lowed by a self-limiting proliferative response. Factors limiting this platelet reactivity and SMC proliferation are unknown.

The direct demonstration of movement of a platelet-specific protein into the vessel wall provides a new tool for studying platelet-vessel wall interactions. Since platelets contain and secrete a variety of other biologically active materials including lysosomal enzymes and a heparin-cleaving enzyme, these materials may also enter the vessel wall and could influence vascular repair. The ability to identify and trace the movement of one platelet protein into the vessel wall should facilitate further study of platelet products in the circulatory system.

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6. Antiserum (100-μl portions) was mixed with equal volumes of buffer containing between 10 and 100 μg of PF4 protein, incubated at 4°C for 72 hours, and centrifuged at 10,000*g*. The precipitate was washed with 150 m*M* NaCl and 10 m*M* phosphate-buffered saline (PBS) (*p*H 7.6), and the proteins in the precipitate were quantified with the Lowry technique. The absorbed antiserum from the equivalence zone of the precipitin curve was analyzed for residual immunoreactivity with the Ouchterlony immunodiffusion technique. Preimmunization, postimmunization, and absorbed antiserums were then used in the immunofluorescence studies described in (*7*). Before being applied onto the vessel sections, the antiserums were absorbed with intima-media preparation at 37°C for 2 hours and cleared in a microcentrifuge (Brinkmann).
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Genetic Variation in the Human Insulin Gene

Abstract. *Four recombinant lambda phages containing nucleotide sequences complementary to a cloned human preproinsulin DNA probe have been isolated from human DNA. Restriction analyses in conjunction with Southern hybridizations reveal two types of gene sequences. One isolate of each type was subjected to complete nucleotide sequence determination. The sequences contain the entire preproinsulin messenger RNA region, two intervening sequences, 260 nucleotides upstream from the messenger RNA capping site, and 35 nucleotides beyond the polyadenylate attachment site. Our results strongly suggest that these two gene types are allelic variants of a single insulin gene.*

Insulin is a polypeptide hormone, consisting of two chains (A and B) that are linked by disulfide bonds. Much is known about the translational and posttranslational events that occur in the biosynthesis of this molecule. The A and B chains are synthesized as a single precursor polypeptide, joined together by a connecting peptide (C peptide). This proinsulin molecule is contained within an even larger precursor molecule (preproinsulin), which includes an additional amino terminal extension or signal peptide to direct the insulin precursor to the cell's secretory machinery. Little is known, however, about the transcription of the insulin gene and posttranscriptional processing of the primary transcript in the pancreatic beta cell. Recombinant DNA technology permits the isolation of single copy chromosomal genes and thus facilitates the experimental approach to investigating these problems. We report here the isolation and characterization of two recombinant lambda phages containing human preproinsulin gene sequences.

A human chromosomal DNA library (*1*) was prepared by partially digesting fetal liver DNA with the restriction endonucleases Hae III and Alu I. The resulting DNA fragments were inserted into phage lambda Charon 4A in a manner which permits excision of the inserted fragment from the phage DNA by Eco RI restriction endonuclease cleavage (*2*). We screened 600,000 recombinant phages and identified four independent isolates containing sequences complementary to a ^{32}P-labeled cloned human preproinsulin complementary DNA (cDNA) probe (*3*). The four recombinant lambda phages were characterized by restriction endonuclease cleavage analysis in conjunction with Southern hybridization (*4*). When the restriction enzyme Pst I was used, the four phages displayed two types of hybridization patterns: two isolates (alpha type) contained hybridizing fragments of length 900, 600, and 310 base pairs; and two (beta type) contained hybridizing fragments of length 900, 520, 310, and 80 base pairs (data not shown).

Specific hybridization probes, representing various regions of the cloned cDNA, were used to correlate the position of these Pst I fragments within the physical map of the human insulin gene. A probe containing sequences from the 3′ end of the preproinsulin cDNA clone hybridized to the alpha-600, beta-520, and beta-80 fragments, localizing the alpha- and beta-type Pst I cleavage differences to the 3′ region. The alpha- and beta-900 base-pair fragments were mapped to the middle of the preproinsulin coding region and the alpha- and beta-310 fragments were mapped to the 5′ end region.

