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Expression and performance in transgenic pigs

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Summary. Recent research clearly shows that fusion genes can be microinjected into a pronucleus of an ovum and integrate into the pig genome. Animals with such fusion genes are called 'transgenic'. The percentage of injected ova that developed into transgenic pigs varied among experiments from 0·31% to 1·73%. The percentage of transgenic pigs that expressed the fusion gene ranged from 17% to 100%.

Eleven different regulatory sequences have been used for fusion genes transferred into pigs. Some of these regulatory sequences directed strong gene expression, but control over level of expression was inadequate. Other regulatory sequences directed weak expression, but imparted only brief spikes of induced expression. The predominant gene coding sequences transferred were for growth-related hormones.

Elevation of growth hormone (GH) in expressing transgenic pigs enhanced plasma concentrations of insulin-like growth factor-I (IGF-I), insulin, and glucose, improved feed efficiency about 15%, and markedly reduced subcutaneous fat compared to non-transgenic siblings. Growth rate was enhanced in some transgenic GH pigs but not in others, possibly due to dietary limits.

The 'over-expression' of GH was detrimental to the general health of most transgenic pigs. The most prevalent problems were lethargy, lameness, and gastric ulcers. Gilts that expressed foreign GH genes were anoestrous. Boars that expressed foreign GH genes lacked libido, but their semen was fertile when used by artificial insemination. Six different fusion genes have been transmitted from transgenic founders to progeny. Most of the transgenic pigs that produced progeny transmitted the fusion gene as an autosomal dominant trait to about half of their progeny. Regulatory sequences that will permit full control of gene expression must be developed before the full potential of gene transfer in pigs can be realized.

Keywords: pig; transgenic; growth; gene transfer; gene expression

Introduction

The fundamental knowledge and technical skills required for the insertion of recombinantly derived genes into the mammalian genome have been available only since 1980, when the first transgenic mice were reported (see reviews, Brinster & Palmiter, 1986; Palmiter & Brinster, 1986). The potential that gene transfer offers was most dramatically demonstrated by the transgenic 'super mouse', which resulted from secretion of a high concentration of rat growth hormone (GH). Palmiter *et al.* (1982) avoided normal GH regulatory mechanisms by transferring a fusion gene composed of the regulatory (promoter) sequences of a mouse metallothionein (MT) gene and the structural gene for rat GH to direct rat GH secretion to several organs and tissues of the mouse. Since then, insertion of genes into mice has become an extraordinarily useful technique for

investigation of gene function, developmental biology, immunology, physiology, and hereditary defects.

The laboratory mouse is clearly the species of choice for most biomedical research. However, extension of gene transfer experiments into other species is desirable for certain physiological studies and is absolutely essential for evaluating the potential of this technology for enhancing the efficiency of animal production, increasing disease resistance in animals, and improving the quality of meat, milk, fibre, and other animal products.

The first application of gene transfer to farm animals was reported by Hammer *et al.* (1985c). It has become evident that several reproductive characteristics of the pig (continuous cyclicity, polytocy, and short generation interval) make it the species of choice for research on genetic engineering of large animals.

This review will examine recent progress on integration of recombinant genes into the pig genome, expression of these genes, effects of expression of growth-related transgenes on the physiology and performance of pigs, and the transmission of transgenes to subsequent generations.

Integration of genes

Microinjection

Three methods of transferring genes into pigs have been under investigation. Petters *et al.* (1989) recently succeeded in infecting pig blastocysts with an avian retrovirus and detected viral sequences in fetal tissues at 6 weeks of gestation; in general, however, progress on use of retroviral insertion has been disappointing. Use of embryonic stem cells is also a method that offers considerable potential, but no progress in the pig is evident.

To date, only microinjection of DNA into a pronucleus or nucleus has been successfully used to produce transgenic pigs. The microinjection process is similar to that used routinely for mice except that the opacity of the cytoplasm of pig ova makes visualization of pronuclei extremely difficult. Wall *et al.* (1985) discovered that centrifugation of pig ova at 10 000–15 000 *g* for 3–5 min stratifies the cytoplasm and results in the pronuclei of 1-cell ova or nuclei of 2-cell ova becoming discernible in the equatorial segment with the aid of interference contrast microscopy. The centrifugation technique has been used in all gene transfers reported for pigs.

