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Transgenic mice containing growth hormone fusion genes

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New genes composed of the mouse metallothionein I promoter–regulator and the rat or human growth hormone structural gene have been introduced into mice. A large proportion of mice containing these genes express them, and some animals grow to be up to twice as large as controls. The technique has been used in part to correct the genetic defect that results in the small stature of the mutant little mouse.

The rapid development of recombinant DNA technology has resulted in numerous new opportunities to study gene activity. A relatively recent group of studies involved the introduction of new genes into animals (E. Wagner *et al.* 1981; T. Wagner *et al.* 1981; Harbers *et al.* 1981; Brinster *et al.* 1981; Palmiter *et al.* 1982*a*, *b*, 1983; McKnight *et al.* 1983; Lacy *et al.* 1983; Brinster *et al.* 1983). These animals have been referred to as transgenic (Gordon & Ruddle 1981). In several cases, expression of the foreign genes occurred. Fusion genes composed of the mouse metallothionein-I (*MT*) promotor–regulator and the herpes simplex virus (*HSV*) thymidine kinase (*TK*) structural gene were expressed in 70% of the animals in which they had integrated into the chromosomes (Palmiter *et al.* 1982*a*).

Success with this gene suggested several other possible experiments employing fusion genes. For one of these series of studies, we fused the *MT* promoter to the rat growth hormone (*rGH*) structural gene (Palmiter *et al.* 1982*b*). When this construction was introduced into mice, a large proportion of animals containing the new gene grew faster and larger than littermate controls without the new gene. Some of these transgenic animals reached twice the normal size. For instance, in the line of animals shown in figure 1, every animal containing the gene grew faster and larger than littermate controls. The new gene was transmitted to half the progeny, which suggests integration into a single chromosome. Furthermore, the gene was expressed in the progeny in a manner similar to that observed in the parent (unpublished observations). This pedigree has been extended to the fifth generation, and it is clear that the gene has become stably integrated into the chromosomes. Whether this gene or other transferred genes will be subject to greater mutation rate or loss than endogenous DNA can only be determined by continued breeding.

For several reasons a fusion gene consisting of the MT promoter–regulator and the human growth hormone gene was constructed (Palmiter *et al.* 1983). Although considerable homology exists between the mouse and rat growth hormone genes, the human hormone differs from the mouse hormone in 67 of 191 amino acids. None the less, animals containing the *MT* promoter–regulator fused to the human growth hormone (*hGH*) gene grew faster and became larger than controls to the same degree as animals with *MT-rGH* genes. Human GH appeared

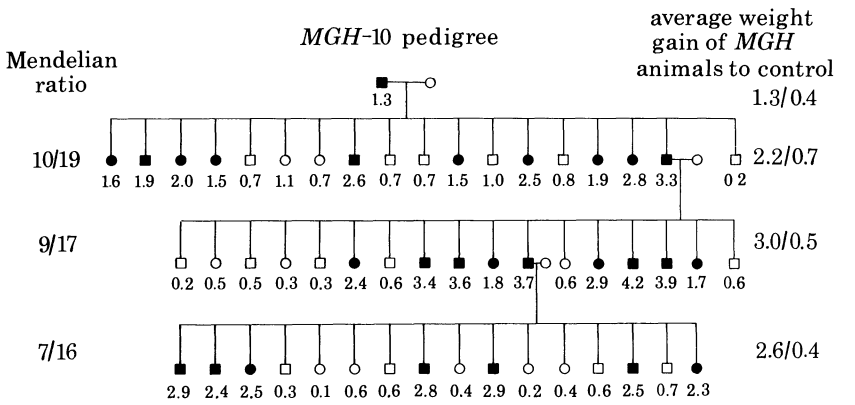

FIGURE 1. Inheritance of growth hormone genes in descendants of transgenic mouse MGH-10 (Palmiter *et al.* 1982*b*). Mice that carry the *MT-rGH* gene are indicated by solid symbols. Squares represent males and circles represent females. Mendelian ratio is the number of animals containing the gene over the total number born. Average weight gain of *MGH* animals to controls compares the average weight gain of animals possessing the gene with those without the gene during the period from 5 to 11 weeks of age. Numbers indicate weight gain (grams per week).

just as effective as rat GH in binding to mouse cell receptors and inducing a physiological response. In these studies we were able to measure accurately the message produced in several tissues by the *MT-hGH* gene and the endogenous *MT* gene. The *MT* gene is expressed in most tissues and provides an ideal control mRNA level against which to compare the mRNA levels from the foreign fusion gene containing the same promoter–regulator. Several important observations can be made from these experiments (Palmiter *et al.* 1983). First, the message levels from the *MT-hGH* gene are lower than from the *MT* gene in all tissues. Second, a hierarchy of MT-hGH relative to *MT* gene expression exists, with the MT-hGH message levels being closest to MT levels in the liver while MT-hGH message levels in kidney, intestine, brain, spleen, and lung are much lower than MT message levels. Third, in certain tissues of some animals, message levels from the new gene are much higher than expected on the basis of levels seen in the same tissue of other animals with the gene, suggesting that integration has occurred in these animals in a region of chromatin that facilitates or allows a higher level of expression (Palmiter *et al.* 1983).

