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Regulation of Expression of a Sheep Metallothionein 1a-Sheep Growth Hormone Fusion Gene in Transgenic Mice

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Transgenic mice containing a sheep metallothionein 1a-sheep growth hormone fusion gene exhibited low, tissue-specific basal levels of transgene mRNA expression, resulting in slightly elevated levels of circulating growth hormone that did not lead to a detectable increase in growth. After zinc stimulation, high levels of transgene mRNA expression were induced in a number of tissues; these levels correlated with increased levels of circulating growth hormone, resulting in growth increases of up to 1.5 times the levels of controls and unstimulated transgenic mice. After removal of the zinc stimulus, transgene expression and circulating growth hormone concentrations returned to basal levels. Additional evidence from the pattern of developmental expression of the transgene suggests that zinc is the main regulator of this promoter in mice. The demonstrated regulation and low basal level of expression of the sheep metallothionein 1a promoter make it a candidate for use in other mouse transgenic studies and for use in transgenic livestock, in which regulation of expression is essential.

Transgenic mice carrying fusion genes composed of the mouse metallothionein I promoter and either human, rat, porcine, or bovine growth hormone structural genes show marked increases in growth (16, 18, 19, 25). This feature of growth hormone in transgenic mice would be of great value in domesticated animals used in commercial meat production; in attempts to exploit this advantage, a number of laboratories have succeeded in creating transgenic pigs, rabbits, and sheep containing various metallothionein-growth hormone transgenes (2, 8, 25).

Metallothionein promoters were chosen initially because of their heavy-metal inducibility; the timing of growth hormone release and thus the growth phases of transgenic animals could be manipulated to occur at a time most beneficial to the farmer. However, problems have arisen in controlling the expression of these transgenes. Although the transgenes remain inducible by heavy metals such as zinc, the basal levels of expression are such that animals are continuously exposed to abnormally high levels of circulating growth hormone. This results in unacceptable side effects, including reduced fertility, arthritic conditions, and impaired immunity to disease (23).

Ideally, a promoter is required that is inducible but produces only low levels of basal expression of its fused structural gene. The sheep metallothionein 1a (MT-1a) promoter differs from that of other metallothionein genes in that it does not contain a recognized basal level expression sequence motif and may also be unresponsive to glucocorticoids (20–22). This promoter has been fused to the sheep growth hormone structural gene, and transgenic mice have been produced by microinjection. The expression of this fusion gene and its phenotypic effects in transgenic mice are described.

MATERIALS AND METHODS

Gene construct. The fusion gene (MTsGH9) was constructed by fusing 861 base pairs of the sheep MT-1a promoter sequence (extending upstream from a region 21 base pairs downstream from the MT-1a cap site [20]), 5′ to the sheep growth hormone gene (3), at a *Bam*HI site present in the native gene at position -59 (Fig. 1).

Microinjection and Southern analysis. Fertilized eggs from superovulated C57/BL6× CBA mice were collected, and 1 to 2 pl of MTsGH9 DNA at a concentration of 5 μg/ml was microinjected into a pronucleus. Surviving eggs were transferred into pseudopregnant Quackenbush females as described by Costantini and Lacy (4).

Founder transgenic mice and positive offspring were identified by Southern and dot blot analysis of extracted tail DNA (17), using a ^{32}P-labeled sheep growth hormone cDNA probe. Copy number was estimated by liquid scintillation counting of hybridized probe on dot blots. DNA concentrations were standardized by fluorimetric analysis and comparison with known concentrations of thymus DNA.

Zinc treatment. Zinc stimulation of transgenic mice was achieved by including 25 mM zinc sulfate in the drinking water.

RNA isolation. Total cytoplasmic RNA was isolated from a number of mouse tissues. Tissues were homogenized on ice in 2× TSM buffer (0.6 M Tris [pH 7.4], 0.3 M NaCl, 3 mM $MgCl_2$) with 0.5 ml of vanadyl ribonucleosides per gram of tissue (10). Nonidet P-40 was added to a final concentration of 0.5% (vol/vol), and the homogenate was left on ice for 3 min. Nuclei were pelleted, and the supernatant was made 1.5% with sodium dodecyl sulfate and extracted twice with Tris-equilibrated phenol. The RNA was precipitated with 1/10 volume of sodium acetate (pH 5.1) and 2.5 volumes of ethanol. The RNA pellet was suspended in water.