Many factors influence survival of microinjected ova. Vize *et al.* (1988) reported that no pregnancies survived to term when an average of 13 injected ova were transferred per recipient; therefore, they subsequently transferred 30 injected ova per recipient. Pursel *et al.* (1988b) found that significantly more injected 2-cell ova survived to term than injected 1-cell ova. Other factors affecting survival are the skill of the micromanipulator, duration of in-vitro culture, concentration and form of the DNA (Brinster *et al.*, 1985), and synchrony of donor and recipient at the time of embryo transfer (V. G. Pursel, unpublished data).

Transferred genes

A number of fusion genes, which are composed of promoter/regulator sequences from one gene fused to the structural sequences of another gene, have now been integrated into the pig genome (Table 1). Some of the structural sequences were cloned from genomic DNA libraries and contain intron sequences; those cloned from RNA libraries are complementary DNA (cDNA) and lack introns. A few genes were mixtures of genomic and cDNA to include an intron. Most of the genes transferred into pigs were growth-related, i.e. GH, growth hormone-releasing factor (hGRF), and insulin-like growth factor-I (hIGF-I). However, other genes recently integrated include the mouse MX gene for investigation of resistance to respiratory diseases (Brem *et al.*, 1988), the mouse whey acidic protein (WAP) gene for investigation of mammary-specific expression (V. G. Pursel, R. J. Wall, C. W. Pittius & L. Hennighausen, unpublished data), and the sheep β^c globin (β^cGLB) gene

Table 1. Fusion genes transferred into the pig genome

Abbreviation	Promoter/regulator	Structural sequence	Type	Reference
ALB-hGRF	Mouse albumin	Human growth hormone-releasing factor	Genomic/cDNA	Pursel *et al.* (1989a)
β^{e}GLB	Sheep β^{e} globin	Sheep β^{e} globin	Genomic	D. King, R. J. Wall, S. G. Shapiro & V. G. Pursel (unpublished)
CMV-pGH	Cytomegalovirus	Pig growth hormone	cDNA	K. M. Ebert (unpublished)
MT-bGH	Mouse metallothionein-I	Bovine growth hormone	Genomic	Pursel *et al.* (1987)
MT-hGH	Mouse metallothionein-I	Human growth hormone	Genomic	Hammer *et al.* (1985c), Brem *et al.* (1985)
MT-hGRF	Mouse metallothionein-I	Human growth hormone releasing factor	Genomic/cDNA	Pinkert *et al.* (1987), Brem *et al.* (1988)
MT-hIGF-I	Mouse metallothionein-I	Human insulin-like growth factor-I	cDNA	Pursel *et al.* (1989b)
MT-MX	Mouse metallothionein-I	Mouse MX	cDNA	Brem *et al.* (1988)
MT-pGH	Human metallothionein-IIA	Pig growth hormone	cDNA	Vize *et al.* (1988)
MLV-pGH	Moloney murine leukemia virus	Pig growth hormone	cDNA	K. M. Ebert (unpublished)
MLV-rGH	Moloney murine leukemia virus	Rat growth hormone	cDNA	Ebert *et al.* (1988)
PEPCK-bGH	Rat phosphoenolpyruvate carboxykinase	Bovine growth hormone	Genomic	Wieghart *et al.* (1988)
PRL-bGH	Bovine prolactin	Bovine growth hormone	Genomic	Polge *et al.* (1989)
TF-bGH	Mouse transferrin	Bovine growth hormone	Genomic	V. G. Pursel, R. R. Behringer, R. D. Palmiter & R. L. Brinster (unpublished)
TF-hGH	Mouse transferrin	Human growth hormone	Genomic	
WAP	Mouse whey acidic protein	Mouse whey acidic protein	Genomic	V. G. Pursel, R. J. Wall, C. W. Pittius & L. Hennighausen (unpublished)

for investigation of juvenile expression (D. King, R. J. Wall, S. G. Shapiro & V. G. Pursel, unpublished data).

