paws—the maximum muscle strength in the forelimbs (measured in grams)—was measured by holding the mice in the air and allowing them to grasp with both forepaws a horizontal wire strain gauge connected to a dynamometer [W. E. Kozachuk, H. Mitsumoto, V. D. Salanga, G. J. Beck, F. Wilber, *J. Neurol. Sci.* **78**, 253 (1987)] (*4*). The differences between the two groups (treated and normal) in quantitative measurements were analyzed with the unpaired *t* test. For multiple group comparisons, analysis of variance (ANOVA) was used. All tests were two-sided; the significance level (α) was set at 0.05.

14. The mean (±SD) biceps muscle weight was 7.9 ± 2.4 mg in cotreated mice ($n = 8$) and 4.4 ± 0.9 mg in vehicle-treated mice ($n = 7$) ($P < 0.005$).
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16. The right biceps muscle was dissected, weighed, rapidly frozen, serially sectioned (10 μm), and stained with acid, intermediate, and routine alkaline phosphatase. The mean diameter of muscle fibers was calculated with a morphometric program (Jandel Sigmascan, San Rafael, CA) and was derived from counts (performed in a blinded fashion) of >1000 muscle fibers in randomly selected areas.
17. For analysis of ventral root fibers, a separate group of six *wobbler* mice was cotreated with CNTF and BDNF as described in the text. At the end of treatment, the animals were perfused intracardially with 3.6% buffered glutaraldehyde. The C5 and C6 ventral roots were embedded in epoxy resin and cut in 0.5-μm sections. Myelinated fibers were counted by a blinded observer as described (*8, 9*) by use of the Jandel Sigmascan histometric system. For motor neuron counts, the entire cervical cord (C1 to C7) was dissected, fixed overnight in 4% paraformaldehyde, and stored in 30% sucrose. Serial 30-μm frozen sections were stained for ChAT with a polyclonal antibody (Chemicon) on the basis of techniques previously described (*6*). Spinal motor neurons were counted in every twelfth section by a blinded observer. Six untreated *wobbler* mice and four healthy littermates were studied separately (*11*).
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TECHNICAL COMMENTS

Phylogenetic Analysis of a Reported Complementary DNA Sequence

In the sequence reported as the complementary DNA (cDNA) of 12-kD human B cell growth factor [(*1*), original GenBank entry accession number M15530], 54 out of 124 codons were predicted to be derived from an Alu fragment. In addition to the originally described Alu element that was predicted to contribute the 32 COOH-terminal amino acids, we identified a second Alu fragment that coded for the 22 NH_2-terminal residues (Fig. 1). The unusually high Alu content suggested that these repeats might be instrumental in the emergence of new proteins. To reconstruct the underlying events, we undertook the phylogenetic analysis of the corresponding sequence. The contributing repeats (Fig. 1) belong to the old Alu subfamilies, Sx and J, which have been present in primates for over 35 million years (*2*). We studied this region in several primates including New World monkeys and prosimians. Six amplification primers based on the reported sequence (*1*) were used in different combinations in order to compensate for possible mismatches between species. DNAs from Old World monkeys (OWM), gibbon, great apes, and humans amplified well with most primer combinations. The apparent length of the PCR products were as predicted from the M15530 clone, indicating that the latter was colinear with the genomic locus. We cloned and sequenced PCR products that were amplified with the use of U-L primer pairs from three humans and from chimpanzee, gorilla, gibbon, baboon, and macaque (*3*).

All human sequences that we sequenced differed from the reported M15530 cDNA (*1*) by two substitutions and two deletions (Fig. 1). Three of these changes interrupted the M15530 open reading frame: the transversion at position 119 created a stop codon TGA, while both deletions resulted in the frameshifts and premature termination. These differences with M15530 also existed in all nonhuman primate sequences analyzed. The authenticity of M15530 cDNA could be thus questioned, unless (i) our PCR products were amplified from an intronless pseudogene, (ii) M15530 was recently derived by duplication from the genomic locus analyzed here, or (iii) the differences represented an edited transcript.

The first possibility is contradicted by the following analysis. The locus in question is at least as old as the phylogenetic divergence of OWM. Therefore, a similar number of changes should separate the intronless pseudogene in OWM, apes, and humans from the original active sequence evolving independently (dotted line in Fig. 2). However, this cannot be the case for M15530, which appears as the closest relative of the genomic human sequence (Fig. 2). It differs at only four positions from the human genomic sequence and at 11, 14, 21, 50, and 51 positions from chimpanzee, gorilla, gibbon, baboon, and macaque genomic sequences, respectively (*4*).

