$\phi + 2\pi k\Delta x$. Thus, $R_D = cAG(k)$ [$\cos(\phi) - \cos(\phi + 2\pi k\Delta x)$]. If $k\Delta x$ is small, then the expression in brackets may be represented as a Taylor series in powers of $k\Delta x$. The coefficient of the first term in the series involving $k\Delta x$ is $2\pi\sin(\phi)$, which has its maximal value at the null position where $\phi_n = \pi/2$ and $\sin(\phi_n) = 1$, and which goes to zero at the peak position where $\sin(p) = 0$. At the peak position, the first nonzero term in the Taylor expansion for R_D is $2\pi^2\, cAG(k)\, k^2\Delta x^2$. M. Sur and S. M. Sherman [*Exp. Brain Research* **56**, 497 (1984)] offered a similar analysis.

10. In general, smooth linespread functions $g(x)$ have Fourier transforms $G(k)$ that for large k are asymptotically bounded by k^{-p} (for $p > 1$) or e^{-k}, depending on the technical conditions used to define smoothness. If we assume $G(k)$ is equal to one or the other of these bounding functions, say $G(k) = k^{-p}$, or $G(k) = e^{-k}$, then, respectively, from Eq. 1a, $(k_{resol})^{-p} = 1/Z$ or, exp $(-k_{resol}) = 1/Z$ implying that k_{resol} equals either $Z^{1/p}$ or $\log(Z)$. Since $G(k)$ is not equal to these functions of Z, but rather is bounded by them, we obtain the result that $k_{resol} = G_{inv}(1/Z)$ is asymptotically bounded by $Z^{1/p}$ or $\log Z$. Both of these rates of increase are substantially shallower than proportional to Z. A stronger result holds for the linespread functions of usual models (Gaussian, difference of Gaussians, Laplacian of Gaussians) and for any linespread function which takes convolution with optical blur into account. In these cases, k_{resol} is asymptotically proportional to $(\log Z)^{1/2}$. For the Gaussian linespread function, these asymptotic limits are reached even when Z is of order 3, well below the physiological range. Thus, $(\log Z)^{1/2}$ is a very good approximation for the dependence of spatial frequency resolution on signal-to-noise ratio.
11. The spatial frequency optimum for displacement, k_D, is calculated by maximizing the function $kG(k)$. This is done by finding the zeroes of the first derivative of $kG(k)$. Thus, for the Gaussian $[kG(k)]' = [1 - 2k^2\pi^2r^2]G(k) = 0$ at $k_D = 1/(\sqrt{2}\,\pi r)$.
12. D. E. Mitchell, personal communication.
13. C. B. Blakemore and F. Vital-Durand, *Trans. Ophthalmol. Soc. U.K.* **99**, 363 (1979); E. Kaplan and R. Shapley, *Invest. Ophthalmol. Suppl.* **25**, 120 (1984); A. M. Derrington and P. Lennie, *J. Physiol. (London)* **357**, 219 (1984).
14. From (*13*), the value of k_{resol} for foveal ganglion cells in monkeys is around 40 cycle/deg and the contrast gain A for the most sensitive monkey ganglion cells is the same as that of cat ganglion cells. Therefore, from Eqs. 6 and 7, one can estimate that the threshold for displacement should be $1/(k_{resol} \cdot 24)$, that is $1/(40 \cdot 24)$ degree, or approximately 4 seconds.
15. A. Parker and M. Hawken, *J. Opt. Soc. Am.* **2**, 1101 (1985).
16. M. J. Morgan and R. J. Watt, *Vision Res.* **22**, 863 (1982).
17. H. B. Barlow, *Proc. R. Soc. London Ser. B.* **212**, 1 (1981); F. H. C. Crick, D. C. Marr, T. Poggio, in *The Organization of the Cerebral Cortex*, F. O. Schmitt, F. G. Worden, G. Edelman, S. G. Dennis, Eds. (MIT Press, Cambridge, MA 1981).
18. K. Nakayama and G. H. Silverman, *J. Opt. Soc. Am.* **2**, 267 (1985).

19. Supported by National Eye Institute grants 2R01-EY 1472 and 1R01-EY 5466, by NINCDS grant NS 877, by the Air Force Office of Scientific Research grant 84-0278, and by the Hartford Foundation and the McKnight Foundation.

