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Pancreatic expression of human insulin gene in transgenic mice

(human C-peptide/pancreatic B cells/RNA transcripts)

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ABSTRACT **We have investigated the possibility of obtaining integration and expression of a native human gene in transgenic mice. An 11-kilobase (kb) human chromosomal DNA fragment including the insulin gene (1430 base pairs) was microinjected into fertilized mouse eggs. This fragment was present in the genomic DNA of several developing animals. One transgenic mouse and its progeny were analyzed for expression of the foreign gene. Synthesis and release of human insulin was revealed by detection of the human C-peptide in the plasma and urine. Human insulin mRNA was found in pancreas but not in other tissues. These findings indicate that (*i*) the 11-kb human DNA fragment carries the sequences necessary for tissue-specific expression of the insulin gene and (*ii*) the human regulatory sequences react to homologous signals in the mouse.**

An accurate method to test the role and tissue specificity of putative regulatory DNA sequences is to introduce them into the germ cell line, so that they are present during development, and observing their expression in various tissues at various steps of differentiation. In the past few years, an increasing number of experiments using gene microinjection into fertilized mouse eggs have shown in the resulting transgenic mice a tissue-specific expression of the nucleotide sequences that have been introduced, independent of their site of integration in the chromosomes (1–18). This suggests that specific nucleotide sequences present either in the gene itself or very close to it interact with regulatory molecules present in the differentiated tissues. These experiments, however, have been conducted mainly with rearranged mouse immunoglobulin genes (1–6) or with composite genes involving regulatory parts of mouse or rat genes (7–12), in which controlling elements not yet identified could have been deleted or displaced. In a few instances, unmodified genes from mouse (13, 14), rat (15, 16), or chicken (17) were correctly expressed in transgenic mice, but up to very recently (18) no instance of transgenic mice expressing a human gene was reported. Also, attempts to obtain expression of the human growth hormone gene failed (19, 20).

The insulin gene was chosen because its product, a key hormone in metabolic homeostasis, is highly conserved in animal species. Its phenotypic activity is very stringently controlled. Furthermore, its transcriptional and translational products can be differentiated from the mouse products without modification of the gene. We show that, upon its introduction into fertilized mouse eggs, the human insulin gene can be correctly expressed in transgenic mice.

MATERIALS AND METHODS

Preparation of the Human Insulin Gene for Microinjection. A *Hin*dIII DNA fragment 11 kilobases (kb) long and containing the human insulin gene [1430 base pairs (bp)] was excised from a recombinant plasmid (pBR322.Hins11) and purified by centrifugation through a sucrose gradient. The plasmid pBR322.Hins11 was itself derived from phage λ Ch4.gH1 14.1 (21). Digestion of human placenta DNA by the restriction enzyme *Hin*dIII generates an 11-kb DNA fragment that hybridizes with the fragment prepared from pBR322.Hins11. For microinjection, dilutions of the purified fragment were made to obtain either 400 or 40 copies per pl.

Production of Transgenic Mice. The day after mating either (C57BL/6 × CBA/He)F_1 or (C57BL/6 × SJL/J)F_1 mice, F_2 fertilized eggs were isolated from oviducts, freed from follicle cells by hyaluronidase treatment, transferred to micromanipulation chambers, and microinjected by following the procedure described by Gordon and Ruddle (22). About 1 pl of the DNA solution was microinjected into the male pronuclei. When microinjections were completed, the eggs were transferred to the oviducts of pseudopregnant mice.

DNA Analysis. DNA was prepared as described (23), from whole fetuses at day 13, from tail biopsy samples from 2- to 4-week-old mice, or from tissues. The presence of the human gene was checked by a dot-blot assay using 10 μg of DNA. DNA samples from animals that were positive were further analyzed by digestion with the restriction enzyme *Bgl* II; the digests were separated on 0.8% agarose slab gels by horizontal electrophoresis at 1.5 V/cm for 16–18 hr. DNA was denatured *in situ* and transferred to nitrocellulose paper (24). For both dot-blot and Southern blot analyses, the DNA probe was the *Hin*dIII 11-kb DNA fragment used for microinjection, labeled with [^{32}P]dATP and [^{32}P]dCTP (3000 Ci/mmol, Amersham; 1 Ci = 37 GBq) by nick translation (25) to a specific activity of 2–3 × 10^8 cpm/μg. The filters were hybridized to the probe, washed (final wash in 15 mM NaCl/1.5 mM sodium citrate at 65°C), and exposed to x-ray film. Under the conditions of stringency used, no hybridization to endogenous sequences was observed.