Integration efficiency

The efficiency of transferring genes into the pig is quite low. Microinjected pig ova represented by offspring at birth has varied from 4·0% to 11·7% (Table 2). Efficiency, expressed as the percentage of injected ova that resulted in transgenic pigs, varied from 0·31 to 1·73 (Table 2). In contrast, transfer of the same fusion genes into mice resulted in an efficiency averaging in excess of 3%.

The number of transgene copies integrated per cell in transgenic pigs varied from 1 to 490 for MT-hGH (Hammer *et al.*, 1985c), <1 to 28 for MT-bGH (Miller *et al.*, 1989), <1 to 15 for MT-pGH (Vize *et al.*, 1988), and <1 to >10 for prolactin-bGH (Polge *et al.*, 1989) with most integrations probably occurring at a single locus. Southern blot analysis showed that transgenic pigs with MT-hGH contained intact copies of the fusion gene with many orientated in tandem head-to-tail arrays and some in a head-to-head configuration (Hammer *et al.*, 1985c). Only 1 of 4 MT-pGH transgenic pigs studied contained the transgene organized in the head-to-tail array (Vize *et al.*, 1988).

Table 2. Efficiency of transferring fusion genes into pigs

Fusion gene	No. ova injected	Offspring		Transgenic		Expressing		Reference
		No.	%	No.	%	No.	%	
MT-hGH	2035	192	9·4*	20	0·98†	11/18	61‡	Hammer *et al.* (1985c)
MT-hGH	286	15	5·6	1	0·40			Brem *et al.* (1985)
MT-bGH	2330	150	6·4	9	0·39	8/9	89	Pursel *et al.* (1987)
MLV-rGH	170	15	8·8	1	0·59	1/1	100	Ebert *et al.* (1988)
MT-pGH	423	17	4·0	6	1·42	1/6	17	Vize *et al.* (1988)
MT-hGRF	2236	177	7·9	7	0·31	2/7	29	Pursel *et al.* (1989b)
MT-hGRF	1041	54	5·4	6	0·60			Brem *et al.* (1988)
MT-MX				6	0·60			Brem *et al.* (1988)
ALB-hGRF	968	108	11·2	5	0·52	3/3	100	Pursel *et al.* (1989a)
PRL-bGH	289	20	6·9	5	1·73			Polge *et al.* (1989)
PEPCK-bGH	1057	124	11·7	7	0·66	5/7	71	M. Wieghart & C. A. Pinkert (unpublished)

*Percentage of injected ova resulting in offspring.
†Percentage of injected ova resulting in a pig with gene integration.
‡Percentage of transgenic pigs expressing the fusion gene.

Table 3. Relationship of integrated copies of gene per cell and expression of MT-hGH gene in transgenic pigs (after Hammer *et al.*, 1985c)

Gene copies per cell	No. of pigs	
	Integrated gene	Expressed gene
1	4	2
2–10	7	5
11–100	4	3
>100	3	2

Expression of integrated genes

Incidence of expression

About 70% of the transgenic pigs that have been tested expressed the integrated gene (Table 2). Failure to express the transgene may be the result of integration in an inactive chromosomal locus or alteration of gene sequences during the integration process. In some cases, the presence of introns in the structural region may greatly influence whether the gene is expressed. Brinster *et al.* (1988) found that many cDNA-based fusion genes were either not expressed or were expressed poorly when integrated into mice.

The lack of introns in the MT-pGH construct used by Vize *et al.* (1988) may have been responsible for only 1 of 6 pigs expressing the gene product (Table 2). Only 2 of 7 pigs and 1 of 7 lambs expressed the MT-hGRF gene, which contained only the first intron (Pursel *et al.*, 1989b; Rexroad *et al.*, 1989). The incidence of expression of the same fusion genes was much higher in transgenic mice than in pigs; 13 of 18 mice expressed MT-pGH (Vize, 1987), and 11 of 14 mice expressed MT-hGRF (Hammer *et al.*, 1985b). Reasons for this discrepancy in incidence of expression among species are unknown.

