developmental stages. If the rabbit sequences are integrated into mouse chromosomes, presumably different strains will contain the rabbit genes at different

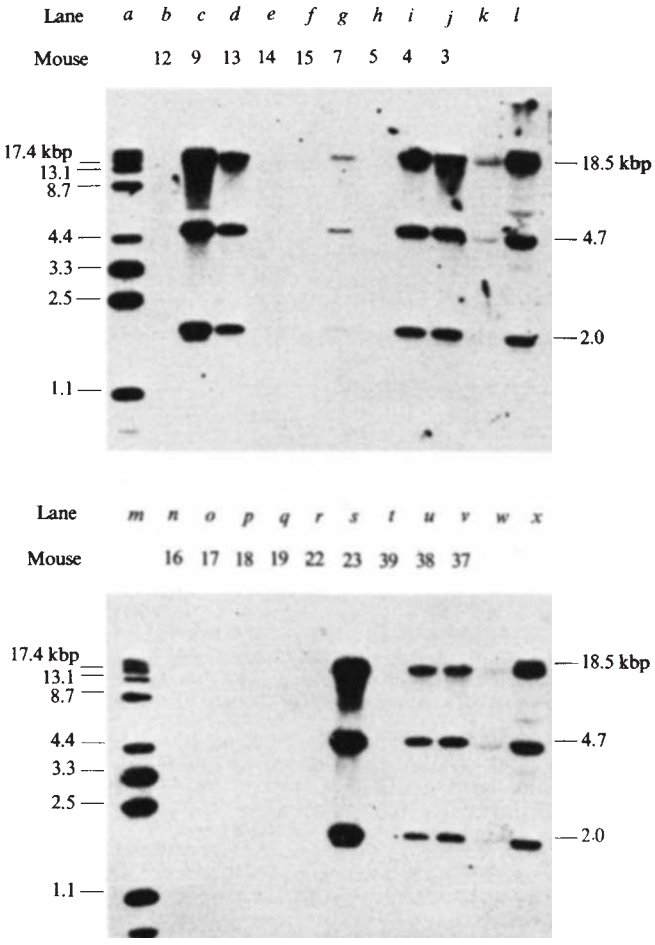

Fig. 2 Detection of λRβG2 sequences in mouse liver DNAs. Liver tissue samples (0.2–0.4 g) were surgically removed from 6–8-week-old mice, frozen in liquid nitrogen and stored at −80 °C. High molecular weight DNA was extracted by the method of Blin and Stafford[25] with the following modifications. Powdered frozen tissue was suspended by pipetting in 0.1 M EDTA, 0.05 M Tris *p*H 8 and subsequently brought to 0.5 M NaCl, 0.5% (v/v) Sarkosyl and 250 μg ml^{-1} proteinase K. Before the final dialysis step, the DNA was precipitated with ethanol. Yields ranged from 200 to 800 μg of DNA from each liver sample. 10 μg of each DNA was digested with *Kpn*I (New England Biolabs), fractionated by electrophoresis on 0.75% agarose gels and transferred to nitrocellulose filters[18] following cleavage by partial depurination[26]. The hybridization probe was a mixture of two plasmids, one containing the β1 gene on a 5.6-kbp *Pst*I fragment and the other containing the Ψβ2 gene on a 2.3-kbp *Eco*RI–*Bgl*II fragment. The regions of λRβG2 corresponding to these probes are shown in Fig. 1. Probe DNA was labelled with ^{32}P by nick translation[27] to a specific activity of 4×10^8 d.p.m. per μg and used at a concentration of 7 ng ml^{-1}. Filters were prehybridized for 6 h and hybridized for 18 h essentially as described by Wahl *et al.*[26]. After hybridization the filters were washed for 12 h at 66 °C in 2 × SSC, 0.5% SDS, 0.01 M sodium phosphate *p*H 6.8, 0.05% sodium pyrophosphate and 0.001 M EDTA, and then for 1 h at 55 °C in a 10-fold dilution of the same buffer. In these conditions, no cross-hybridization between the rabbit β-globin probe and normal mouse DNA is detected. Lanes *a* and *m*, molecular weight markers. Lanes *b–j* and *n–v*, *Kpn*I-digested liver DNA from 18 mice derived from injected eggs. Lanes *k* and *w*, 80 pg of *Kpn*I-digested λRβG2 DNA plus 10 μg of salmon sperm DNA. Lanes *l* and *x*, 800 pg of *Kpn*I-digested λRβG2 DNA plus 10 μg of salmon sperm DNA. These amounts correspond to 1 and 10 copies of λRβG2 per diploid genome in 10 μg of mouse DNA, and the limit of detection on the autoradiographs was 0.1–0.2 copies per diploid genome. The 18.5-, 4.7- and 2.0-kbp fragments of λRβG2 hybridize strongly to the probe whereas the 1.8-kbp fragment hybridizes weakly. Other bands visible in the digest of λRβG2 (lanes *l* and *x*) are partial digestion products.