Northern (RNA) analysis. Total cytoplasmic RNA (30 μg) was electrophoresed in 1.5% agarose gels containing 2.2 M formaldehyde in a buffer containing 20 mM 3-(*N*-morpholino)propanesulfonic acid, 1 mM EDTA, 5 mM sodium acetate (6), and 0.5 μg of ethidium bromide per ml. The integrity of RNA was visualized by UV illumination of gels before transfer to GeneScreen Plus as specified by the manufacturer. Slot blots, loaded with 20 μg of total cytoplasmic

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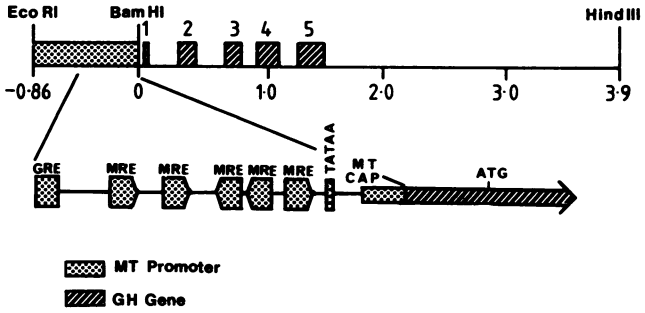

FIG. 1. MTsGH9 fusion gene construct. The construct included approximately 2.2 kilobase pairs of native sequence 3′ to the sheep growth hormone gene. MRE, metal-responsive element; GRE, glucocorticoid-responsive element (this has not been shown to be active in the MT-1a promoter [21]).

TABLE 1. MTsGH9 transgenic mice

Founder	Line	Insertions		No. of copies	% H-T[a]
		No.	Type[b]		
MG 72 (male)		1	ND	49	100
MG 10 (female)		1			
	10	1	A	43	100
	101	1	A	5	100
MG 75 (male)		4			
	751	1	A	20	40
	752	1	A	24	50
	753	1	A	28	60
	754	1	Y	32	20

[a] Percentage of MTsGH9 copies arranged head to tail as determined by *Bam*HI digestion of the DNA.
[b] ND, Not determined; A, autosomal; Y, Y chromosome.

RNA, were prepared by using GeneScreen Plus as specified by the manufacturer.

Filters were hybridized at 65°C for 16 h in 55% formamide–2× SSPE (1 mM EDTA, 10 mM $NaH_2PO_4 \cdot 2H_2O$, 0.15 M NaCl [pH 7.0]–1% sodium dodecyl sulfate–0.5% nonfat skim milk powder–10% dextran sulfate–0.5 mg of salmon sperm DNA per ml–1 × 10^5 to 5 × 10^5 cpm of a sheep growth hormone ^{32}P-labeled RNA probe per ml. The probe was generated from the T7 promoter of the vector pGEM3 (Promega Biotec) into which had been cloned a 1.9 kilobase-pair genomic sheep growth hormone fragment. Total sheep pituitary RNA (4.0 μg) was used as a positive control and, together with 18S and 28S RNAs, as a size marker. Northern (RNA) blots were reprobed with α-tubulin cDNA (5) to confirm the integrity and amount of RNA transferred. Relative amounts of growth hormone mRNA were quantified by densitometer scanning of slot blot autoradiographs after normalization for differences in RNA loaded.

Growth hormone assay. Ovine growth hormone (oGH) was measured in a homologous radioimmunoassay, using a double-antibody method adapted from that of Wallace and Bassett (26) in which mouse plasma was diluted with either assay buffer or hypophysectomized sheep plasma. Half-maximal displacement of the radioligand occurred at 0.6 ng per tube, and the sensitivity of the assay was 0.1 ng. Highly purified preparations of mouse growth hormone (A. F. Parlow, Harbor-UCLA Medical Center) did not cross-react in the assay. Nonspecific binding was <2.9 and 5.7% of bound zero in the presence of mouse and sheep plasma, respectively. Furthermore, mouse plasma containing a high concentration of oGH did not dilute in parallel with ovine plasma. This finding indicated that some interference in the homologous assay system occurred when mouse plasma was used, which may account for the low values obtained for control mice containing no oGH.