RNA Analysis. RNA was prepared from mouse tissues by the guanidium thiocyanate procedure (26), denatured by glyoxal and dimethyl sulfoxide (27), and purified on 1% agarose slab gels by horizontal electrophoresis at 6 V/cm for 3 hr. RNA was transferred to GeneScreen membranes (New England Nuclear). The human-insulin cDNA (28) probe used was a *Pvu* II–*Pst* I 158-bp fragment including the last 21 coding bp, the 76 bp corresponding to the 3′ nontranslated RNA, 45 bp of poly(A)·poly(T), and 16 bp of the G·C homopolymeric tail. The probe was labeled with [^{32}P]dATP and [^{32}P]dCTP by nick-translation to a specific activity of 2–3 × 10^8 cpm/μg. The filters were hybridized to the probe at 42°C in 50% (vol/vol) formamide, washed (final wash 15 mM NaCl/1.5 mM sodium citrate, 42°C) and exposed to x-ray film. Under the conditions of stringency used, no hybridization to mouse pancreas RNA was observed. The probe

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Abbreviations: kb, kilobase(s); bp, base pair(s).

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hybridized to human pancreas total RNA and poly(A)$^+$ RNA (the latter kindly provided by J. C. Dagorn).

Determination of Human C-Peptide, Insulin, and Glucose Levels. The human C-peptide (the fragment excised from proinsulin during insulin biosynthesis) was determined by a radioimmunoassay (29) with ^{125}I-labeled human C-peptide and an antiserum to human C-peptide raised in goat (kindly provided by M. A. Root and B. H. Frank, Eli Lilly). The level of detection is 50 pg/ml. Insulin was measured by a radioimmunoassay with ^{125}I-labeled porcine insulin and guinea-pig antibodies to porcine insulin according to the supplier of the assay kit (Behring, Marburg, F.R.G.). Glycemia was measured by a glucose oxidase assay (Peridochrom, Boehringer Mannheim). Each determination required 100, 50, and 10 μl of plasma for the human C-peptide, insulin, and glucose, respectively.

Glucose Injection. Fasting mice were anesthetized with Nembutal and injected intraperitoneally with 20 mg of glucose immediately after the first drawing of blood. Each mouse was bled twice, at time 0 and either 30 or 60 min later. Blood (300 μl) was drawn from the orbital sinus into heparin and centrifuged, and plasma samples were either immediately used for assay or frozen at −72°C. Glucose and C-peptide levels were determined on each sample.

RESULTS

Production of Transgenic Mice Carrying the Human Insulin Gene. Approximately 40 or 400 copies of an 11-kb *Hin*dIII DNA fragment containing the human insulin gene (Fig. 1) were microinjected into male pronuclei of fertilized eggs. The microinjected eggs were transferred into oviducts of pseudopregnant females. Since previous attempts to obtain transgenic mice with the human insulin gene were negative (30), in a first series of experiments pregnancy was interrupted at day 13, and DNA was prepared from whole fetuses. The presence of the human gene was checked by a dot-blot assay using the 11-kb ^{32}P-labeled DNA fragment as a probe. Southern blot analysis confirmed the positive results and showed the presence of multiple copies of the intact 11-kb fragment. In subsequent experiments, development of the mice was allowed to proceed normally. DNA prepared from tissues or tail biopsy samples was analyzed as described above for fetuses. All the positive mice also had intact copies of the gene (Fig. 1*A*). Of a total of 2807 eggs microinjected, 2104 were transferred into pseudopregnant females, and 13 transgenic animals (6 fetuses and 7 pups) were found among the 200 microinjected eggs that developed. Six transgenic newborns died within 24 hr, but negative littermates also died, suggesting that the death of transgenic mice was not related to the presence of the human insulin gene. (We have now overcome this technical problem by performing cesarian sections and using foster mothers.)