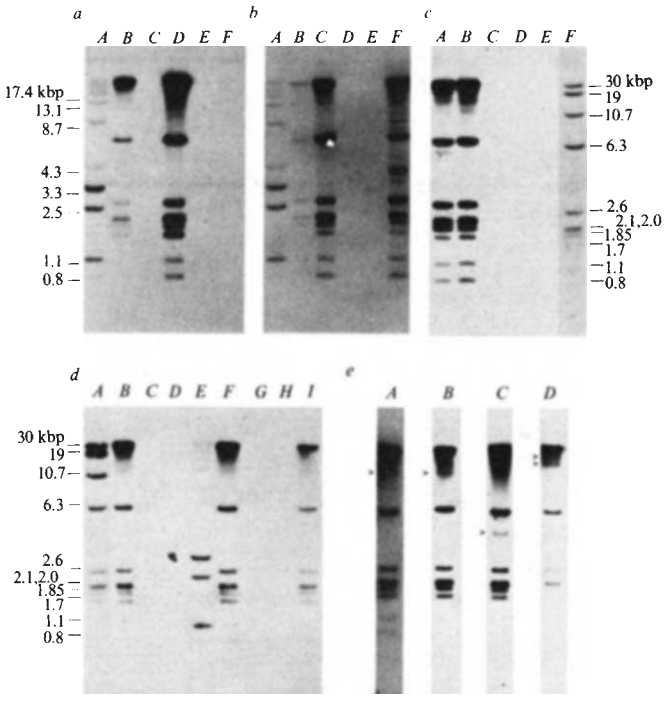

Fig. 3 Transmission of λRβG2 sequences through the male germ line. Four male mice containing λRβG2 sequences in liver DNA were mated to normal CBA/H females and total DNA was isolated from their whole newborn progeny. Liver DNAs from the four males (10 μg) and total DNAs from their progeny (10 μg) were digested with *Eco*RI (Boehringer), fractionated on 0.5% agarose gels and blotted. Filters were hybridized to ^{32}P-labelled λRβG2 DNA ($2–4 \times 10^8$ d.p.m. per μg; 10 ng ml^{-1}). Panel *a*: lane *A*, molecular weight markers whose sizes are indicated on the left; lane *B*, liver DNA from mouse 4; lanes *C–F*, total DNAs from four progeny of mouse 4. Panel *b*: lane *A*, molecular weight markers; lane *B*, liver DNA from mouse 7; lanes *C–F*, total DNAs from four progeny of mouse 7. The DNA sample in lane *F* is incompletely digested by *Eco*RI. Panel *c*: lane *A*, liver DNA from mouse 23; lanes *B–E*, total DNAs from four progeny of mouse 23; lane *F*, a mixture of linear monomers and concatemers of λRβG2 digested with *Eco*RI. The 19- and 10.7 kbp fragments are the λ vector arms of monomeric linear λRβG2 (see Fig. 1). The 30-kbp fragment derives from concatemers and consists of the 19- and 10.7-kbp fragments joined by the λ cohesive ends. The smaller fragments are the *Eco*RI digestion products of the rabbit DNA insert in λRβG2. Panel *d*: lane *A*, same as lane *F* of panel *c*; lanes *B–D* and *F–H*, total DNAs from six progeny of mouse 13; lane *E*, molecular weight markers; lane *I*, liver DNA from mouse 13. Panel *e*: *Eco*RI-digested liver DNAs from four of the positive mice hybridized to ^{32}P-labelled λRβG2 DNA. Each lane is exposed to show one or more new, faint bands not characteristic of λRβG2. Lane *A* (a longer exposure of lane *I*, panel *d*) contains liver DNA from mouse 13 and shows a faint band at ~12 kbp, which is also visible in two of the progeny of mouse 13 (lanes *B* and *F*, panel *d*). Lane *B* contains liver DNA from mouse 7 and shows a faint band of ~13 kbp, which is visible in at least one of the progeny of mouse 7 (lane *C*, panel *b*). Lane *C* contains liver DNA from mouse 37 which shows a new band at ~4.5 kbp. Lane *D* contains liver DNA from mouse 38 which shows two new bands at roughly 15 and 20 kbp. Progeny from mice 37 and 38 have not been analysed.

chromosomal locations. This will allow us to investigate how the host chromosomal environment influences the expression of a foreign gene[23].

