between $\beta$-strands depends on loop size and specific interactions of the loop back to the $\beta$-sheet. However, in the same class of variable domains ($V_h$, $V_e$ or $V_i$) these interactions are usually conserved (ref. 5 and A. M. Lesk and C. Chothia, personal communication).

While human monoclonal antibodies have therapeutic potential in human disease, they can be difficult to prepare$^{17}$ and treatment of patients with mouse monoclonal antibodies often increases the titre of circulating antibody against the mouse immunoglobulin$^{18}$. As chimaeric antibodies containing human constant domains$^{19,20}$ and variable domains made by grafting mouse CDRs into human FRs, could have therapeutic potential, we wondered whether the HuVNP-IgE antibody loses antigenic determinants associated with the MVNP variable region (idiotypes). The binding of HuVNP-IgE and MVNP-IgE to both monoclonal and polyclonal anti-idiotypic antibodies directed against the MVNP domain was examined by using inhibition assays. As shown in Fig. 3d, the HuVNP-IgE antibody has lost the MVNP idiotype determinant recognized by antibody Ac146 (ref. 21). Furthermore, HuVNP-IgE also binds the antibody Ac38 (ref. 20) less well (Fig. 3e), therefore it is not surprising that HuVNP-IgE has lost many of the determinants recognized by a polyclonal rabbit anti-idiotypic antiserum (Fig. 3e). While the loss of idiotype determinants that accompanies 'humanising' of the $V_h$ region is reassuring in view of potential therapeutic applications, it does suggest that the recognition of the hapten and of anti-idiotypic antibodies is not equivalent. Thus the HuVNP-IgE antibody retains hapten binding but has lost idiotype determinants, indicating that the immunoglobulin uses different sites to bind hapten and anti-idiotypic antibodies. It appears, therefore, that both FR and CDR side chains form the binding site for these anti-idiotypes, but mainly CDR side chains interact with hapten.

We thank C. Milstein for suggesting this project, K. Rajewsky and M. Reth for the anti-idiotypic antibodies Ac38 and Ac146, and A. M. Lesk, C. Chothia, R. J. Leatherbarrow and C. Milstein for helpful discussions. J.F. is a Fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

Regulation of human insulin gene expression in transgenic mice

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Insulin is a polypeptide hormone of major physiological importance in the regulation of fuel homeostasis in animals (reviewed in refs 1, 2). It is synthesized by the $\beta$-cells of pancreatic islets, and circulating insulin levels are regulated by several small molecules, notably glucose, amino acids, fatty acids and certain pharmacological agents. Insulin consists of two polypeptide chains (A and B, linked by disulphide bonds) that are derived from the propeptide cleavage of proinsulin, generating equimolar amounts of the mature insulin and a connecting peptide (C-peptide). Humans, like most vertebrates, contain one proinsulin gene$^{19}$, although several species, including mice$^{20}$ and rats$^{21}$, have two highly homologous insulin genes. We have studied the regulation of serum insulin
levels and of insulin gene expression by generating a series of transgenic mice containing the human insulin gene. We report here that the human insulin gene is expressed in a tissue-specific manner in the islets of these transgenic mice, and that serum human insulin levels are properly regulated by glucose, amino acids and tolbutamide, an oral hypoglycaemic agent.

The human insulin gene that we have used to generate transgenic mice is contained within a 12.5-kilobase (kb) EcoRI fragment that was isolated from a genomic library. Of 46 mice born after one series of single-cell embryo microinjections, three contained human insulin gene sequences as detected by Southern hybridization analysis (Fig. 1a). A human C-peptide radioimmunoassay (Behringwerke) was used to detect expression of the human insulin gene in the transgenic mice and their offspring. Several hundred transgenic and control mice have been analysed under a variety of physiological circumstances (see below), and the transgenic mice show variable levels of human C-peptide in their sera, whereas control mice show no such expression.