Transmission of the Human Insulin Gene to the Progeny. A transgenic male [Tg (OHIns)JM74, hereafter referred to as Tg74 G_0] originating from a (C57BL/6 × SJL/J)F_2 egg and its progeny were examined. Tg74 G_0 was mated with C57BL/6 and SJL/J females and the G_1 mice obtained were tested for the presence of the human genomic DNA fragment by dot-blot analysis of tail DNA. Forty-one out of the 75 G_1 mice examined were transgenic (55%, Tg74 G_1). Crosses between G_1 transgenic mice resulted in 23 transgenic G_2 out of 29 mice (79%, Tg74 G_2). These figures suggest that the human chromosomal DNA fragment is integrated in one chromo-

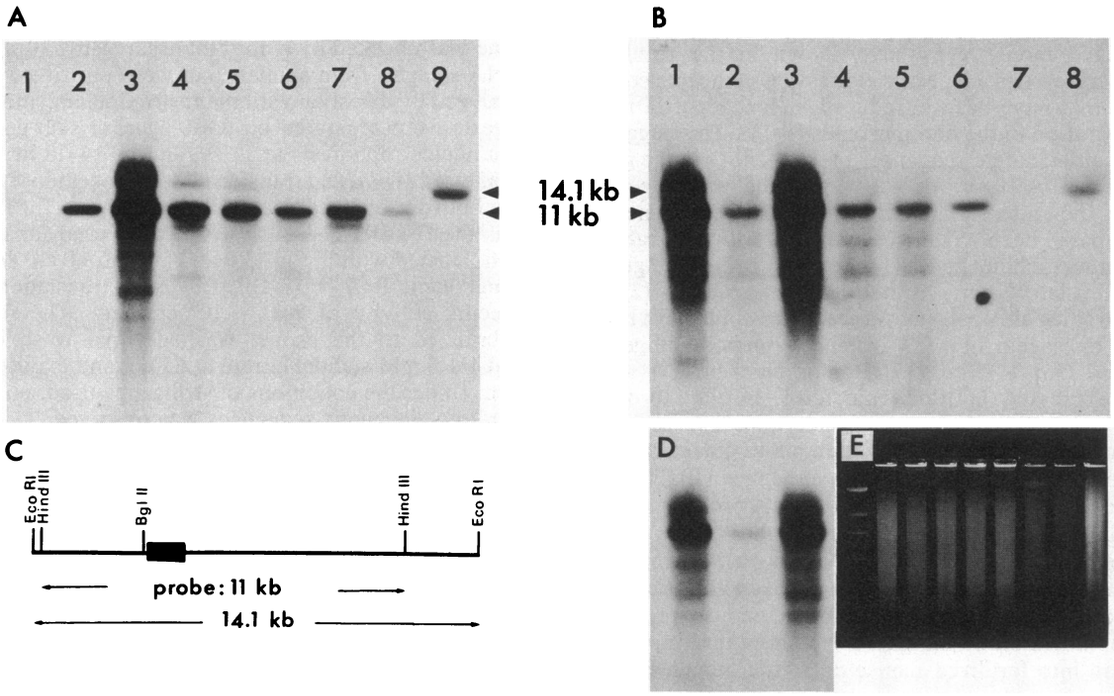

FIG. 1. Analysis of transgenic mice for the human insulin gene. DNA (20 μg) from transgenic and control mice was digested with *Bgl* II (one site in the microinjected human chromosomal DNA fragment). The digests were electrophoresed. DNA was denaturated *in situ* and transferred to nitrocellulose paper. The ^{32}P-labeled DNA probe was the *Hin*dIII 11-kb DNA fragment used for microinjection. (*A*) Lane 1, control mouse; lane 2, control mouse + the 11-kb human insulin fragment (two copies); lanes 3 to 8, six independent transgenic animals; lane 9, human placenta DNA digested by *Eco*RI. (*B*) Tg74, four G_1 offspring, and controls. Lane 1, Tg74; lane 2, Tg74.4; lane 3, Tg74.7; lane 4, Tg74.30; lane 5, Tg74.34; lane 6, control mouse + two copies of the 11-kb human insulin fragment; lane 7, control mouse; lane 8, human placenta DNA digested by *Eco*RI. Tg74 and Tg74.7 mice had about 50 copies of the human chromosomal DNA fragment and the other mice had about 5 copies, as determined by dot-blot analysis. (*C*) Scheme of the 11-kb human genomic DNA fragment used in these experiments. The dark box represents the insulin gene (1430 bp), which includes three exons and two introns (21). (*D*) The same gel transfer as in B with a shorter x-ray film exposure (16 hr). (*E*) Ethidium bromide staining of the gel corresponding to *B* and *D*. The leftmost lane contains size markers.

some. Transgenic G_2 and control mice have been crossed to determine which transgenic G_2 mice are homozygous for the human insulin (*HIns*) locus introduced into the mouse line, but the results are not yet available. Both males and females were found among transgenic offspring of the Tg74 male, indicating that the *HIns* locus is on an autosomal chromosome.