In the rat, mouse, and several fish species there are two insulins (I and II), which are the products of nonallelic genes (*5*); these two insulins are almost identical in their amino acid sequences. In the rat, however, where the two insulin genes have been isolated and sequenced (*6*), the structure of the two genes is different. In brief, the rat I gene has one intervening sequence, whereas the rat II gene has two intervening sequences (*6*). To detect fine-structure differences between the alpha and beta sequence types of human insulin genes, we

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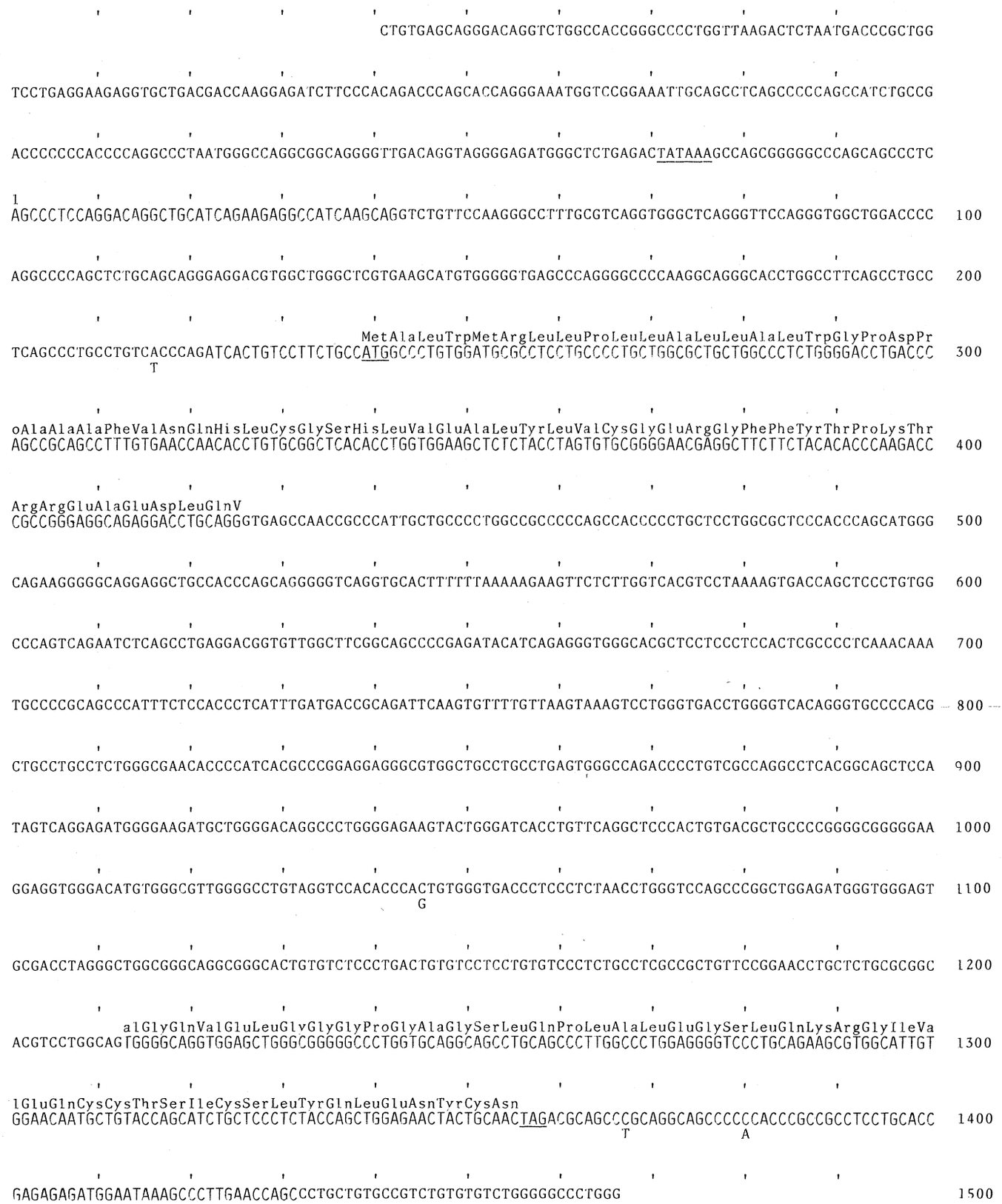