Since the submission of our manuscript, chromosomal integration of the λRβG2 sequences in mouse 23 has been demonstrated by *in situ* hybridization to metaphase chromosome spreads prepared from peripheral blood. The probe was ^{125}I-labelled λRβG2 DNA, and hybridization was performed as described by Robins *et al.*[22]. In every metaphase spread examined (19), intense labelling was observed at a site in the middle of one homologue of chromosome 1. This indicates that most if not all of the copies of λBβG2 in this mouse are integrated into chromosome 1.

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1. Wigler, M. *et al. Cell* **16,** 777–785 (1979).
2. Pellicer, A. *et al. Science* **209,** 1414–1422 (1980).
3. Hamer, D. H., Kaehler, M. & Leder, P. *Cell* **21,** 697–708 (1980).
4. Mulligan, R. C., Howard, B. H. & Berg, P. *Nature* **277,** 108–114 (1979).
5. Gruss, P. & Khoury, G. *Proc. natn. Acad. Sci. U.S.A.* **78,** 133–137 (1981).
6. Capecchi, M. R. *Cell* **22,** 479–488 (1980).
7. Anderson, W. F., Killos, L., Sanders-Haigh, L., Kretschmer, P. J. & Diacumakos, E. G. *Proc. natn. Acad. Sci. U.S.A.* **77,** 5399–5403 (1980).
8. Dierks, P., Van Ooyen, A., Mantei, N. & Weissman, C. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1411–1415 (1981).
9. Benoist, C. & Chambon, P. *Nature* **290,** 304–310 (1981).
10. Osborne, T. F., Schell, R. E., Burch-Jaffe, E., Berget, S. J. & Berk, A. J. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1381–1385 (1981).
11. Jaenisch, R. & Minitz, B. *Proc. natn. Acad. Sci. U.S.A.* **71,** 1250–1254 (1974).
12. Gordon, J. W., Scangos, G. A., Plotkin, D. J., Barbosa, J. A. & Ruddle, F. H. *Proc. natn. Acad. Sci. U.S.A.* **77,** 7380–7384 (1980).
13. Jähner, D. & Jaenisch, R. *Nature* **287,** 456–458 (1980).
14. Lacy, E., Hardison, R. C., Quon, D. & Maniatis, T. *Cell* **18,** 1273–1283 (1979).
15. Hardison, R. C. *et al. Cell* **18,** 1285–1297 (1979).
16. Lacy, E. & Maniatis, T. *Cell* **21,** 545–553 (1980).
17. Tarkowski, A. J. *Acta theriol.* **2,** 251–267 (1959).
18. Southern, E. *J. molec. Biol.* **98,** 503–517 (1975).
19. Wang, J. C. & Davidson, N. *J. molec. Biol.* **15,** 111–123 (1966).
20. Bendig, M. M. *Nature* **292,** 65–67 (1981).
21. Perucho, M., Hanahan, D. & Wigler, M. *Cell* **22,** 309–317 (1980).
22. Robins, D. M., Ripley, S., Henderson, A. S. & Axel, R. *Cell* **23,** 29–39 (1981).
23. Jaenisch, R. *et al. Cell* **24,** 519–529 (1981).
24. Maniatis, T. *et al. Cell* **15,** 687–701 (1978).
25. Blin, N. & Stafford, D. W. *Nucleic Acids Res.* **3,** 2303–2308 (1976).
26. Wahl, G., Stern, M. & Stark, G. *Proc. natn. Acad. Sci. U.S.A.* **76,** 3683–3687 (1979).
27. Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72,** 1184–1188 (1975).

Transcriptional regulation of the prolactin gene by ergocryptine and cyclic AMP

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A large body of evidence suggests that the synthesis[1,2] and secretion[3–7] of the pituitary hormone prolactin is inhibited by the hypothalamic hormone dopamine. The finding that dopamine inhibits adenylate cyclase activity of rat pituitary[8] and human prolactin-secreting adenoma[9] suggests that the dopaminergic inhibition of prolactin synthesis may be mediated by decreased levels of cyclic AMP. Recently, the dopaminergic inhibition of prolactin synthesis has been shown to involve decreased concentrations of prolactin mRNA[2,10]. Furthermore, monobutyryl cyclic AMP increases prolactin mRNA levels in pituitary cells treated with the potent dopaminergic agonist ergocryptine[11]. Such changes in prolactin mRNA levels could involve transcriptional or post-transcriptional events. Here we report that treatment of pituitary cells with ergocryptine leads to rapid inhibition of prolactin gene transcription and that addition of monobutyryl cyclic AMP to ergocryptine-pretreated cells results in a rapid stimulation of prolactin gene transcription.

We first examined the effect of ergocryptine on the levels of nuclear precursors of prolactin mRNA. Previous work has demonstrated the presence of large, potential precursors of prolactin mRNA in pituitary cell nuclei[12], the largest of these being 7.0 kilobases (kb); the mature prolactin mRNA is 1.0 kb.

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