RESULTS

Insertion of the MTsGH9 transgene. Three mice containing the MTsGH9 construct were produced by microinjection and designated lines MG 10, MG 72, and MG 75. MG 72 failed to produce any offspring. MG 75 was found to contain four separate insertions, from which four distinct lines were established (MG 751 to 754). MG 754 presumably carried a Y-chromosome insertion (because of direct male-to-male transmission over a number of generations and failure of females to inherit this insert). MG 10 contained only a single insertion, and a number of these animals were bred to homozygosity (MG 10/10). During this process, a third-generation male offspring was found to have a reduced number of copies of MTsGH9; this loss possibly occurred during an unequal crossover event, and subsequent offspring from this male were designated line MG 101. Detailed analyses of all lines are shown in Table 1. Large numbers of animals representing all of these transgenic lines (except MG 72) have been bred for at least six generations, and expression has been analyzed in more than 80 animals covering all generations.

Expression of oGH mRNA in transgenic mice. Seven lines of transgenic mice were tested for expression of MTsGH9 mRNA (oGH). Of the six lines for which large numbers of hemizygous animals were available (MG 10, 101, 751, 752, 753, and 754), general expression patterns were very similar except for 754, the Y-insertion line. Tissue specificities of the transgene were consistent for animals within a line and were unchanged over successive generations.

Basal expression and response to zinc. Figure 2 shows Northern blot analyses of a number of tissues from zinc-induced and uninduced MG 10 transgenic mice. MG 75-derived lines (751, 752, and 753) exhibited similar expression patterns. MTsGH9 produced a transcript of approximately 850 base pairs. Basal expression was detected in brain, testis, lung, and occasionally ovary in MG 10-derived lines but only in brain and testis in MG 75-derived lines. A double transcript in the testis was common to all lines (arrowhead in Fig. 2B). The larger transcript in the testis was due to an alternate transcription start site, as demonstrated by primer extension analysis (results not shown). Occasionally, very low levels of basal expression were observed in the kidneys of some animals, but this expression was not consistently present.

Expression in all other tissues could be detected only after zinc stimulation. Small intestine and kidney showed the greatest response to zinc, and liver showed a much lower response. The intestinal expression varied among animals, ranging from no response to a 30-fold increase above a standardized basal level based on noninduced lung expression in MG 10 mice (Fig. 2). Expression in lung, heart, and ovary increased in MG 10 lines, whereas heart and lung failed to express at all in MG 75 lines. Brain and testis showed no detectable response to zinc stimulation. Other tissues tested that did not produce a detectable transcript were spleen, thymus, large intestine, stomach, uterus, epididymis, prostate, skeletal muscle, pancreas, salivary gland, and skin.