7 June 1985; accepted 25 October 1985

Gonadotroph-Specific Expression of Metallothionein Fusion Genes in Pituitaries of Transgenic Mice

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Transgenic mice expressing a metallothionein-somatostatin fusion gene contain high concentrations of somatostatin in the anterior pituitary gland, a tissue that does not normally produce somatostatin. Immunoreactive somatostatin within the anterior pituitaries was found exclusively within gonadotrophs. Similarly, a metallothionein–human growth-hormone fusion gene was also expressed selectively in gonadotrophs. It is proposed that sequences common to the two fusion genes are responsible for the gonadotroph-specific expression.

FOREIGN GENES UNDER THE CONTROL of either their own or heterologous promoters are expressed in various tissues in transgenic mice. Expression of genes may be limited to specific tissues depending on the particular promoter, enhancer sequences, or undetermined elements (*1*). Several experiments involving transgenic mice have made use of fusion genes containing the mouse metallothionein I (MT) promoter (*2, 3*). Recently we have shown that a fusion gene (MT-SS) consisting of the MT promoter, the protein-coding region of rat pre-prosomatostatin (pre-proSS), and exon 5 plus the 3′ flanking region of the human growth-hormone (hGH) gene was actively expressed in the anterior pituitaries of transgenic mice (*4*). This tissue does not normally produce somatostatin. We report here the distribution of somatostatin-containing cells in the anterior pituitaries of these transgenic mice and show that expression is restricted to gonadotrophs. Additionally, we demonstrate that, in MT-hGH transgenic mice, expression of the fusion gene within the pituitary also occurs exclusively in gonadotrophs. We propose that the MT promoter, either alone or in concert with other sequences common to the two fusion genes, results in gonadotroph-specific expression.

Fusion genes containing the mouse MT promoter, rat pre-proSS complementary DNA or the hGH structural gene, and the hGH 3′ flanking region are depicted in Fig. 1. A portion of exon 5 and the 3′ flanking region of the hGH gene were retained in the somatostatin construction to provide polyadenylation and transcriptional termination signals. The fusion genes were microinjected into the male pronuclei of fertilized eggs from C57 × SJL hybrid mice, and the eggs were transplanted into the reproductive tracts of random-bred Swiss mouse foster mothers (*5*). Founder mice with integrated fusion genes were outbred to produce several pedigrees of heterozygous transgenic mice

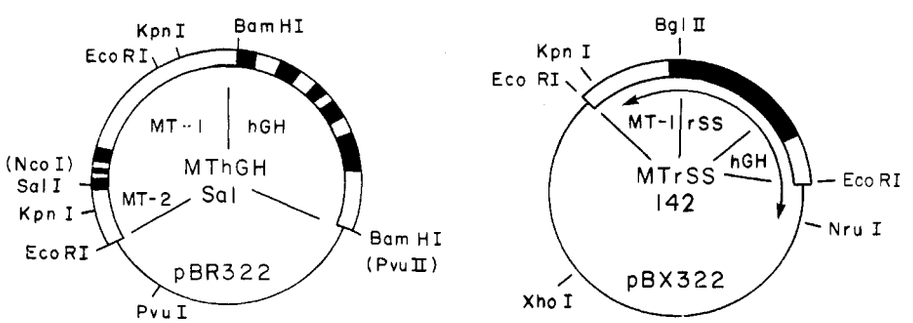

Fig. 1. Structures of the metallothionein-somatostatin and metallothionein–human growth-hormone fusion genes. The plasmid MTrSS 142 was constructed as described (*4*). MThGH Sal has an 8000-bp Kpn fragment of the mouse MT gene (*12*) inserted into MThGH 111 (*3*). Plasmid pBX322 or pBR322 sequences are denoted by a solid line, introns and flanking sequences by open boxes, and exons by closed boxes. The linearized fragment of MTrSS used for microinjection is indicated by a double-headed arrow. MThGH Sal was linearized at the Pvu I site. Both injected fragments contain portions of the MT-1 promoter and first exon (*12*) and portions of the hGH exon 5 and 3′ flanking regions (*13*). The two gene constructions differ in the structural coding sequences.