Fig. 1. Nucleotide sequence of two human insulin genes. The complete sequence of the alpha-type gene is shown (5′ to 3′); differences found in the beta-type gene are shown beneath the alpha sequence. The nucleotide sequence was determined for both complete genes, with Eco RI, Pst I, and Pvu II fragments subcloned into the plasmid pBR322. Most of the sequence was determined on both strands; all of the sequences were verified at least twice. The TATAAA box and the initiation and termination codons are underlined. The intervening and flanking sequences are shown in small capital letters; the mRNA regions are displayed in larger capital letters. The coding region of preproinsulin mRNA is shown with the corresponding amino acid sequence.

submitted one isolate of each type to complete nucleotide sequence determination by the procedure of Maxam and Gilbert (*7*). The complete nucleotide sequence of these two types of insulin genes is shown in Fig. 1. These 1725 nucleotides contain the complete preproinsulin messenger sequence (*3, 8*), two intervening sequences (IVS 1 and 2), 260 nucleotides upstream from the messenger region (5′ flanking region), and 35 nucleotides in the 3′ flanking region. The alpha and beta isolates were found to be different at only four positions: nucleotide 216 in IVS 1, nucleotide 1045 in IVS 2, and nucleotides 1367 and 1380 in the 3′ untranslated region. The T (thymidylate) to C (cytidylate) transition at position 1367 creates a new Pst I site in the beta isolate, which we detected in our Southern hybridizations.

It is unlikely that these sequence variations were introduced during the cloning procedures because we were able to identify two different isolates of the variant type (*9*). Since the flanking and intervening sequences of the alpha and beta types are otherwise identical, these sequence differences probably represent allelic variation. There are only a few reports on the frequency of nucleotide changes within specific alleles. Most of these reports are based on restriction endonuclease cleavage analysis of cloned genes or of total genomic DNA (*10*). In the chicken ovalbumin gene and the human beta, gamma, and delta globin genes (*10*), allelic sequence variations occur in IVS regions. However the complete nucleotide sequences of two rabbit beta-1 globin genes (*11*) reveal four nucleotide changes that lead to amino acid differences, in addition to two nucleotide differences in the large IVS of that gene. The four nucleotide differences between the alpha and beta human insulin genes are found in IVS regions and in a portion of the 3′ untranslated region that is not highly conserved between insulin genes of different species. A completely independent human insulin cDNA clone [derived from insulinoma tissue (*12*) obtained from a different individual] contains the same C residue at position 1367 as the alpha gene type, but has an A residue at position 1380, like the beta gene type (*13*). These site-specific base changes, indicative of nonrandom and independent mutation events, provide further evidence that allelic variation occurs in the human insulin gene.

Several structural features became evident when the nucleotide sequence of the human insulin gene was examined. First, the preproinsulin messenger RNA (mRNA) sequence (*3, 8*) fits readily within the sequence of the alpha-type gene. Some differences were observed in the 5′ untranslated region, however, and are probably due to errors caused by the enzymatic technique used to sequence this region of the RNA (*3*). We have been able to identify the site of mRNA capping by using the length that we determined for the mRNA 5′ untranslated region (*3*), the sequence homology with other capping sites (*6, 14*), and the presence of the sequence TATAAA (A, adenylate) 24 nucleotides upstream. This distance between TATAAA sequence and the capping site is characteristic of many eukaryotic structural gene 5′ flanking regions (*6, 14*). We have numbered the gene sequence in Fig. 1 beginning with the cap site.