Hammer *et al.* (1985c) found no evidence of a relationship between the number of gene copies per cell integrated into the genome and the proportion of transgenic pigs that expressed MT-hGH (Table 3).

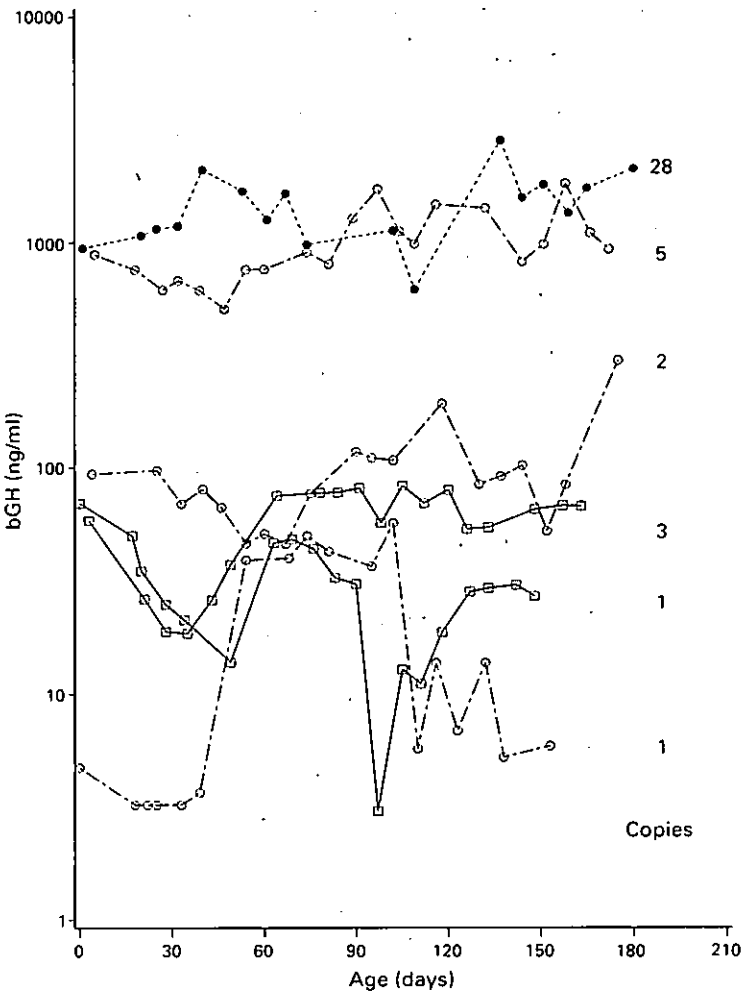

Fig. 1. Each line identifies the concentration of bovine growth hormone (bGH) in plasma for a single MT-bGH founder transgenic pig. The number of integrated gene copies per cell for each pig is indicated at the right (after Miller *et al.*, 1989).

The MT promoter directed expression of fusion genes to the expected tissues in transgenic pigs. However, the concentrations of foreign GH mRNA in pig tissues were considerably lower than in tissues of transgenic mice harbouring the same fusion genes (Palmiter *et al.*, 1983; Hammer *et al.*, 1985a). In pigs, MT-hGH and MT-bGH genes produced high levels of messenger RNA in liver, kidney, testis, adrenal and pancreas, with low levels in several other tissues (Pursel *et al.*, 1989b). Ebert *et al.* (1988) reported that rat GH mRNA was high in spleen, lung, colon and jejunum with lesser amounts in the kidney, lymph nodes, and bone marrow of a transgenic pig with MLV-pGH.

Level of gene expression

Since fusion genes integrate in a different locus in each transgenic founder (G_0), the rate of gene transcription is probably determined by the general activity at the locus of integration and the characteristics of enhancer sequences in genes located on either side of the fusion gene.