Table 2 summarizes the expression patterns of MG 10- and

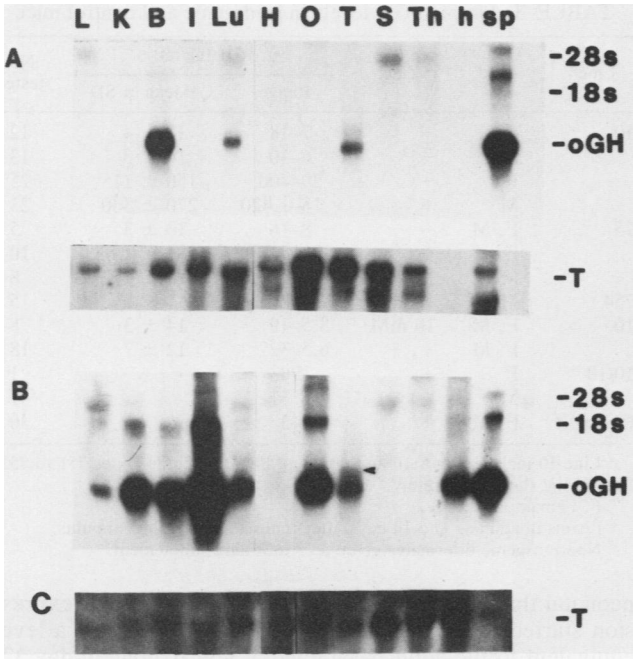

FIG. 2. Expression of MTsGH9 in tissues of MG 10 mice in the presence and absence of zinc. (A) Noninduced expression pattern; (B) expression after zinc induction. ◀, Double testis transcript. oGH indicates the position of the MTsGH9 transcript. Positions of 28S and 18S rRNAs are also indicated. Lanes are designated as follows, with the numbers in parentheses indicating the relative tissue levels of oGH mRNA before and after zinc treatment: L (liver) (0 to 3); K (kidney) (0 to 18); B (brain) (3 to 7, basal range); I (small intestine) (0 to 30); Lu (lung) (1 to 4); H (heart) (0 to <1); O (ovary) (0 to 4); T (testis) (3 to 4, basal range); S (spleen) (0 to 0); Th (thymus) (0 to 0). Other lanes: h, homozygous female heart (gel B only); sp, sheep pituitary. (C) α-Tubulin reprobes.

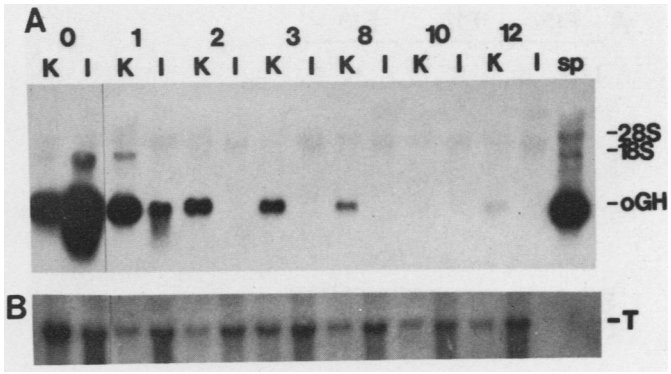

FIG. 3. (A) Northern analysis of MTsGH9 expression in the kidneys (K) and small intestines (I) of transgenic mice after removal of the zinc stimulus. Numbers refer to days after removal of the zinc stimulus, with day 0 representing mice on zinc. (B) α-Tubulin reprobes. Relative levels of oGH in tissues at times after zinc removal were as follows: for day 0, K, 7.0, I, > 9.0; for day 1, K, 8.0; I, 5.0; for day 2, K, 5.0; I, 1.0; for day 3, K, 3.0; I, 0.0; for day 8, K, 4.0; I, 0.0; for day 10, K, 0.0; I, 0.0; for day 12, K, 1.0; I, 0.0. Plasma levels of oGH at times after zinc removal were >64.0, 15.0, 8.5, 9.0, 6.5, 6.5, and 8.5 ng/ml on days 0, 1, 2, 3, 8, 10, and 12, respectively.

MG 75-derived lines, including MG 754, in which only testicular expression was high; all other tissues from MG 754 showed no or barely detectable levels of transgene mRNA even after zinc stimulation. Expression in MG 10/10 homozygotes was tested in only two animals (one male and one female). The only difference detected between hemizygous and homozygous animals was increased expression in the heart of the homozygous female (Fig. 2).

Control of expression. In transgenic mice, MTsGH9 showed low, tissue-specific expression in the absence of zinc and a high level of stimulation with zinc. To confirm whether expression returned to basal levels after zinc removal, kidneys and intestines of male and female transgenic mice were examined at various times after removal of the zinc stimulus. After removal of zinc, expression in the intestine disappeared within 24 h, whereas most kidney expression ceased within 3 days, although trace expression could still be detected in the kidney for up to 10 days (Fig. 3).

The concentration of zinc in the drinking water also affected expression levels. Kidney and intestinal transgene mRNA could be induced with 10 mM zinc sulfate in the drinking water, but the response to this low level of zinc was more variable between transgenics than the response to 25 mM zinc. This variability was also reflected in the growth rates and plasma oGH levels; these results are shown in later comparisons.

Developmental expression. Without zinc, fetal mice did not express MTsGH9 mRNA in the liver and kidney at days 15, 16, 17, and 18 of gestation. However, brain expression could be detected at days 17 to 18 of gestation. Fetuses whose mothers had been given 10 mM zinc sulfate in the drinking water during pregnancy expressed transgene mRNA in the brain and to a much lesser extent in liver and kidney during these days of gestation. However, all neonatal mice ex-

TABLE 2. Tissue specificity and relative levels of transgene mRNA in mice lines before and after zinc stimulation

Line	Zinc	Expression[a]							
		L	K	B	I	Lu	H	O	T
MG 10	−	−	−	+++	−	+	−	±	++
	+	+	+++	+++	++++	++	±	++	++
MG 75	−	−	−	+++	−	−	−	−	+++
	+	+	+++	+++	++++	−	−	++	+++
MG 754	+	−	±	±	−	−	−	—[b]	+++
10/10									
Female	+	+	+++	++	++	+	++[c]	++	—
Male	+	+	+++	+++	+++	+	±	—	+

[a] L, Liver; K, kidney; B, brain; I, small intestine; Lu, lung; H, heart; O, ovary; T, testis; −, no detectable expression; ±, expression very low and only detected in some animals; +, expression detected.