Like the rat II insulin gene (*6*), the genomic mRNA region is interrupted twice by intervening sequences. The 59-nucleotide 5′ untranslated region is interrupted at position 42 by the 179-nucleotide IVS 1. The 786-nucleotide IVS 2 interrupts the C peptide coding region at position 425. The total length of a putative precursor RNA molecule (from the cap site to the site of polyadenylation) would be 1431 nucleotides.

The entire nucleotide sequence is rather high (65 percent) in its content of G (guanylate) and C residues. In contrast to other genes examined (*14–16*), the content of GC base pairs within the intervening sequence regions is also very high. In IVS 1, GC residues constitute 69 percent of the nucleotide sequence, uniformly. In IVS 2, the GC residues average 64 percent of the sequence; but at both the 5′ and 3′ ends, they constitute 70 percent, which may be important in the processing of primary RNA transcripts. The middle part of IVS 2 shows an almost random distribution of nucleotides. A stretch of about 40 nucleotides particularly rich in AT residues (68 percent) is found near position 544. The TATAAA box is surrounded by a region of DNA that is extremely high in its content of GC residues; this structural feature may play a role in the initiation of transcription.

The nucleotide sequences at the boundaries of the intervening sequences are very closely related to those found in other genes (*6, 15, 17*). These sequences may interact with small nuclear RNA's during RNA processing (*17*), and the insulin gene IVS boundaries are consistent with this hypothesis. The nucleotide sequences of the IVS 1 and 2 boundaries permit four and two ways, respectively, for an accurate splicing event to occur. It is interesting that the presence of a G residue at position 425 reduces the number of possible splicing frames from four to two, which may indicate that there is no selective pressure in favor of greater splicing frame options. Alternatively, the as yet unknown splicing mechanisms may utilize the multiple phase options, unhindered by the presence of the extra G residue. The presence of the T residue in the variant gene may reduce the optimal homology postulated to be involved in the splicing mechanism (*17*).

When the nucleotide sequence of the human preproinsulin gene is compared with the rat I and II genes, striking homologies can be found in regions of probable functional importance. We have already discussed the interesting homologies found in the mRNA sequences (*3*). If single base insertions and deletions are permitted, extremely high levels of homology (70 percent) can be found in the region of the gene between the 20th nucleotide upstream from the TATAAA box and the 10th nucleotide of IVS 1. The 3′ end boundary of IVS 1 also shows a similarly high level of homology with the corresponding rat gene sequences, stretching 30 nucleotides into IVS 1 itself. In contrast, the boundaries of IVS 2 have a much shorter region of homology with the rat II gene. The polyadenylate [poly(A)] addition site at nucleotide 1431 was identified by the location of poly(A) residues at the 3′ end of the mRNA (*3*). We have already noted the extensive homology upstream from this site. A limited degree of homology between rat and human sequences extends beyond this site in the gene. The actual functional importance of these homologous regions awaits further experimentation (*18*).

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Rapid Eye Movement Sleep PGO-Type Waves Are Present in the Dorsal Pons of the Albino Rat

Abstract. *We have found rapid eye movement sleep central phasic activity in the form of episodic, repetitive, monophasic waves in the albino rat. This activity is recorded in discrete areas of the dorso-lateral pons, including the nucleus locus ceruleus. The vast majority of these waves occur during rapid eye movement sleep. Their distribution and electrophysiological characteristics are similar to those of ponto-geniculo-occipital waves in the cat.*

Rapid eye movement (REM) sleep is identified by the simultaneous appearance of characteristic phasic and tonic physiological events within behavioral sleep. The defining macroelectrode brain signal of REM sleep in cat and monkey is the repetitive ponto-geniculo-occipital (PGO) wave, which is readily recordable in pons, lateral geniculate nucleus, and cortex (*1, 2*). Studies in the cat suggest that PGO waves reflect a phasic discharge generated in the hindbrain during REM sleep (*3*). This PGO generating system has been viewed as essential in all mammals to the inception and maintenance of the REM sleep state (*4*). This hypothesis is consistent with the findings that (i) REM sleep episodes in the cat are always preceded and accompanied by PGO waves (*1*) and (ii) careful prevention of all PGO waves in a REM sleep deprivation procedure results in even greater REM sleep rebound after deprivation than does deprivation of REM sleep alone (*5*).