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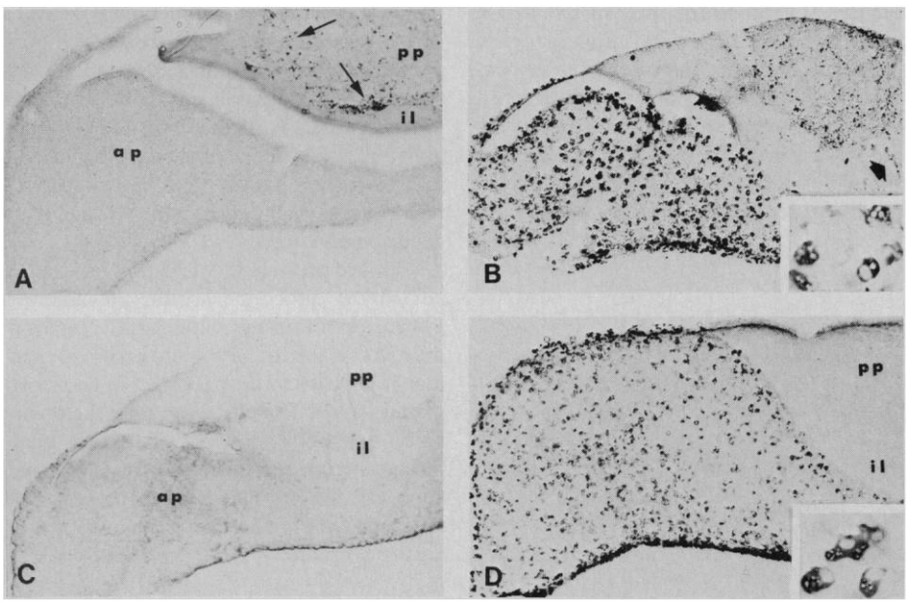

Fig. 2. Localization of somatostatin and luteinizing hormone (LH) in mouse pituitary glands. Eighteen hours after induction of the metallothionein promoter with $CdSO_4$ (1 mg of Cd per kilogram body weight, injected intraperitoneally at 1800 and 0800)(*14*), animals were decapitated. The pituitary glands were rapidly removed and fixed for 2 hours in 5 percent acrolein (*15*). Free-floating Vibratome sections (50 μm) from a control C57 B1 (A) or MT-SS transgenic mouse (B) were reacted with antiserum to cyclic somatostatin at a titer of 1:1000 (*16*), based on the unlabeled peroxidase-antiperoxidase (PAP) technique of Sternberger and co-workers (*6*). The reaction product was developed with 0.025 percent diaminobenzidine (DAB). Sections were counterstained with methyl green, dehydrated, and permanently mounted in Histomount. Abbreviations: (pp) posterior pituitary; (ap) anterior pituitary; (il) intermediate lobe. Specificity of the reaction product was demonstrated by the loss of all somatostatin immunoreactivity when the primary somatostatin antiserum was preadsorbed with excess ($10^{-5}M$) synthetic cyclic somatostatin (C). Sections of pituitaries from MT-SS transgenic mice were stained by the same method with antiserum to rat LH (1:500), rat growth hormone (1:500), rat prolactin (1:500), rat thyroid-stimulating hormone (1:500), and rat adrenocorticotropic hormone (1:750). Each antiserum identified a characteristic population of cells. Only immunoreactive LH cells in the ap (D) are shown. Original magnifications: (A, B, C, and D) ×156; (insets) ×500.