Upon digestion of DNA from Tg74 and offspring with the enzyme *Bgl* II, which cuts once in the human chromosomal DNA fragment, Southern blot analysis revealed a predominant 11-kb band and several bands of lower molecular weight (Fig. 1*B*). This suggests that multiple copies of the 11-kb human chromosomal DNA fragment were tandemly integrated head to tail, likely at two different loci. Also, in some mice, there was a band larger than 11 kb. There is a striking difference in the total numbers of copies in Tg74 G_0 and Tg74.7 G_1 mice, on one hand, and in Tg74.4, Tg74.30, and Tg74.34 G_1 mice, on the other hand (Fig. 1*B*).

Tg74 Mice Secrete Human Insulin. Tg74 mice had plasma levels of glucose (mean ± SD = 1.09 ± 0.15 mg/ml, $n = 6$) and of insulin (0.81 ± 0.10 ng/ml, $n = 8$) similar to those of control mice (glucose = 1.12 ± 0.31 mg/ml, $n = 7$; and insulin = 0.76 ± 0.07 ng/ml, $n = 7$).

To test for gene expression, we looked for the presence of human insulin. Insulin is synthesized in pancreatic B cells as preproinsulin, which is translocated as proinsulin to the endoplasmic reticulum; proinsulin is cleaved into mature insulin and the C-peptide, which are secreted into the blood stream in equimolar quantities. Human and mouse insulin cannot be differentiated by antibodies against insulin. Therefore, we assayed for the human C-peptide because it is well known (31) that there is a high divergence between animal species, and it was likely that specific antibodies could discriminate between the human and mouse C-peptides. Discrimination was achieved by using a highly sensitive radioimmunoassay (29) with an anti-human C-peptide antiserum. Plasma and urine from control mice and littermates of Tg74 G_1 mice were negative. Transgenic mice had plasma levels of human C-peptide ranging between 60 and 170 pg/ml. As expected (32), their urine contained concentrations of human C-peptide 5–15 times higher (Table 1). This clearly indicates that the human insulin gene is expressed in Tg74 mice.

If the human insulin gene is expressed in pancreatic B cells of transgenic mice, there should be an increase in circulating hormone upon challenge with glucose. Six G_1 transgenic mice were each injected intraperitoneally with 20 mg of glucose. The resulting elevation of the plasma glucose level is accompanied by an increase in circulating human C-peptide (Fig. 2).

Presence of Human Insulin Gene Transcripts in Pancreas of Tg74 Mice. Correct phenotypic expression of the human insulin gene was further demonstrated by the presence of human insulin gene transcripts in total RNA from pancreas and their absence from other tissues. The transcripts found in total RNA prepared from pancreas of the two Tg74 G_1 mice that were analyzed (Fig. 3*A*, lanes 4 and 5) have the size of the transcripts found in poly(A)$^+$ RNA prepared from human pancreas (Fig. 3*A* lane 6 and 3*B* lane 1). Human insulin mRNA was specifically discriminated from mouse insulin mRNA by using a cDNA probe corresponding mainly to the 3′ nontranslated human mRNA, which does not form stable hybrids with mouse insulin mRNA under stringent conditions (Fig. 3*A* lane 3 and 3*B* lane 8). This indicates (since there is no apparent size difference between the human mRNA made in mouse and human) that the transcript initiates at or near the correct site. No hybridization to the probe was obtained with total RNAs from the other organs that were tested [liver,

Table 1. Presence of human C-peptide in transgenic mice

Mouse	Presence of human insulin gene*	Human C-peptide, pg/ml	
		Plasma	Urine
Controls			
($n = 5$)	(−)	0	ND
($n = 9$)	(−)	ND	0
G_0 Tg74	+	+†	ND
G_1 Tg74-3	+	ND	740
Tg74-5	−	ND	0
Tg74-11	−	0	ND
Tg74-12	−	0	ND
Tg74-13	−	0	ND
Tg74-14	+	170	1700
Tg74-15	+	155	1250
Tg74-16	+	120	900
Tg74-26	−	ND	0
Tg74-31	+	115	ND
Tg74-32	−	0	0
Tg74-33	+	145	ND
Tg74-35	+	105	480
Tg74-36	+	60	900
G_2 Tg74-2-1	−	0	0
Tg74-2-2	+	95	ND
Tg74-2-3	+	62	350

ND, not determined.
*As determined by tail DNA dot-blot analysis.
†Plasma from the mouse Tg74 (G_0) was assayed with an anti-human C-peptide antiserum different from the antiserum used for all the other mice and less sensitive; the values found (600 pg/ml) cannot be considered accurate.