The tissue specificity of human insulin gene expression in these transgenic mice was examined by both RNA analyses and pancreatic islet function studies. Northern hybridizations using a human insulin complementary DNA probe demonstrated that total pancreatic RNA from both transgenic (Fig. 1b, lane 11) and control (lane 1) mice hybridized to the human insulin cDNA probe, whereas RNAs from transgenic spleen, kidney, brain, lung, islet, salivary gland, intestine, heart and muscle (lanes 2–10, respectively) showed no hybridization. Because no transgenic tissues other than pancreas were found to express detectable levels of insulin RNA, insulin expression in both transgenic and control pancreas was studied to determine whether the transgenic pancreas is a site of human insulin expression. Pancreatic islets from six transgenic and six control mice were isolated by collagenase digestion as described previously and cultured in groups of 80–100 islets per tissue culture well. The following day, aliquots of media were taken and human C-peptide levels measured. The samples from the transgenic islet wells contained 250–650 ng ml⁻¹ of human C-peptide, but the control islet wells contained no detectable human C-peptide. The cultured transgenic islets continued to express human C-peptide for several days. From these experiments, we conclude that the major site of human insulin expression in these transgenic mice is the endocrine pancreas.

Transgenic and control pancreas were stained with immunoperoxidase using a guinea pig anti-porcine insulin antibody and a goat anti-human C-peptide antibody (Fig. 2). The anti-porcine insulin antibody cross-reacted with both human and mouse insulin, and islets from both transgenic (Fig. 2a) and control (Fig. 2b) mice were stained. The size, distribution and number of islets were essentially the same in transgenic and control mice. The anti-human C-peptide antibody showed little or no cross-reactivity with mouse C-peptide, however, and the transgenic islets (Fig. 2c) were stained using this antibody whereas the control islets (Fig. 2d) were not. These immunohistochemistry data are consistent with the Northern analysis and islet function studies presented above, and demonstrate that the transgenic islets were specifically expressing human insulin.

Glucose and human C-peptide levels in the transgenic mice were studied under a variety of physiological conditions to determine whether normal glucose homeostasis was being preserved and whether expression of the human insulin gene was being regulated appropriately in these mice. Blood glucose regulation was studied by glucose tolerance tests. Transgenic offspring of mouse 16 and non-transgenic siblings were fasted overnight, given an intraperitoneal (i.p.) injection of glucose, and bled at various times after injection to determine serum glucose levels. The glucose tolerance curve from the transgenic mice was similar to that from the control mice (Fig. 3a). Of particular importance is the finding that the fasting and maximally stimulated glucose levels as well as the kinetics of"
Fig. 2 Immunoperoxidase staining of transgenic and control pancreas. Pancreas samples were immersed in liquid nitrogen and 4-μm tissue sections were prepared using a cryostat. Immunoperoxidase staining of serial sections was performed as described previously11, using either guinea pig anti-porcine insulin antibody (a and b) or goat anti-human C-peptide antibody (c and d). a, Transgenic pancreas stained with anti-insulin antibody (Arnel Products). b, Control pancreas stained with anti-insulin antibody. c, Transgenic pancreas stained with anti-human C-peptide antibody (Behringwerke). d, Control pancreas stained with anti-human C-peptide antibody (photographically enhanced to allow visualization of islets). Other transgenic tissues, including liver, adrenal and thyroid, are not stained using the guinea pig anti-porcine insulin antibody (not shown).