[b] —, Tissue not applicable.

[c] Female heart showed enhanced expression.

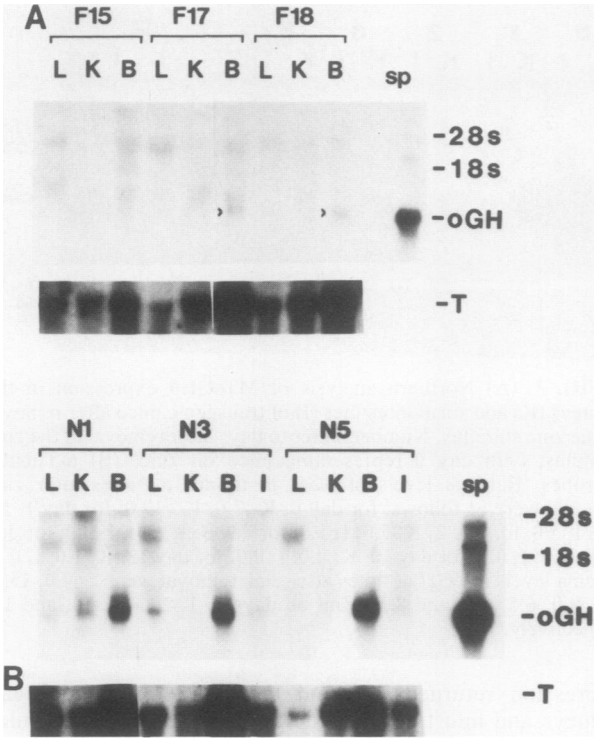

FIG. 4. (A) Northern analysis of MTsGH9 expression in unstimulated fetuses and neonates. Shown are results for fetuses (F) sampled at days 15, 17, and 18 of gestation and for neonates (N) sampled at days 1, 3, and 5 after birth. Lanes: L, liver; K, kidney; B, brain; I, small intestine. >, Fetal brain transcripts. (B) α-Tubulin reprobes. Ethidium bromide staining of parallel gels showed that equal amounts of RNA were loaded for each tissue. The excess of α-tubulin RNA in brain tissue was due to differential expression of α-tubulins in this tissue during development (5).

pressed transgene mRNA in the brain, liver, kidney, and intestine at day 1. This expression disappeared from all tissues except brain after 3 to 5 days (Fig. 4). In brain, expression was continued into adulthood.

Testes of unstimulated neonatal mice showed increasing levels of MTsGH9 mRNA expression from day 8 to approximately day 21 after birth (Fig. 5). At least two different transcripts were produced as in the adult testes, but the higher-molecular-weight transcripts were more abundant in

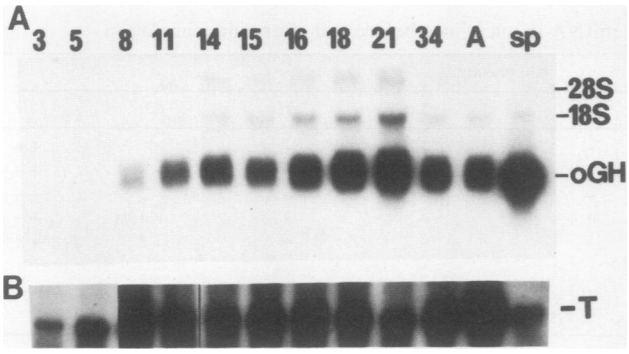

FIG. 5. (A) Testicular expression of MTsGH9 in unstimulated transgenic mice. Numbers refer to days after birth. Lane A contains adult testis cytoplasmic RNA. (B) α-Tubulin reprobes.