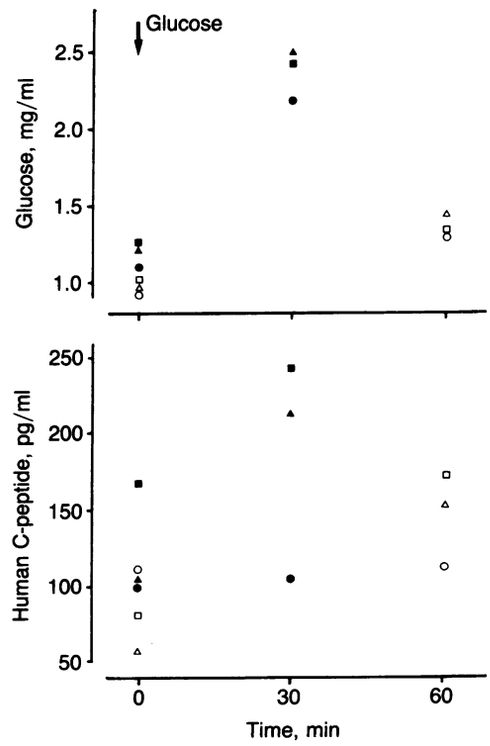

FIG. 2. Increase of plasma levels of human C-peptide upon glucose injection into transgenic mice. Fasting mice were anesthetized with Nembutal and injected intraperitoneally with 20 mg of glucose immediately after the first drawing of blood. Each mouse was bled twice, at time 0 and either 30 or 60 min later. Each mouse is represented by a different symbol.

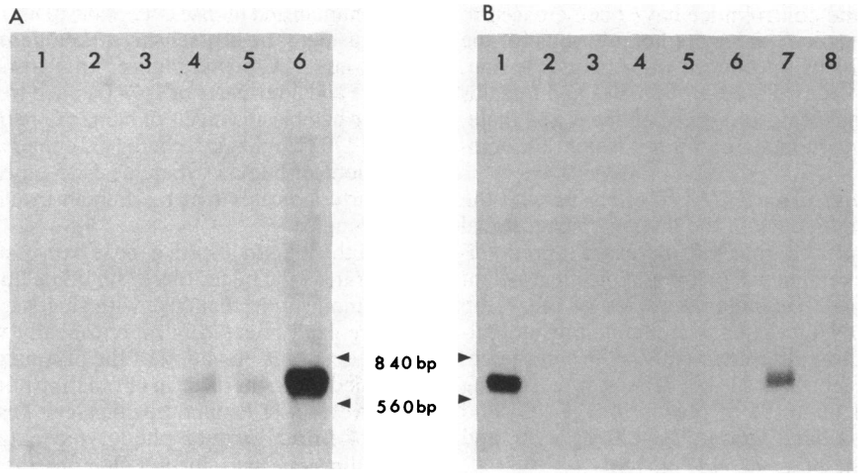

FIG. 3. Analysis of transgenic mice for human insulin gene transcripts. RNA (30 μg) from transgenic and control mice was electrophoresed and transferred to GeneScreen membranes. The ^{32}P-labeled probe used was a *Pvu* II–*Pst* I 158-bp fragment from the human insulin cDNA including the last 21 coding bp, the 76 bp corresponding to the nontranslated RNA, 45 bp of poly(A)·poly(T), and 16 bp of the G·C homopolymeric tail. (*A*) Lane 1, Tg74.7 liver, lane 2, Tg74.4 liver; lane 3, control mouse pancreas; lane 4, Tg74.7 pancreas; lane 5, Tg74.4 pancreas; lane 6, poly(A)$^+$ RNA (1.5 μg) from human pancreas. (*B*) Lane 1, poly(A)$^+$ RNA (0.6 μg) from human pancreas; lane 2, Tg74.7 kidney; lane 3, Tg74.7 testis; lane 4, Tg74.7 heart; lane 5, Tg74.7 salivary glands; lane 6, Tg74.7 liver; lane 7, Tg74.7 pancreas; lane 8, control mouse pancreas. The migration of the DNA fragments used as size markers overestimates the size of the mRNA. The human insulin mRNA is about 550 nucleotides long, including the poly(A)$^+$ tail (21). Time of exposure: *A*, 3 days; *B*, 6 days. Pancreatic RNA was prepared from whole pancreas, in which B cells represent only 1% of the cell population. Demonstration that human-insulin RNA is restricted to the B cells is supported by the human-insulin secretory response to glucose stimulus (see Fig. 2).