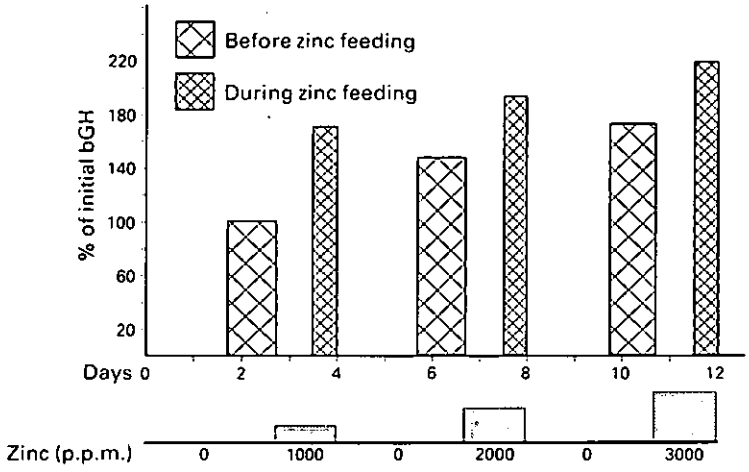

Fig. 2. Relative bovine growth hormone (bGH) concentrations in plasma of 2 MT-bGH transgenic pigs before and after consumption of 1000, 2000 or 3000 p.p.m. zinc ($ZnSO_4$) in the diet. Each bar for pre-zinc feeding is based on mean of 2–5 plasma samples per pig collected between 0 and 27 h before zinc feeding. Each bar during zinc feeding is based on mean of 6 or 7 plasma samples per pig collected between 19 and 31 h after zinc intake began. Concentration of bGH in plasma during the pre-zinc period was significantly lower than during zinc feeding ($P < 0{\cdot}01$).

Additionally, DNA methylation is a known but poorly understood factor that influences gene expression (Jahner & Jaenisch, 1985).

The level of gene expression varied greatly among transgenic pigs that had integrated the same fusion gene. Plasma concentrations at birth ranged from 3 to 949 hGH ng/ml and 5 to 944 bGH ng/ml in MT-hGH and MT-bGH transgenic pigs, respectively (Hammer *et al.*, 1985c; Miller *et al.*, 1989). The single transgenic pig that expressed the MLV-rGH gene had 500–1300 ng rat GH/ml plasma (Ebert *et al.*, 1988), and the pig expressing MT-pGH had 28 ng pGH/ml plasma (Vize *et al.*, 1988). Polge *et al.* (1989) reported that 2 of 4 prolactin-bGH transgenic pigs secreted detectable levels of bovine GH.

Plasma concentrations of hGH were unrelated to the number of gene copies per cell in MT-hGH transgenic pigs (Hammer *et al.*, 1985c). However, Miller *et al.* (1989) found that, in transgenic pigs with MT-bGH, the concentration of bGH in plasma at birth or at 30–180 days of age was positively correlated ($P < 0{\cdot}05$) with the number of gene copies per cell. This relationship is evident in Fig. 1.

Regulation of expression in transgenic pigs has only recently been investigated. In transgenic mice harbouring fusion genes with the MT promoter, concentrations of hGH and bGH in plasma were frequently elevated more than 10-fold after zinc was added to the drinking water (Palmiter *et al.*, 1983; Hammer *et al.*, 1985a). In contrast, addition of 1000–3000 p.p.m. zinc to the feed of transgenic pigs resulted in little more than a doubling of the bovine GH concentration in plasma (Fig. 2). Polge *et al.* (1989) reported that several transgenic pigs with a prolactin-bGH fusion gene released bovine GH in an episodic fashion after pigs were injected with thyrotrophin-releasing hormone (TSH) or after infusion of a dopamine antagonist, sulpiride. Both studies with pigs demonstrated that the promoter sequences of fusion genes can respond to induction.