TABLE 3. Plasma oGH levels in transgenic and control mice

Line[a]	Sex[b]	Zinc	oGH (ng/ml)		No. tested
			Range	Mean ± SD	
10	F	−	7–18	12 ± 4	12
	M	−	6–30	14 ± 8	13
	F	+	29–400	150 ± 115	25
	M	+	18–1,420	270 ± 360	23
75	F, M	−	8–16	10 ± 3	5
	F	+	20–5,000	884 ± 1,653	10
	M	+	13–4,600	895 ± 1,593	8
754	M	+	4–8.5	5 ± 2	5
10	F, M	10 mM	8.5–19	14 ± 3	8
	F, M	+, −	6.5–32[c]	12 ± 7	18
10/10	F	+	903		1
	M	+	31		1
Control[d]	F, M	+	1–3		30

[a] Line 10 includes lines 10 and 101, and line 75 includes lines 751 to 753. Line 754 is shown separately.
[b] F, Female; M, male.
[c] Levels in animals 1 to 10 days after removal of the zinc stimulus.
[d] Nontransgenic littermates given a 25 mM zinc supplement.

neonatal than in adult testes. The level of testicular expression started to decline after day 21 until it reached a level equivalent to the adult basal level at approximately day 32.

oGH levels. Table 3 shows levels of oGH in the plasma of transgenic mice. Unstimulated transgenic mice demonstrated slightly higher levels of growth hormone than did controls. However, zinc-stimulated transgenics exhibited a range of elevated growth hormone levels generally above those of unstimulated mice. Although it is difficult to compare mRNA levels and growth hormone levels directly, there was a general tendency for mice with high levels of intestinal expression to have highly elevated growth hormone levels. After zinc was removed, transgenic mice showed elevated growth hormone levels for 1 day before levels returned to within the unstimulated range. These growth hormone levels also correlated with the presence or absence of intestinal transgene mRNA in these animals.

The MG 754 line, with only testicular expression, showed oGH levels generally below those of unstimulated transgenic mice but above those of controls, suggesting that testis and other tissues that exhibit basal expression (lung and brain) in other lines can release oGH into the plasma.

Growth of transgenic mice. Figure 6 shows growth curves for male and female transgenic animals and controls both with and without zinc stimulation. Growth of stimulated transgenic mice was highly repeatable, with only a few mice in each line failing to show a response (6 of 59 in MG 75 and 3 of 63 in MG 10). When females from the MG 10 and MG 75 lines were treated with zinc, they grew significantly larger (up to 1.5 times) than negative control littermates and untreated transgenic mice. There was no significant difference between growth of unstimulated transgenic mice and negative control littermates. Similarly, after zinc treatment, MG 75 males (except 754) showed growth increases corresponding to those of the females. However, MG 10 males did not exhibit increased growth when given zinc. Interestingly, zinc-treated MG 101 males, derived from the MG 10 line by a deletion of approximately 40 gene copies, showed the same significant growth increase as MG 10 females and MG 751 to 753 males and females. Males from the MG 754 line had normal growth rates, in keeping with their low levels of circulating growth hormone.

MG 10 females on 10 mM zinc sulfate showed a significant growth response when compared with controls; however,