kidney, testis, submaxillary salivary glands, and heart (Fig. 3) and muscle, brain, ovary, lung, thymus, and spleen (results not illustrated)].

All together, these data suggest that the human insulin gene is specifically expressed in pancreatic B cells of Tg74 mice and that both the transcript and the protein are normally processed.

DISCUSSION

Various genes have already been introduced into the mouse genome, and specific expression in the corresponding specialized tissues has been found (1–6, 13–18). Attempts to obtain transgenic mice with the human insulin gene were restricted to the finding of two transgenic fetuses that have integrated the insulin gene linked to the pBR322 vector (33) and one transgenic mouse that did not transmit the human insulin sequence to any of its 10 offspring (30). Expression has been reported only with composite genes including the upstream region of the rat insulin gene and the sequences coding for the large tumor antigen of simian virus 40 (11). Contrary to what was the case with the other genes (1–6, 13–18) and because of its major role in metabolic regulation, expression of the microinjected insulin gene could have deleterious effects during intrauterine development, after birth, or both. We show that transgenic mice expressing the human insulin gene can be obtained. The demonstration is supported by the existence of a signal when pancreatic RNA is hybridized to a specific human insulin cDNA probe and by the increase of the plasma human C-peptide in response to glucose administration to transgenic mice. It was not possible to measure the mouse C-peptide, but the relative level of expression of the human insulin gene can be indirectly estimated. The plasma human C-peptide concentration in transgenic mice is 5–20% of that found in humans (32), and the plasma insulin concentrations in transgenic mice are similar to those of control mice (our results) and of humans (34). All together, these facts suggest that the order of magnitude of the human insulin secretion in Tg74 mice is 10% of the total insulin secretion.

All the transgenic mice that were tested produced the human C-peptide (not shown). However, Southern blot (Fig. 1) and dot-blot (not shown) analysis on 42 G_1 mice showed a distribution into two groups. Mice in the first group had about 50 copies of the human chromosomal DNA fragment, and those in the second had about 5 copies. This is compatible with the existence of two loci of recombination in the Tg74 G_0 mouse and their segregation in the progeny.

Our results indicate that the expression of intact human gene with its own controlling elements incorporated into transgenic mice can be expressed, as it was recently reported for the human β-globin gene (18). It appears that the human regulatory sequences present in the inserted DNA fragment correctly respond to the corresponding heterospecific signals in the mouse, and one can assume that the regulatory factors involved are conserved between mouse and human. The target nucleotide sequences for these factors may display a higher degree of homology than the surrounding nonregulating sequences. Transient expression experiments with cell cultures in which the chloramphenicol acetyltransferase gene linked to the 5′-flanking region of the human insulin gene was preferentially expressed when introduced into simian virus 40-transformed hamster pancreatic β cells (35) suggested that some tissue-specific regulatory DNA sequences are located within the 300 bp upstream of the initiation site of transcription. Comparison of the DNA fragments, particularly on the 5′ side of the insulin gene, should reveal the nucleotide sequences that are common and facilitate their isolation and the characterization of their role.