The concentration of GRF in plasma of transgenic pigs with MT-hGRF or Alb-hGRF were 130–380 pg/ml and 400–8000 pg/ml, respectively (Pursel *et al.*, 1989a, b). These values are 10- to 500-fold higher than concentrations of GRF in plasma of litter-mate control pigs. However, most of the assayable GRF in plasma of the MT-hGRF pigs was the 3–44 metabolite rather than the

Table 4. Average daily gain and feed efficiency of pigs harbouring growth hormone fusion genes

Reference (pigs)	Dietary protein (%)	No of pigs	Daily gain (g/day)		Feed efficiency (kg feed/kg gain)	
			Mean	s.e.	Mean	s.e.
Hammer *et al.* (1986) (30–90 kg body weight)						
Control	16	7	780	30	nd*	
MT-hGH G_0	16	5	630	40	nd*	
Pursel *et al.* (1989b) (30–60 kg body weight)						
Control	16	6	743	32	3·12	0·15
MT-bGH G_0	16	6	690	65	$2{\cdot}62^{P=0{\cdot}026}$	0·12
Pursel *et al.* (1988a) (30–90 kg body weight)						
Siblings	18†	8	760	24	2·99	0·12
MT-bGH G_2	18†	5	$874^{P=0{\cdot}016}$	30	$2{\cdot}46^{P=0{\cdot}026}$	0·16
Vize *et al.* (1988) (20–90 kg body weight)						
Control	nr‡	nr‡	781	44	nd*	
MT-pGH G_0	nr‡	1	1273		nd*	
Ebert *et al.* (1988) (2·5–5·5 months of age)						
Control	nr‡	3	737		nd*	
MLV-rGH G_0	nr‡	1	810		nd*	

*Not determined.
†Diet contained 18% crude protein plus 0·25% lysine, additional minerals and vitamins.
‡Information not reported.

Table 5. Transmission of fusion genes from transgenic founders to progeny

Gene	No. of transgenic founders		Transmission frequency (%)	Reference
	With progeny	Transmitted gene		
MT-hGH	6	5	3–63	Pursel *et al.* (1987)
MT-bGH	3	2	32–73	V. G. Pursel (unpublished)
MT-hGRF	1	1	50	V. G. Pursel (unpublished)
MT-pGH	2	2	20–75	Vize *et al.* (1988)
PRL-bGH	4	4	22–56	Polge *et al.* (1989)
PEPCK-bGH	1	1	50	M. Wieghart & C. A. Pinkert (unpublished)

native peptide (1–44), which may explain why the concentration of pig GH in plasma was not elevated in the transgenics compared to the litter-mate controls (Pursel *et al.*, 1989a).

Performance and physiological characteristics

The enhanced growth rate and body size of transgenic mice that expressed foreign GH genes (Palmiter *et al.*, 1982, 1983) provided both the impetus for transfer of similar fusion genes into pigs and the expectation that the rate of growth might be greatly enhanced. This expectation was not realized in the (G_0) population of MT-hGH and MT-bGH transgenic pigs (Table 4) even though

convincing evidence indicates that the transgenic pigs produced a biologically active form of GH. Transgenic pigs expressing the hGH gene product rarely had detectable concentrations of plasma pig GH (Miller *et al.*, 1989), which indicates that the negative feedback mechanism was functioning. Furthermore, insulin-like growth factor-I (IGF-I) concentrations were 2-fold to 7-fold higher in pigs transgenic with human, rat or bovine GH than in litter-mate control pigs (Ebert *et al.*, 1988; Miller *et al.*, 1989), which also indicates that foreign GH exerted a biological effect by binding to GH receptors of hepatocytes to stimulate IGF-I synthesis.

The MT-hGH and MT-bGH (G_0) transgenic pigs were fed a diet containing only 16% protein that may not have provided sufficient protein for them to gain weight faster than their litter-mate controls. Studies of pigs injected with exogenous pig GH indicate that maximal growth rate is attained only if the diet contains adequate protein and, particularly, lysine (Goodband *et al.*, 1988; Newcomb *et al.*, 1988). Subsequently, Pursel *et al.* (1988a, 1989b) increased the levels of dietary protein and lysine during the 30 to 90 kg growth period and reported that MT-bGH transgenic pigs gained weight 16·5% faster than did sibling control pigs (Table 4). In addition, Vize *et al.* (1988) reported that a transgenic pig expressing MT-pGH gained 492 g per day faster than litter-mate control pigs during the 20 to 90 kg growth period.