However, the necessity of PGO activity in the REM sleep process has recently been questioned because PGO waves have not been found during REM sleep in the albino rat in structures in which they are prominent in the cat and monkey, that is, the lateral geniculate nucleus and visual cortex (*6, 7*). Gottesmann (*6*) did show, in the rat, PGO-type waves recorded during REM sleep in other areas where they are found in the cat—the oculomotor nuclei and parasagittal pons. However, the distribution of these waves within the sleep stages was not presented. Further, the failure of the waves in that study to be affected by reserpine, which predictably alters the PGO-wave pattern in the cat, left the matter of PGO activity inconclusive in the rat. The lack of a clear demonstration until now of PGO activity in the albino rat has led some to the conclusion that such activity is not a fundamental component of REM sleep in mammals, but, rather, a visual system process appearing during REM sleep in some species (*7*).

We have hypothesized that systems other than the visual are also the recipients of REM sleep phasic activation. The expression of this activation by a macroelectrode event, the PGO wave, may be determined by the cytoarchitectonics of the investigated structures and also by species specialization. Given the aberrations found in the visual systems of albinos of some species (*8*), we reasoned that the search for REM sleep phasic activity in the visually poor albino rat would be more fruitful in brain regions that subserve other, highly utilized, waking state functions. We now report on the existence and distribution of REM sleep phasic activity monitored in the dorso-lateral pons of the albino rat.

Eleven male albino Sprague-Dawley rats (350 to 500 g) had electrodes implanted for long-term recording of electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG). In all rats, a twisted bipolar stainless steel electrode (0.20 to 0.25 mm in diameter), which was completely insulated except at the cross section of the tips, was aimed at the dorsal tegmentum of the pons, into the region of the nucleus locus ceruleus. After a 7- to 10-day recovery period, the subject was placed in a sound-attentuated cage and connected for polygraphic recording. After a 24-hour adaptation period, electrophysiological data were collected on an ink-writing polygraph (Grass 78). Monitoring sessions usually lasted 4 to 24 hours. The polygraphic recording time was divided into 30-second epochs for analysis. These epochs were characterized as either awake (AW), slow wave (SW) sleep, or REM sleep, according to conventional criteria in the rat based on EEG, EOG, and EMG activity. REM sleep was identified by EEG desynchronization, hippocampal theta, the loss of resting muscle tone, and the occurrence of rapid eye movements.

The electrophysiological output of the pontine placement was examined for evidence of discrete phasic activity associated with REM sleep. Since our hypothesis was that PGO waves can be recorded in the pons of the albino rat, the pontine tracings were initially explored for waves with characteristics similar to those of the PGO waves in the cat. In the cat, the waves typically (i) are monophasic, of the same polarity and having a duration of 60 to 120 msec; (ii) are present in every REM sleep episode; and (iii) occur primarily in REM sleep but also in the SW sleep immediately preceding REM sleep.

In 7 of the 11 rats, we found associated with REM sleep the unmistakable appearance of such wave forms in the pontine recordings. The waves were monophasic, always of the same polarity, 60 to 100 msec in duration, and 25 to 150 μv in amplitude. Figure 1A is an oscilloscope tracing of a representative phasic wave recorded in REM sleep from an electrode in the region of the nucleus locus ceruleus.

A majority of the waves occurred in REM sleep. Every REM sleep episode contained the pontine waves. Single

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