Fig. 3 a, Intraperitoneal glucose tolerance test. Transgenic offspring of mouse 16 and non-transgenic siblings were fasted overnight, then given an i.p. injection of glucose (1 mg glucose per g body weight). Each mouse was then arbitrarily assigned to a group of four for serum sampling at 5, 10, 20, 30, 45, 60 or 90 min post-injection. Four mice did not receive glucose and were bled to determine fasting serum glucose levels. Each time point therefore represents an average of values from four animals, and standard deviation half-bars are indicated. Solid line, transgenic mice; dashed line, control mice. Serum glucose determinations were performed using a commercially available kit (Worthington) under the conditions recommended by the manufacturer. Intravenous and oral glucose tolerance tests performed as described above showed no statistically significant difference between the transgenic and control mice (data not shown). b, Human C-peptide levels during an i.p. glucose tolerance test. The experiment was performed as described in a, with each transgenic point representing an average of values from six animals (i.p. injection of 2 mg glucose per g body weight). Serum human C-peptide levels were measured using a commercially available kit (Behringwerke) under the conditions recommended by the manufacturer. Solid line, transgenic mice; dashed line, control mice. These data are similar to glucose tolerance results previously reported for both mice22 and humans2. Preliminary experiments suggest that mouse 20 also expresses human C-peptide and responds to a glucose tolerance test, and more extensive studies will be performed when mouse 20 and mouse 38 have generated large colonies. An assay specific for mouse C-peptide is not available, and we were unable to compare levels of endogenous mouse C-peptide with levels of human C-peptide. c, Human C-peptide levels during an i.v. amino-acid tolerance test. Transgenic and control mice were fasted overnight, infused with a solution containing 0.5 mg arginine and 0.5 mg leucine, and bled at the indicated times for human C-peptide determination. Each time point represents the average of values from four animals. Solid line, transgenic mice; dashed line, control mice. d, Human C-peptide levels during an i.v. tolbutamide infusion test. Transgenic and control mice were fasted overnight, infused with a solution containing 0.5 mg of tolbutamide (Upjohn) and bled at the indicated times for human C-peptide determination. Each time point represents the average of values from two animals. Solid line, transgenic mice; dashed line, control mice.
the return to basal glucose levels were similar for the transgenic and control animals. In addition, intravenous (i.v.) administration of glucagon increased serum glucose levels by ~50% within 15 min in both transgenic and control mice. Taken together, these results strongly suggest that serum glucose levels were appropriately modulated in the transgenic mice. The weights of the transgenic mice, growth rates, feeding behaviour, reproducibility and longevity appeared normal.

The role of human insulin in the regulation of blood glucose levels in transgenic mice was investigated by performing a glucose tolerance test on transgenic and control mice (Fig. 3b). No human C-peptide was detected in the sera of fasting transgenic mice, but within 10 min of i.p. administration of M. Chloramphenicol acetyltransferase results indicate that drugs thought to affect human insulin metabolism can now be tested in an in vivo animal system. In a more general sense, the in vivo effects of various pharmacological agents on human gene expression and protein function can therefore be evaluated in a non-human setting.

Finally, it is noteworthy that a 12.5-kb DNA fragment contains sufficient information for the appropriate physiological regulation of insulin levels in these transgenic mice. The organism's ability to modulate foreign DNA sequences and proteins on a minute to minute basis clearly has important implications for both molecular biology and gene therapy.

We thank Dr Tom Wagner for instruction in microinjection, Victoria Roman for technical assistance and Patrick Mattoon for animal care. The human C-peptide assay was the gift of Dr H. H. Schoene and Behringwerke, AG. This work was supported by grants from Hoechst AG and the NIH (AM-07055).

Received 17 December 1985; accepted 18 March 1986.

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Genetic recombination between RNA components of a multipartite plant virus

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Genetic recombination of DNA is one of the fundamental mechanisms underlying the evolution of DNA-based organisms and results in their diversity and adaptability. The importance of the role of recombination is far less evident for the RNA-based genomes that occur in most plant viruses and in many animal viruses. RNA recombination has been shown to promote the evolutionary variation of picornaviruses1,2,3,4,5,6,7,8 and is implicated in the synthesis of the messenger RNAs of influenza virus9 and coronavirus10. However, RNA recombination has not been found to date in viruses that infect plants. In fact, the lack of RNA recombination ability to demonstrate recombination in mixedly infected plants has been regarded as evidence that plants do not support recombination of viral RNAs. Here we provide the first molecular evidence for recombination of plant viral RNA. For brome mosaic virus (BMV), a plus-stranded, tripartite-genome virus of monocytes, we show that a deletion in the 3' end region of a single BMV RNA genomic component can be repaired during the development of infection with recombination involving the homologous region of either of the two remaining wild-type BMV RNA components. This result clearly shows that plant viruses have available powerful recombinatory mechanisms that previously were thought to exist only in animal hosts, thus they are able to adapt and diversify in a manner comparable to animal viruses. Moreover, our observation suggests an increased versatility of viruses for use as vectors in introducing new genes into plants.