Supporting information for Farris *et al.* (2003) *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.0230450100

Supporting Text

Generation and Genotyping of the Insulin-Degrading Enzyme (IDE) –/– Mice. Integration of a single copy of the gene targeting vector in the mouse genome of the embryonic stem (ES) cell line used to generate the IDE –/– mice was determined by quantitative PCR of the neomycin gene. In addition, the observed Mendelian inheritance pattern of the neomycin gene measured by quantitative PCR performed on F_1 het × het progeny also indicate that only one targeting cassette is integrated into the mouse genome. The insertion site of the targeting vector was mapped to intron 1 of the IDE gene. Two chimeric messages are generated from the insertion of the targeting cassette into intron 1 of the IDE gene. The first message is an in-frame fusion of exon 1 of the IDE gene with the targeting cassette neomycin gene, allowing for selection of neomycin resistance. The second message is a fusion transcript of exon 1 of Bruton's tyrosine kinase gene and the IDE exons downstream of the gene-targeting cassette. Stop codons in all three reading frames were introduced in the 3' end of BTK exon 1 to prevent translation of the downstream IDE exons (Fig. 4*A*). cDNA sequence, known as the Omnibase Sequence Tag (OST), was obtained from a 5' RACE reaction performed on RNA isolated from the IDE gene targeted ES cell clone by using nested primers directed to the BTK sequence (round 1, 5'-GCCATGGCTCCGGTAGGTCCAGAG-3'; round 2, 5'-CCAGAGTCTTCAGAGATCAAGTC-3'). OST132429 shows that the IDE sequence fused to BTK exon 1 sequence starts with amino acid 34 of the mouse IDE gene product and corresponds to exon 2 and part of exon 3 of the mouse IDE gene (Fig. 4*B*). The gene structure of the IDE mouse gene was obtained by performing a pairwise BLAST search (www.ncbi.nlm.gov/BLAST), using the mouse IDE cDNA sequence (NM_031156) and a chromosome 19 mouse genomic sequence obtained from the Celera database, and with a pairwise blast search using the exon 1/intron 1 human IDE junction obtained from www.ensembl.org/perl/geneview. The site of integration of the targeting cassette in the 31.3-kb intron 1 of the mouse IDE gene was mapped by PCR and localized to nucleotide 1862 of intron 1 by sequencing (Fig. 4*C*) of a 3-kb PCR product obtained using a sense exon 1 primer (5'-GAACGGGCTCGTGTGGCT-3') directed toward intron 1, and an antisense Neo primer directed toward exon 1 (5'-TTGGTGGTCGAATGG GCAGGT-3'). Mouse genotyping was performed by PCR using primers in intron 1 of the IDE gene flanking the targeting cassette (5'-ATCTGTGTCAGGAGGAGGGAC-3' and 5'-CAGGGTAGGGAAGTCAAGGTTAC-3'), which detect the WT allele, and primers directed against the neomycin gene (5'-GGGCGCCCGGTTCTTTTTGTC-3' and 5'-TTGGTGGTCGAATGGGCAGGT-3'), which detect the integrated targeting cassette. No message was detected in RT-PCR assays performed on IDE –/– mouse liver or kidney RNA when using exonic primers flanking the insertion site (Tom Lanthorn, Lexicon Genetics; personal communication). Furthermore, IDE protein was absent from liver (not shown) and brain (Fig. 4*D*) of IDE –/– animals. In addition, IDE levels were significantly diminished in IDE +/– vs. WT brains (not shown).