Several recent studies of pigs treated with exogenous pGH have revealed that appetite depression accompanies elevated GH in pigs (Campbell *et al.*, 1988; Evock *et al.*, 1988) and may explain why growth rates of GH-treated pigs are not increased as dramatically as in transgenic mice or in rats with a GH-secreting tumour, which have enhanced feed intake (McCusker & Campion, 1986). In comparison to litter-mate or sibling controls, feed intake was depressed 20% in MT-bGH (G_0) and 17% in MT-bGH (G_2) transgenic pigs fed *ad libitum* (Pursel *et al.*, 1989b). These results are comparable to a 14% and 17% depression in feed intake reported for pigs injected with pig GH (Campbell *et al.*, 1988; Evock *et al.*, 1988).

MT-bGH (G_0) transgenic pigs were 16% more efficient and MT-bGH (G_2) transgenic pigs were 18% more efficient in converting feed into body weight gain than were litter-mate or sibling controls (Table 4). Similar improved feed efficiencies of 23% (Campbell *et al.*, 1988) and 25% (Evock *et al.*, 1988) were reported for pigs injected with exogenous pGH in comparison to litter-mate controls.

The elevation of foreign GH in transgenic pigs with MT-hGH and MT-bGH have resulted in marked repartitioning of nutrients away from subcutaneous fat and into other carcass components, including muscle, skin, bone, and certain organs. Ultrasonic estimates or slaughter measurements of backfat thickness at the 10th rib of hGH and bGH transgenic pigs at ~90 kg body weight averaged 7·0 mm and 7·9 mm, respectively, while litter-mate control pigs averaged 18·5 and 20·5 mm, respectively ($P < 0·01$) in each case; Hammer *et al.*, 1986; Pursel *et al.*, 1989b). Additionally, the backfat measurements do not adequately reflect the lack of subcutaneous fat in the transgenic pigs because the skin over the 10th rib was about 1 mm thicker for transgenic pigs than for control pigs (V. G. Pursel & M. B. Solomon, unpublished data).

Ebert *et al.* (1988) reported that by 9 months of age a transgenic boar with MLV-rGH was 26% heavier, and linear bone growth of fore and hind limbs was greater than for a litter-mate control boar. In contrast, at 8 and 10 months of age, MT-hGH and MT-bGH transgenic pigs had not grown to a larger body size, nor were femur, tibia or humerus longer in 4 MT-bGH transgenic pigs than in sibling controls (Pursel *et al.*, 1989b). Additional investigation is required to determine whether this difference is due to structural differences of bGH and rGH that affect binding to GH receptors in epiphyseal chondrocytes, or to some other physiological factor, or whether the single MLV-rGH transgenic boar represents a unique occurrence.

Transgenic pigs expressing MT-hGH and MT-bGH were moderately hyperglycaemic, averaging 10 to 40 mg/dl above litter mates, and insulin concentrations in fasted MT-bGH transgenics were elevated about 20-fold above those in siblings (Pursel *et al.*, 1989b). Pigs injected daily with exogenous pig GH had average increases in serum glucose ranging from 8% to 48% and concentrations of serum insulin that were 2- to 7-fold higher than in control pigs (Campbell *et al.*, 1988,

1989; Evock *et al.*, 1988). In contrast, an MLV-rGH transgenic pig had glucosuria and consistently had serum glucose levels more than 3-fold higher than normal (Ebert *et al.*, 1988).

The MT-hGH and MT-bGH transgenic pigs exhibited a number of notable health problems, including lameness, susceptibility to stress, peptic ulcers, parakeratosis, lethargy, anoestrus in gilts, and lack of libido in boars (Pursel *et al.*, 1987, 1989b). Pathology in joints, characteristic of osteochondritis dissecans, was also observed in the MLV-rGH transgenic pig (Ebert *et al.*, 1988) and in some pigs treated with exogenous pig GH for 57 days (Evock *et al.*, 1988). In contrast, no increase in the incidence of these pathological conditions was observed in non-expressing MT-hGH or MT-bGH transgenic pigs (Pursel *et al.*, 1987) or in prolactin-bGH transgenic pigs that expressed only low levels of bGH (Polge *et al.*, 1989).