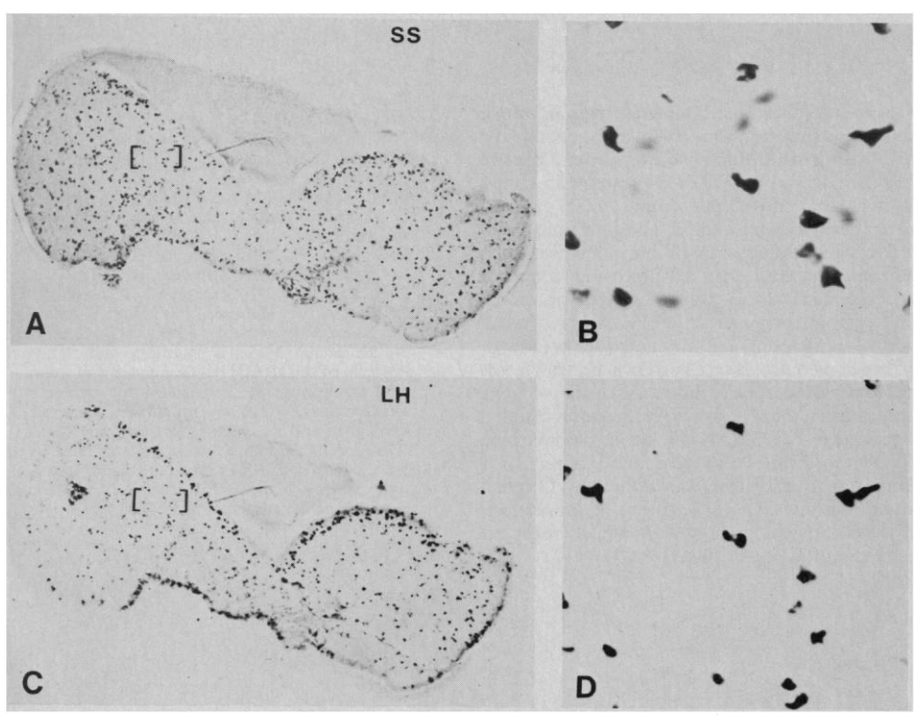

Fig. 3. Colocalization of somatostatin and luteinizing hormone in anterior pituitary cells. Pituitary sections were reacted with antiserum to somatostatin with 4-chloro-1-naphthol as the chromogen, then wet-mounted in glycerol and photographed (A and B). The reaction product was removed in acetone, and the antibody complex was eluted as described (*8*). Complete elution was established by the absence of immunostaining when tissues were reincubated with goat antiserum to rabbit immunoglobulin G, the PAP complex, and 4-chloro-1-naphthol. The same sections were then reacted with the other antisera listed in Fig. 2, and the reaction product was developed with DAB and photographed again (C and D). Tissue shrinkage occurred because of the dehydration step for permanent mounting. The brackets in (A) and (C) outline areas of enlargement shown in (B) and (D). All the somatostatin cells (A and B) can be matched with LH cells (C and D), allowing for the differences in focal planes in these 50-μm Vibratome sections. No correspondence was identified between SS cells and the other anterior pituitary cell types. Original magnifications: (A and C) ×40; (B and D) ×400.

with stable high levels of either somatostatin or growth-hormone production.

We determined the distribution of somatostatin in the transgenic mice by immunohistochemical techniques involving the peroxidase-antiperoxidase procedure (*6*). The posterior pituitaries of both control (Fig. 2A) and transgenic mice (Fig. 2B) showed the expected distribution of somatostatin-immunoreactivity in nerve fibers and terminals (arrows). Unlike the controls, the anterior pituitaries of the transgenic mice also contained a discrete population of somatostatin-immunopositive cells (Fig. 2B). These cells were distributed predominantly along the dorsal and ventral surfaces of the rostral half of the gland and were less densely distributed within the central areas of the lateral wings. These polygonal, vacuolated cells varied in size from 7 to 15 μm in diameter and frequently occurred in small clusters centered around capillaries. In one transgenic mouse, we observed rare somatostatin-positive cells within the intermediate lobe (arrow, Fig. 2B). Specificity of the reaction product was demonstrated by an adsorption control (Fig. 2C). Because the distribution (*7*) and the morphology of these cells were similar to gonadotrophs (Fig. 2D), we investigated the possibility that the MT-SS fusion gene was expressed only in gonadotroph cells.

Pituitary sections from three independent lines of MT-SS transgenic mice were reacted sequentially with antiserum to somatostatin and to each of the anterior pituitary hormones by means of the antibody elution method of Tramu and colleagues (*8*) as described in the legend to Fig. 3. These studies demonstrated that somatostatin pro-

duction was limited to the luteinizing hormone–producing cells of the anterior lobe. Although immunoreactive gonadotrophic cells are occasionally found within the intermediate lobe (*9*), we have been unable to demonstrate colocalization of somatostatin and luteinizing hormone in the intermediate lobes of any of the transgenic mice.