In experiments done during the past two years, we have attempted to correct the genetic defect known as little (*lit/lit*) by gene transfer (Hammer *et al.* 1984). This mutant is characterized by small stature (one-half normal size) and poor reproductive performance. These animals contain an intact growth hormone gene, but have decreased levels of growth hormone mRNA in the pituitary and growth hormone in the serum. Following introduction of the *MT-rGH* gene, mutant little mice grow faster than *lit/lit* mice and reach a size three times that of their mutant control littermates (figure 2). Thus the growth defect can be corrected by gene transfer. However, GH is not regulated by the normal hypothalamic control mechanism, and the animals thus grow larger than even normal mice. The mutant males, which are relatively infertile, become more fertile when they express the *MT-rGH* gene. However mutant females, which are normally fertile, become more infertile when they express the *MT-rGH* gene. In fact, we have noted reduced fertility in most female mice that produce elevated levels of GH from either the *MT-rGH* or *MT-hGH* gene (Hammer *et al.* 1984). The manner in which elevated growth hormone affects fertility is unknown but might reflect the result of an endocrine inbalance.

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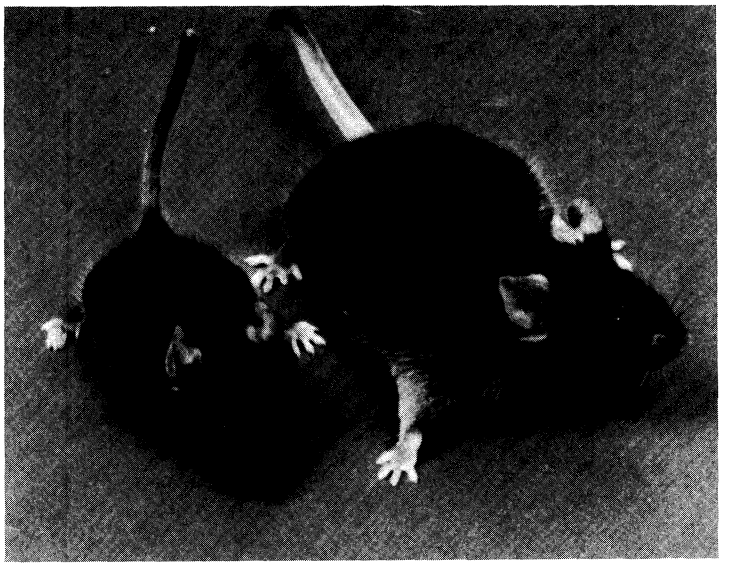

FIGURE 2. Correction of small size in mutant little mouse by introducing a new gene into the germ line (Hammer *et al.* 1984).

Mouse on left is a male mutant little mouse weighing 12 g. On the right is a male littermate weighing 32 g that has inherited the *MT-rGH* gene from the father of both mice. The gene had been injected into the pronucleus of the egg from which the father developed. The father was large and was mated to a homozygous mutant *lit/lit* mouse to produce these two littermates. The new gene results in a marked increase in size when it is present in this line of animals even though they are homozygous for the *lit/lit* gene.

Transgenic animals provide an important new experimental tool to examine gene expression *in vivo*. The studies described with growth hormone fusion genes are one example of how this system may be used.

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REFERENCES

Brinster, R. L., Chen, H. Y., Trumbauer, M., Senear, A. W., Warren, R. & Palmiter, R. D. 1981 Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. *Cell* **27**, 223–231.

Brinster, R. L., Ritchie, K. A., Hammer, R. E., O'Brien, R. L., Arp, B. & Storb, U. 1983 Expression of a microinjected immunoglobulin gene in the spleen of transgenic mice. *Nature, Lond.* **306**, 332–336.

Gordon, J. W. & Ruddle, F. H. 1981 Integration and stable germ line transmission of genes injected into mouse pronuclei. *Science, Wash.* **214**, 1244–1246.

Hammer, R. E., Palmiter, R. D. & Brinster, R. L. 1984 Partial correction of a mouse hereditary disorder by introducing a new gene into the germ line. *Nature, Lond.* (In the press.)

Harbers, K., Jähner, D. & Jaenisch, R. 1981 Microinjection of cloned retroviral genomes into mouse zygotes: integration and expression in the animal. *Nature, Lond.* **293**, 540–542.

Lacy, E., Roberts, S., Evans, E. P., Burtenshaw, M. D. & Costantini, F. D. 1983 A foreign β-globin gene in transgenic mice: integration at abnormal chromosomal positions and expression in inappropriate tissues. *Cell* **34**, 343–358.

McKnight, G. S., Hammer, R. E., Kuenzal, E. A. & Brinster, R. L. 1983 Expression of the chicken transferrin gene in transgenic mice. *Cell* **34**, 335–341.

Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans, R. M. 1982*b* Dramatic growth of mice that develop from eggs microinjected with metallothionein-growth hormone fusion genes. *Nature, Lond.* **300**, 611–615.

Palmiter, R. D., Chen, H. Y. & Brinster, R. L. 1982*a* Differential regulation of metallothionein-thymidine kinase fusion genes in transgenic mice and their offspring. *Cell* **29**, 701–710.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. 1983 Metallothionein-human growth hormone fusion genes stimulate growth of mice. *Science, Wash.* **222**, 809–814.

Wagner, E. F., Stewart, T. A. & Mintz, B. 1981 The human β-globin gene and a functional viral thymidine kinase gene in developing mice. *Proc. natn. Acad. Sci. U.S.A.* **78**, 5016–5020.

Wagner, T. E., Hoppe, P. C., Jollick, J. D., Scholl, D. R., Hodinka, R. L. & Gault, J. B. 1981 Microinjection of a rabbit β-globin gene into zygotes and its subsequent expression in adult mice and their offspring. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6376–6380.

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