Structure of the 5′ Portion of the Human Plakoglobin Gene

To the Editor:

Plakoglobin, a member of the arm-repete family of proteins, binds to classical cadherins and desmosomal cadherins (Zhuirinsky et al., 2000). Mutations in plakoglobin cause Naxos disease, which involves arhythmogenic right ventricular cardiomyopathy, palmoplantar keratoderma, and woolly hair (McKoy et al., 2000). Plakoglobin is also required for the cell separation effect of pemphigus autoantibodies (Caldelari et al., 2000).

In recent studies, we found a discrepancy between the exon/intron structure of the human plakoglobin gene as described in Whittock et al. (2000) and in the public (NCBI) and Celera human genome databases. Using sequence information from plakoglobin cDNA, Whittock et al. amplified virtually the entire plakoglobin gene by polymerase chain reaction (PCR). They found the gene to contain 13 exons spanning approximately 328 bp of cDNA existed in the genome as a single exon, as shown in Figure 1 ("single exon"). In contrast, the plakoglobin gene sequence in the Celera database is annotated and indicates that this 328 bp sequence is split into two exons separated by a 20 bp intron (see Figure 1, "two exons"). The plakoglobin sequence in the public database is incomplete (exons 2–13 are not present) and has not yet been annotated, but exon 1 and exons 2–13 are all present and are virtually identical to the corresponding sequences in the Celera database. The only exception is a 20 bp region within intron 1, about 2.7 kb downstream of exon 1, which differs in sequence between the Celera and public databases.

We verified the "two exons" structure by performing PCR on human genomic DNA (kindly provided by Richard Wenstrup, MD, Children's Hospital, Cincinnati, OH) with a series of primer pairs, as shown in Figure 1. All primer pairs generated fragments with sizes that were consistent with the "two exon" structure, and inconsistent with the "single exon" structure. Further, one PCR product was sequenced on both strands and matched the database sequences at the 5′- and 3′-ends of exon 1. The presence of the 14.7 kb intron between exons 1 and 2 is clearly significant for analyses of the effects of plakoglobin mutations and for studies of plakoglobin gene regulation. For example, Potter et al. (2001) noted that the immediate 5′ flanking region of the plakoglobin gene (5′ in Figure 1) has a high CpG dinucleotide content and demonstrated that expression of plakoglobin is downregulated in some cell lines as a result of hypermethylation in this region. Analysis of the intron sequence shows that the 1 kb immediately downstream of the first exon has an exceptionally high CpG content, strongly supporting the suggestion of Potter et al. (2001). Furthermore, regulogram analysis (Jegga et al., in press) of the plakoglobin gene reveals a significant cis-acting element cluster within the 14.7 kb intron (Figure 2). In particular, there is a 300 bp block of sequence within the first intron that contains a cluster of cis-regulatory elements, which is highly conserved in human and mouse plakoglobin genes. The split portions of the exon and, in retrospect, it seems likely that the 5′ portion of this oligonucleotide primed synthesis from within the actual exon 2, misleadingly suggesting these sequences constituted a single exon.

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Figure 1. Structure of the 5′-end of the human plakoglobin gene. The "single exon" model for exon 1 shown at the top is based on evidence presented in Whittock et al. (2000). The "two exons" model is based on sequence information in the Celera human genome database. Exon bp numbers are derived from Whittock et al. (2000) and Potter et al. (2001); bp 1 represents the proximal transcription start site mapped by 5′-RACE (Potter et al., 2001). The translation start site (arrow with ATG) is located at bp 120, 5′ flanking sequences (marked 5′ in both models) are identical in Whittock et al. (2000), Potter et al. (2001), and both public and Celera databases. Arrows indicate upstream primers U2 (5′-CAT GGT GAT GTG GGA G-3′), U3 (5′-AGG TCA GAT ATC CAA GCC-3′), U4 (5′-ATG ACC CTG ATG CAG C-3′), U5 (5′-CTA CAA TCT GCC TCC TAT CAG-3′), D1 (5′-CTA CAA TCT GCC TCC TCC TAT CAG-3′), D2 (5′-CTA CAA TCT GCC TCC TCC TAT CAG-3′), and D3 (5′-CTT GCT CAG ATC TCT GGT TC-3′), used in PCR reactions with human genomic DNA. A and B signify primers used by Whittock et al. (2000).
Figure 2. Potential cis-acting regulatory regions in phylogenetically conserved regions of the mouse and human plakoglobin genes. This “regulogram” (Jegga et al, in press) shows a comparison of the mouse (top) and human (bottom) plakoglobin gene sequences aligned by BlastZ (Schwartz et al, 2000). Exons are shown as dark boxes. Homologous conserved regions are connected by shaded polygons. Potential cis-elements were identified using MatInspector (Werner, 2000) and the TRANSFAC professional transcription factor binding site database. Cis-elements contained within a 200 bp window of the homologous regions are scored as a hit. Hits per 200 bp window are shown on the Y-axis. Further detailed analyses of implicated cis-elements can be viewed at: http://trafac.chmcc.org/

many possible cis-acting elements and the high CpG content underscore the potential significance of this region for gene regulation, and the importance of a precise understanding of the gene in this region.

REFERENCES

Isolated Hemoperfused Porcine Skin as a Valid Model to Assess Percutaneous Absorption

To the Editor:
The passage of topically administered dermatologic drugs across the human skin and consecutive systemic adverse reactions can significantly limit the use of topical drugs and represents one of the main problems in the development of new topical compounds (Zucchi et al, 2001). Also, transdermal absorption is a major pathway for environmental toxins or allergens to reach the systemic circulation, and how drugs such as analgesics may be administered systemically (Levy, 1996). The passage through cutaneous membranes chiefly occurs according to the laws of passive diffusion or carrier-mediated transport mechanisms and the extent of absorption of drugs and toxins is thus largely determined by their physicochemical properties (Hadgraft and Pugh, 1998). Until now, most studies on skin penetration have been carried out using living laboratory animals (Brown et al, 1999) or humans (Leopold and Maibach, 1999) and, therefore, dermatologic drug development is partly the focus of ethical controversy.

In this study, a model of isolated perfused porcine skin was developed to assess transdermal absorption using porcine forelegs (average organ weight 1514.5 ± 101 g) of eight female pigs. The organs were harvested after desanguination for the collection of autologous blood from commercial abattoir pigs to reduce animal experiments as previously suggested for perfusion studies of the pig ear (VaniRooij et al, 1995). After transfer to the laboratory using cold preservation, the organs were placed in a perfusion system and normothermic pressure-controlled perfusion was performed through the brachial artery with a pressure of 100 mmHg and perfusates were periodically collected for analysis. The perfusion system consisted of a blood and a dialysis circuit (Fig 1A). The dialyse (composition in mmol per liter: 142.5 Na+, 2.9 K+, 1.5 Ca2+, 0.5 Mg2+, 109.9 Cl−, 37 HCO3−, 2.5 CH3COO−, 3.55 glucose) reservoir with a capacity of 10 l was permanently enriched with...