To determine whether the high level of expression in gonadotrophs is specific for MT-SS, we examined the pituitaries of transgenic mice containing MT-hGH. The hGH fusion gene differs from the SS fusion gene in the substitution of the pre-proSS coding region for the four introns and the first four of the five exons of the hGH gene. MT-hGH Sal also contains an additional 8000 base pairs (bp) of the mouse MT gene. In contrast to the uniform population of small, round somatotrophs in the control animals (Fig. 4A), pituitaries from MT-hGH transgenic mice showed two populations of growth-hormone–immunopositive cells (Fig. 4B). One population of cells (open arrowhead) had characteristics of normal somatotrophs. These cells were substantially reduced in number compared to normal pituitaries; this is consistent with an earlier report that the number of acidophilic cells decreases in the pituitaries of mice containing an MT-hGH fusion gene (*3*). A second population of hGH-immunoreactive cells (numbered arrows and small inset) was distinguished by their large, pleomorphic, vacuolated appearance (Fig. 4B), which is characteristic of gonadotrophs. This second population of cells contained both growth hormone and luteinizing hormone (Fig. 4C).

By Northern blot analysis with a metallothionein antisense RNA hybridization probe, we determined that anterior pituitaries do contain detectable levels of MT-I RNA (*10*). However, we were unable to determine whether MT expression, like that of the fusion genes, was restricted to a specific cell type in the anterior pituitary by either immunohistochemistry or by in situ hybridization.

The mechanism underlying the cell-specific expression of the two fusion genes in gonadotrophs is unknown. The location of integrated foreign genes in the genome of transgenic animals is presumably a random process (*11*), so that it is unlikely that insertion of two different genes in several founder mice always occurs in a chromosomal site actively transcribed in gonadotroph cells. A more plausible explanation is that the MT promoter used in the fusion genes is activated by *trans*-acting factors in gonadotrophs. This interpretation is consistent with the finding that the endogenous MT gene is expressed at a low level in the anterior pituitary. However, the level of expression of the MT-SS fusion gene in various tissues does not correlate well with the level of expression of endogenous MT. This observation suggests that the MT promoter within the fusion gene is influenced by a different set of controls than the natural MT promoter. A further possibility is that gonadotroph-specific enhancer sequences may be present within the 3′ flanking hGH region common to both the SS and hGh gene constructions.

We have previously found that pre-proSS is correctly processed within the anterior pituitaries of transgenic mice to the biologically active forms of somatostatin (*4*) and that it appears to be packaged in secretory granules (*10*). These findings raise the possibility that secretion of MT fusion gene products from the pituitaries of transgenic mice can be regulated by hypothalamic-releasing factors such as gonadotropin-releasing hormone.

