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THE STRUCTURE OF THE HUMAN ENDOTHELIAL PLASMINOGEN ACTIVATOR INHIBITOR (PAI-1) GENE: NON-RANDOM POSITIONING OF INTRONS.
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The endothelium plays a crucial role in the regulation of the fibrinolytic process, since it synthesizes and secretes tissue-type plasminogen activator (t-PA) as well as the fast-acting plasminogen activator inhibitor (PAI-1). Molecular cloning of full-length PAI-1 cDNA, employing a human endothelial cDNA expression library, and a subsequent determination of the complete nucleotide sequence, allowed a prediction of the amino-acid sequence of the PAI-1 glycoprotein. It was observed that the amino-acid sequence is significantly homologous to those of members of the serine protease inhibitor ("Serpin") family, e.g. α1-antitrypsin and antithrombin III. Serpins are regulators of various processes, such as coagulation, inflammatory reactions, complement activation and share a common functional principle and a similar structure, indicative for a common primordial gene. The intron-exon arrangement of Serpin genes may provide a record for the structure of a primordial gene. A comparison of the location of introns among members of the Serpin family reveals that some introns are indeed present at identical or almost identical positions, however in many other cases there is no correspondence between the intron positions among different Serpin genes.
Obviously, more data on the chromosomal gene structure of members of this family are required to formulate a scheme for the evolutionary creation of the Serpins. To that end, we have established the number and the precise location of the introns in the PAI-1 gene and have compared these data with those reported on other Serpin genes. For that purpose a human genomic cosmid DNA library of about 340.000 independent colonies was screened with radiolabelled full-length PAI-1 cDNA as probe. Two clones were found which contain the entire PAI-1 gene. Restriction site mapping, electron microscopic inspection of heteroduplexes and nucleotide sequence analysis demonstrate that the PAI-1 gene comprises about 12.2 kilo basepairs and consists of nine exons and eight introns. Intron-exon boundaries are all in accord with the "GT-AG" rule, including a cryptic acceptor splice site found in intron 7. Furthermore, it is observed that intron 3 of the PAI-1 gene occupies an identical position as intron E of chicken ovalbumin and intron E of the ovalbumin-related gene Y. The location of the other seven introns is unrelated to the known location of introns in the genes encoding the Serpins, rat angiotensin, chicken ovalbumin (and gene Y), human antithrombin III and human α1-antitrypsin. The 3' untranslated region of the PAI-1 gene is devoid of introns, indicating that the two mRNA species detected in cultured endothelial cells which share an identical 5' untranslated segment and codogenic region, but differ in the length of the 3' untranslated region, arise by alternative polyadenylation. An extrapolation of the position of the introns to the amino-acid sequence of PAI-1, and adaption of the view that the subdomain structure of the Serpins is analogous, shows that the introns of PAI-1 are non-randomly distributed. Except for intron 7, the position of the other seven introns corresponds with random-coil regions of the protein or with the borders of β-sheets and α-helices. Extrapolation of the position of introns in the genes of other Serpins to their respective amino-acid sequences and subdomain structures also reveals a preference for random-coil regions and borders of subdomains. These observations are reminiscent of an evolutionary model, called "intron sliding", that accounts for variations in surface loops of the same protein in different species by aberrant splicing (Craik et al., Science 220 (1983) 1125). The preferential presence of introns in gene segments, encoding these variable regions, and absence in regions determining the general folding of these proteins would explain conservation of the structure during the evolution of those genes.

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SYNTHETIC PEPTIDE PROBES OF FACTOR VIII IMMUNOLOGY AND FUNCTION.
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In order to develop specific immunologic reagents for mapping functionally important sites on FVIII, we have prepared rabbit polyclonal antibodies against synthetic peptides of FVIII derived from regions along the entire FVIII amino acid sequence. To date, a total of 70 peptides have been synthesized and characterized by amino acid and HPLC analysis. The peptides were coupled to keyhole limpet hemocyanin with glutaraldehyde as a linkage reagent and used to immunize rabbits. Antisera were tested by ELISA assay on polystyrene microtiter plates coated with either the peptide immunogen, or purified FVIII. The antisera were also tested for their ability to inhibit FVIII clotting activity and to react with separated FVIII polypeptides on immunoblots. Of the 70 peptides, all reacted with the peptide immunogen, 45 reacted with purified FVIII and 33 reacted with FVIII on immunoblots. Because we had obtained evidence that cleavage of the amino terminal region of the 80 kDa polypeptide may play a role in FVIII activation by thrombin, a series of partially overlapping peptides, 15 residues in length, were synthesized in this area. After affinity purifying these antibodies on columns of FVIII immobilized on agarose, adjusting the antibodies to equal antigen binding titers by dot immunoblotting and testing for inhibition of FVIII activity, only one antibody could strongly inhibit FVIII clotting activity. This inhibition could be blocked by the peptide itself at nanomolar concentrations and no significant inhibition could be shown by antibodies to partially overlapping peptides individually, or in combination. These data suggest that a site important to FVIII function can be localized to a 15 amino acid residue region of the 80 kDa polypeptide of FVIII. In addition, a second inhibitory antibody was identified which was produced against a peptide in the carboxy terminal region of the 54 kDa thrombin fragment of FVIII and this area is currently being studied in a similar manner. In addition, two monoclonal anti-FVIII synthetic peptide antibodies have been produced which react with purified FVIII on immunoblots. One of these antibodies also functions as an immunoadsorbent when linked to agarose and FVIII can be purified in this manner, using the synthetic peptide as eluant. It is evident that antibodies to synthetic peptides of FVIII can be useful probes of FVIII structure, function and interactions as well as being of use in FVIII purification.