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1. Brinster, R. L., Ritchie, K. A., Hammer, R. E., O'Brien, R. L., Arp, B. & Storb, U. (1983) *Nature (London)* **306,** 332–335.
2. Storb, U., O'Brien, R. L., McMullen, M. D., Gollahon, K. A. & Brinster, R. L. (1984) *Nature (London)* **310,** 238–241.
3. Grosschedl, R., Weaver, D., Baltimore, D. & Costantini, F. (1984) *Cell* **38,** 647–658.
4. Ritchie, K. A., Brinster, R. L. & Storb, U. (1984) *Nature (London)* **312,** 517–519.
5. Stewart, T. A., Pattengale, P. K. & Leder, P. (1984) *Cell* **38,** 627–637.
6. Rusconi, S. & Kohler, G. (1985) *Nature (London)* **314,** 330–334.
7. Palmiter, R. D., Brinster, R. L., Hammer, R. E., Trumbauer, M. E., Rosenfeld, M. G., Birnberg, N. C. & Evans, R. M. (1982) *Nature (London)* **300,** 611–615.
8. Palmiter, R. D., Norstedt, G., Gelinas, R. E., Hammer, R. E. & Brinster, R. L. (1983) *Science* **222,** 809–814.
9. Low, M. J., Hammer, R. E., Goodman, R. H., Habener, J. F., Palmiter, R. D. & Brinster, R. L. (1985) *Cell* **41,** 211–219.
10. Ornitz, D. M., Palmiter, R. D., Hammer, R. E., Brinster, R. L., Swift, G. H. & MacDonald, R. J. (1985) *Nature (London)* **313,** 600–602.
11. Hanahan, D. (1985) *Nature (London)* **315,** 115–122.
12. Chada, K., Magram, J., Raphael, K., Radice, G., Lacy, E. & Costantini, F. (1985) *Nature (London)* **314,** 377–380.
13. Le Meur, M., Gerlinger, P., Benoist, C. & Mathis, D. (1985) *Nature (London)* **316,** 38–42.
14. Yamamura, K., Kikutani, H., Folsom, V., Clayton, L. K., Kimoto, M., Akira, S., Kashiwamura, S., Tonegawa, S. & Kishimoto, T. (1985) *Nature (London)* **316,** 67–69.
15. Swift, G. H., Hammer, R. E., MacDonald, R. J. & Brinster, R. L. (1984) *Cell* **38,** 639–646.
16. Shani, M. (1985) *Nature (London)* **314,** 283–285.
17. McKnight, G. S., Hammer, R. E., Kuenzel, E. A. & Brinster, R. L. (1983) *Cell* **34,** 335–341.
18. Townes, T. M., Lingrel, J. B., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. (1985) *EMBO J.* **4,** 1715–1723.
19. Wagner, E. F., Covarrubias, L., Stewart, T. A. & Mintz, B. (1983) *Cell* **35,** 647–655.
20. Hammer, R. E., Palmiter, R. D. & Brinster, R. L. (1984) *Nature (London)* **311,** 65–67.
21. Bell, G. I., Pictet, R. L., Rutter, W. J., Cordell, B., Tischer, E. & Goodman, H. M. (1980) *Nature (London)* **284,** 26–32.
22. Gordon, J. W. & Ruddle, F. H. (1983) *Methods Enzymol.* **101,** 411–433.
23. Gross-Bellard, M., Oudet, P. & Chambon, P. (1973) *Eur. J. Biochem.* **36,** 32–38.
24. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
25. Rigby, P. W. J., Dieckmann, M., Rhodes, C. & Berg, P. (1977) *J. Mol. Biol.* **113,** 237–251.
26. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **24,** 5294–5299.
27. McMaster, G. K. & Carmichael, G. G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4835–4838.
28. Bell, G. I., Swain, W. F., Pictet, R., Cordell, B., Goodman, H. M. & Rutter, W. J. (1979) *Nature (London)* **282,** 525–527.
29. Heding, L. G. (1978) *Diabetologia* **11,** 541–548.
30. van der Putten, H., Botteri, F. & Illmensee, K. (1984) *Mol. Gen. Genet.* **198,** 128–138.
31. Steiner, D. F. (1984) *Harvey Lect.* **78,** 191–228.
32. Kuzuya, T., Matsuda, A., Sakamoto, Y., Tanabshi, S. & Kajinuma, H. (1978) *Diabetes* **27,** Suppl. 1, 210–215.
33. Burki, K. & Ullrich, A. (1982) *EMBO J.* **1,** 127–131.
34. Johnston, D. G., Alberti, K. G., Wright, R., Smith-Laing, G., Stewart, A. M., Sherlock, S., Faber, O. & Binder, C. (1978) *Diabetes,* **27,** Suppl. 1, 201–206.
35. Walker, M. D., Edlund, T., Boulet, A. M. & Rutter, W. J. (1983) *Nature (London)* **306,** 557–561.