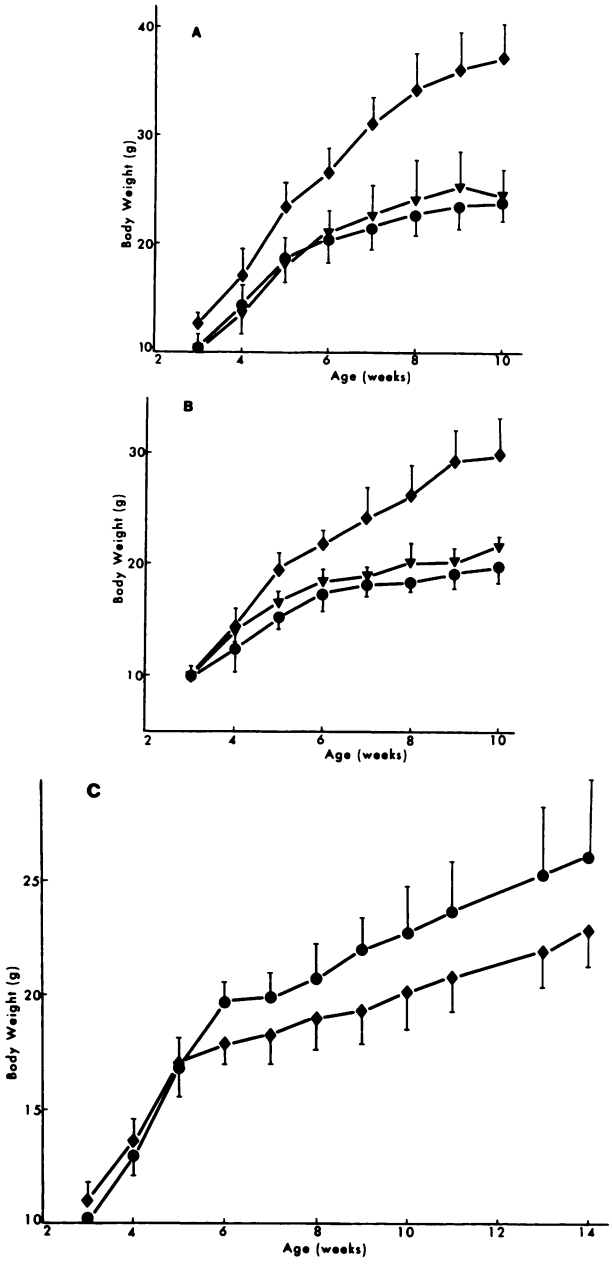

FIG. 6. Growth curves for zinc-stimulated and unstimulated male and female transgenic mice and controls. Animals were weighed weekly from weaning to 100 days. Each group contained between 6 and 16 animals. Standard deviations are indicated. (A) Male growth curves. Symbols: ◆, MG 75, zinc stimulated; ▼, MG 10, zinc stimulated; ●, control littermates. *t* tests: MG 75 versus controls, $P < 0.001$ from week 5; MG 10 versus controls, no significant difference. Data for unstimulated MG 10 and MG 75 mice are not shown, but *t* tests showed no significant difference in comparison with controls. (B) Female growth curves. Symbols: ◆, MG 10, zinc stimulated; ▼, MG 10, not stimulated; ●, control littermates. *t* tests: MG 10 (zinc stimulated) versus MG 10 (unstimulated), $P < 0.01$ from week 5, $P < 0.001$ from week 7; MG 10 (zinc stimulated) versus controls, $P < 0.001$ from week 5; MG 10 (unstimulated) versus controls, no significant difference. Data for zinc-stimulated MG 75 mice are not shown, but *t* tests showed no significant difference in comparison with zinc-stimulated MG 10 mice. (C) Growth curve for MG 10 females on 10 mM zinc (●) and controls (◆). *t* test showed a significant difference in growth from week 6 ($P < 0.01$).

this was not as marked as the response of mice on 25 mM zinc sulfate (Fig. 6C).

DISCUSSION

Sheep MT-1a promoter is regulated by zinc in transgenic mice. The sheep MT-1a promoter has shown the greatest level of control with zinc of any metallothionein promoter used in transgenic mice to date. Six presumably different integration sites of MTsGH9 were studied in a large number of animals. Despite minor differences in tissue specificity between MG 10- and MG 75-derived lines, all transgenic mice showed low levels of basal expression (chiefly in brain and testis) and high levels of expression in intestine and kidney after zinc stimulation. The one exception to this general expression pattern was the MG 754 Y-insertion line, in which expression, even after zinc stimulation, was almost totally suppressed in all tissues except testis. This is an extreme example of the effect of integration site on transgene is probably related to the specialized function of genes on the Y chromosome. Expression of MTsGH9 mRNA in homozygous and hemizygous MG 10 animals was identical in all tissues except heart, where expression in the homozygous female was enhanced. Whether this is a general effect of homozygosity in the female is not yet known.

Evidence suggests that MTsGH9 expression in transgenic mice is extremely sensitive to, and may be solely dependent on, the bioavailability of zinc. The basal expression in the brain and testis can be correlated with the requirement and accumulation of zinc in these tissues during development (9, 12). Provided the mother is not fed a zinc supplement, fetal mice do not express MTsGH9 in the liver and kidney, whereas brain expression can be detected only at day 18. However, neonatal mice express MTsGH9 in liver, kidney, intestine, and brain immediately after birth. Neonatal expression correlates with a high natural zinc intake provided by maternal milk and especially colostrum (15). After 3 to 5 days, all but brain expression has disappeared, perhaps because of a fall in milk zinc levels and less active uptake by the neonatal tissues (7, 24). Fetal zinc concentration increases with gestational age, peaking at day 18 (11), which may account for initiation of brain expression. Although zinc function in the brain is not fully understood, this tissue continues to actively require, accumulate, and use zinc (9), which may account for the continued expression of MTsGH9.