The second scenario is improbable as it would require a series of fortuitous events during a short evolutionary time period (Fig. 2). Duplication of the genomic locus analyzed here would have to be followed by nucleotide substitution and two deletions extending the reading frame, gain of a tissue-specific promoter, and an intron capture, which would render the resulting locus refractory to PCR.

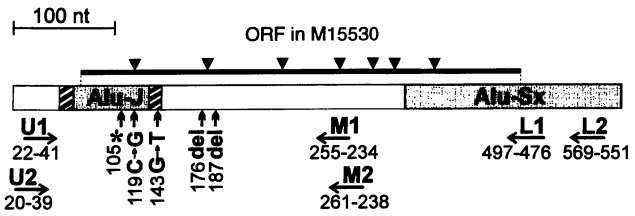

Fig. 1. Structure of the 12 kD BCGF cDNA reported by Sharma *et al.* (*1*). Alu segments are shown as shaded boxes, hatched blocks represent direct repeats, and the horizontal line above the locus corresponds to the open reading frame in M15530 cDNA. Horizontal arrows show localization of PCR primers; vertical arrowheads, STOP codons; vertical arrows, substitutions and deletions with respect to the orginally reported M15530 sequence. The asterisk indicates the location of a six-nucleotide sequence CGCGGC present in OWM and in the corresponding Alu-J consensus sequence.

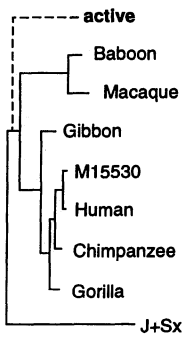

Fig. 2. Phylogenetic analysis of the M15530 cDNA and the corresponding genomic sequence with the use of the neighbor-joining method (*Phylip* package v.3.5). The dotted line indicates the tree typology expected under the assumption that analyzed sequences represent an intronless pseudogene derived from an **active** sequence before OWM divergence.

The third option would require a complex and as-yet unidentified editing mechanism to introduce a nucleotide transversion and two independent insertions. We therefore concluded that the M15530 sequence did not correspond to a protein-coding mRNA and likely represented an error.

Our results demonstrate the power and utility of the phylogenetic analysis in order to judge whether a putative coding region corresponds to a transcriptionally active gene, a pseudogene, or has been misassigned as a functional segment. The latter problem may be especially important in open reading frames of tissue-specific cDNAs sequenced at random (*5*), when either no coding counterparts are found in the database or spurious homology has been identified. "Contamination" of cDNAs by Alu or other repeats may be a frequent cause of a misinterpretation of sequence homology. Conversely, Alu-containing cDNAs must not be automatically classified as artifacts or nonlegitimate transcripts. In some cases, Alu segments may be integral to the coding or regulatory region, or may occur in a variant allele or in an alternatively spliced transcript (*6*). In the absence of reliable biological tests, interspecies sequence comparison is an efficient tool when investigators must choose from among these possibilities.

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Response: Sequencing of the genomic fragment (*1*) has revealed three errors in the cDNA sequence we originally published (*2*): C→G (nucleotide 119) and G→T (nucleotide 143) are incorrectly given and the C and A residues present in M15530 cDNA at positions 176 and 187 are absent [M15530 cDNA was originally sequenced by the biotechnology division of Phillips Petroleum Company; see reference 22 in our original report (*2*)]. These corrections, which have been received by GenBank (*3*), do not alter the basic conclusions of our studies, namely that the 12-kD BCGF gene represents a functional domain. The nucleotide changes described above do not alter the major portion of the coding region, except that the AUG initiation codon is now placed seven nucleotides downstream from that which we originally reported (*2*). Consequently, the amino acid composition of the first 37 residues at the NH_2-terminal is altered. The remaining 83 amino acids are identical to those reported in the M15530 coding region. Recent studies on the expression and chromosomal localization of 12-kD BCGF suggest that the originally cloned cDNA is a protein-coding and transcriptionally active domain (*1*) irrespective of the presence of Alu repeats within and flanking the coding sequence.

In their comment, Zietkiewicz *et al.* further suggest that the M15530 cDNA is associated with phylogenetic and evolutionary improbabilities. Although an extensive investigation into phylogenetic and evolutionary linkage of the M15530 cDNA locus needs to be carried out before we can make any conclusive statements, we are considering the data (*1*) and find that it may be fruitful rather than evolutionarily improbable.

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