Many of the health problems observed in pigs exposed to high concentrations of GH are quite prevalent in the general pig population but at a lower incidence and with less severity. Many necropsy surveys conducted on market hogs at slaughter indicate that 10–30% have gastric ulcers (O'Brien, 1986) and up to 90% have lesions of osteochondrosis (Reiland *et al.*, 1978; Carlson *et al.*, 1988), which is primary to degenerative joint disease, the major cause of lameness in swine. Additional investigation is required to determine whether these ailments would be exhibited in pigs expressing GH fusion genes if the foundation stock was not predisposed to such conditions.

Reproductive capacity of transgenic pigs expressing MT-hGH and MT-bGH genes was seriously impaired. Gilts failed to exhibit oestrus, and at necropsy their ovaries were devoid of corpora lutea or corpora albicantia (V. G. Pursel, unpublished data). Boars totally lacked libido; spermatozoa were recovered by electroejaculation or by flushing them from the epididymis at necropsy to use for artificial insemination to obtain germ-line transmission of the transgene (Pursel *et al.*, 1987). In recent experiments, we found that plasma concentrations of oestrone sulphate did not increase between 80 and 125 days of age in boars expressing an MT-bGH transgene while levels tripled in sibling control boars at this stage of sexual development (H. D. Guthrie, V. G. Pursel & D. J. Bolt, unpublished data). Profiles of follicle-stimulating hormone and luteinizing hormone in plasma were similar for transgenic and sibling control gilts or boars, a finding that suggests the libido problems of the boars may be more closely related to altered steroid metabolism than to pituitary function.

A major difference between a transgenic pig with elevated GH and a normal pig injected daily with exogenous GH is that, in the normal pig, GH is elevated episodically, while in the transgenic pig, GH is elevated continuously (Miller *et al.*, 1989). Rats infused continuously with a high concentration of GH do not attain a maximal rate of growth (Robinson & Clark, 1989). We suggest that continuous exposure to elevated concentrations of GH contributes to the multiple health problems reported in the MT-hGH, MT-bGH and MLV-rGH transgenic pigs and also may prevent them from growing to their full potential. Use of promoters that permit expression of GH fusion genes only during the rapid growth phase or promoters that can induce the release of large episodic doses of GH may be essential to achieve only the positive aspects of elevated GH for pigs. Future research on growth regulation with transgenic pigs will most certainly be directed toward this goal, along with investigation on the potentials of IGF-I, GH receptors, and other structural genes involved in growth.

Transmission of transgenes

If transgenic livestock of economic importance are produced, the transgenes must be consistently transmitted to progeny. Of 17 transgenic pigs harbouring 6 different fusion genes tested to date, 15 pigs successfully transmitted the transgene to one or more progeny (Table 5). Most of the transgenic pigs transmitted the fusion gene to about 50% of the progeny as expected with simple Mendelian inheritance. One of 6 MT-hGH transgenic pigs (Pursel *et al.*, 1987) and 1 of 3 MT-bGH (Pursel *et al.*, 1989a) transgenic boars that failed to transmit the transgene to their progeny were

probably mosaic for the gene, with integration only in somatic cells. In another MT-hGH boar, integration was evidently mosaic in the germ line since the transgene was only transmitted to 1 of 33 progeny (Pursel *et al.*, 1987). Mosaicism in the germ line was found in 25–36% of transgenic mice produced by microinjection (Wilkie *et al.*, 1986).

Germ line transmission has been obtained from both expressing and non-expressing pigs. All transgenic progeny produced from expressing transgenic boars with MT-hGH, MT-bGH or MT-hGRF fusion genes have also expressed the transgene (Pursel *et al.*, 1987; V. G. Pursel, unpublished data). Of 4 MT-hGH non-expressing transgenics, 1 produced 17 transgenic progeny of which 2 expressed the gene; plasma concentrations were < 10 ng hGH/ml (V. G. Pursel, K. F. Miller & D. J. Bolt, unpublished data). The transgenic progeny of the other 3 non-expressing transgenic (G_0) pigs did not express the gene. Such results suggest that most of the fusion genes become stably integrated into the pig genome and usually function in progeny in the same manner as they did in the founder.

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