Similarly, the testis requires and uses zinc during spermatogenesis and sperm formation. Zinc levels are high in parts of the prostate and epididymis, and lack of zinc causes infertility in rats (12). In transgenic mice, testis expression commences 8 days after birth, continues at a high level until day 21, and then declines gradually to the adult basal level. The start of transcription at day 8 coincides with the start of meiosis in neonatal mice (14). The gradual decline beginning at day 21 corresponds to weaning, when mice no longer receive zinc from the maternal milk. Therefore, the active use of zinc in this tissue together with, possibly, testis-specific factors present during meiosis initiates transgene expression in this tissue. A full analysis of the cells that express the two transcripts will be required to further clarify the nature of control of this expression.

The enhancement of expression in intestine, kidney, liver, heart and lung correlates with zinc uptake by these tissues. When mice are given supplemental zinc, the intestine, kidney, liver, heart, and lung all actively take up zinc (1; unpublished observations), which is released after the zinc

supplement is withdrawn, returning tissues to homeostatic levels. The age and reproductive status of animals affect tissue zinc uptake and metabolism (1, 9, 24), which in turn may affect levels of transgene mRNA expression and result in variation among animals. The brain and testis, however, are very slow to respond to supplemental zinc (1, 23; our unpublished observations), and this may account for the lack of enhancement of transgene mRNA in these tissues after zinc stimulation. Occasional kidney expression in unstimulated transgenic mice may be related to periodic release of sequestered zinc associated with general zinc metabolism.

Growth hormone levels and growth. Transgenic mice containing various metallothionein-growth hormone fusion genes have suffered from female infertility (16). However, unlike other growth hormone transgenic mice, the oGH transgenic animals are fully fertile and appear to have suffered no detrimental effects from the high levels of circulating growth hormone. This may be due either to the different structure of the oGH protein or to the greater control exerted on transgene expression. MTsGH9 transgenic mice are not exposed to very high growth hormone levels until after zinc stimulation, unlike other growth hormone transgenic mice, in which high levels of growth hormone are present during early development.

The growth response in zinc-stimulated transgenic mice containing the oGH gene was similar to that observed in other metallothionein-growth hormone transgenic mice (16, 17, 19). The absolute levels of growth hormone could not be fully correlated with the amount of growth. Indeed, considering the sensitivity of MTsGH9 induction to zinc, the amount of circulating growth hormone might be expected to fluctuate considerably in phase with dietary zinc ingestion. This view is supported by the observation that mice on a 10 mM zinc supplement had levels of circulating growth hormone in the range of the levels of unstimulated mice and much lower than the levels of mice on a 25 mM zinc supplement. The former showed significant but only partial response in growth, suggesting that surges of oGH production, capable of stimulating growth, may have been occurring in these animals.

The sex-limited growth response seen in the MG 10 line and the subsequent correction of the growth response in males by the deletion of approximately 40 copies of the transgene (in MG 101) could be explained if insertion of the transgene affected a gene whose function is restored when most copies are removed. This sex-limited mutation is under further investigation.

Future use of the sheep MT-1a promoter. The sheep MT-1a promoter may be useful in other transgenic systems in which control is required, assuming that it maintains its ability to be regulated when fused to other structural genes. The tissue distribution of expression of the MTsGH9 transgene in mice differs markedly from the normal expression of the endogenous MT-1a gene in sheep. In sheep, the principal tissues expressing the gene are liver and ovary, with lower levels of message observed in the kidney and brain (J. Mercer, personal communication). Thus, the change in tissue specificity and the presence of tissue-specific elongated transcripts suggest that the sheep MT-1a promoter is controlled differently in transgenic mice. This view is supported by our recent observations that the same MTsGH9 construct in transgenic sheep is expressed at high levels in the absence of a dietary zinc supplement (13). However, ruminants are known to differ from other mammals in copper and zinc metabolism (21), and hence this promoter may be useful for gene transfer into nonruminant livestock.

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