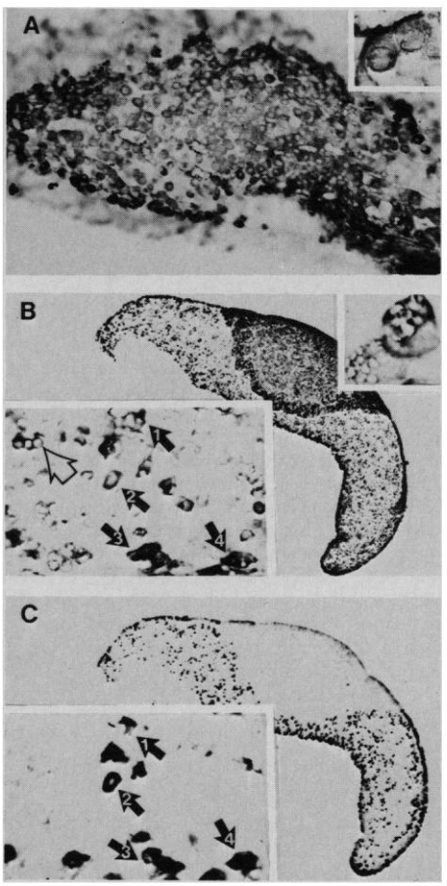

Fig. 4. Colocalization of human growth hormone and luteinizing hormone in mice expressing the MT-hGH gene. Pituitary sections from a control C57 Bl (A) and an MT-hGH transgenic mouse (B) were reacted with antiserum to hGH (1:200) as described in Figs. 2 and 3. The antiserum cross-reacts with mouse growth hormone despite a difference of more than 50 percent in sequence between mouse and human growth hormone. The open arrowhead identifies a cluster of three normal somatrophs. The numbered arrows identify larger pleomorphic cells shown under oil immersion in the smaller inset. After the sections were subjected to the antibody elution technique described in Fig. 3, only the larger, pleomorphic population of hGH cells were found to stain with antiserum to luteinizing hormone (C). Original magnifications: (A) ×390, (inset) oil immersion; (B) ×40, (large inset) ×400, (small inset) oil immersion; (C) ×40, (inset) ×400.

REFERENCES AND NOTES

1. G. S. McKnight, R. E. Hammer, E. A. Kuenzel, R. L. Brinster, *Cell* **34**, 335 (1983); G. H. Swift, R. E. Hammer, R. L. MacDonald, R. L. Brinster, *ibid.* **38**, 639 (1984); R. Grosschedl, D. Weaver, D. Baltimore, F. Constantini, *ibid.*, p. 647; R. L. Brinster *et al.*, *ibid.* **37**, 367 (1984); T. A. Stewart, P. K. Pattengale, P. Leder, *ibid.* **38**, 627 (1984).
2. R. D. Palmiter *et al.*, *Nature (London)* **300**, 611 (1982); R. D. Palmiter, H. Y. Chen, R. L. Brinster, *Cell* **29**, 701 (1982); R. D. Palmiter, T. M. Wilkie, H. Y. Chen, R. L. Brinster, *ibid.* **36**, 869 (1984).
3. R. D. Palmiter, G. Norstedt, R. E. Gelinas, R. E. Hammer, R. L. Brinster, *Science* **222**, 809 (1983).
4. M. J. Low *et al.*, *Cell* **41**, 211 (1985).
5. R. L. Brinster *et al.*, *ibid.* **27**, 223 (1981).
6. L. A. Sternberger, P. H. Hardy Jr., J. T. Cuculis, H. G. Meyers, *J. Histochem. Cytochem.* **18**, 315 (1970).
7. H. D. Purves and W. E. Griesbach, *Endocrinology* **49**, 244 (1951).
8. G. Tramu, A. Pillez, J. Leonardelli, *J. Histochem. Cytochem.* **26**, 322 (1978).
9. B. L. Baker, in *Handbook of Physiology*, S. R. Geiger, Ed. (Waverly, Baltimore, 1974), sect. 7, vol. 4, part 1, p. 73.
10. M. J. Low, unpublished data.
11. E. Lacy, S. Roberts, E. P. Evans, M. D. Burtenshaw, F. D. Constantini, *Cell* **34**, 343 (1983); E. F. Wagner, L. Covarrubias, T. A. Stewart, B. Mintz, *ibid.* **35**, 647 (1983).
12. P. F. Searle *et al.*, *Mol. Cell Biol.* **4**, 1221 (1984); N. Glanville, D. M. Durnam, R. D. Palmiter, *Nature (London)* **292**, 267 (1981).
13. P. H. Seeburg, *DNA* **1**, 239 (1982).
14. D. M. Durnam and R. D. Palmiter, *J. Biol. Chem.* **256**, 5712 (1981).
15. J. C. King, R. M. Lechan, G. Kugel, E. L. P. Anthony, *J. Histochem. Cytochem.* **31**, 62 (1983).
16. R. M. Lechan, R. H. Goodman, M. Rosenblatt, S. Reichlin, J. F. Habener, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2780 (1983).

17. We thank the National Hormone and Pituitary Program of the NIADDKD for antisera to anterior pituitary hormones; S. Reichlin for antisera to somatostatin; P. C. Huang for antisera to rat metallothionein; and R. Palmiter for providing the MT cDNA probe. Supported by grants from NIH (AM 01313, AM 31400, AM 30457, AM 34928, and HD 17321) and by a research award from the Medical Foundation, Inc., Boston, MA (G.M.).

12 June 1985; accepted 11 October 1985