Rapid Paper

A New cDNA Clone Relating to Larger Molecular Species of Rat Insulin-like Growth Factor-I mRNA

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A new cDNA clone for rat IGF-I mRNA was obtained. The cDNA contained a new base sequence as cDNA in the 3' region. The sequence coincided with a part of the sequence reported earlier by Shimatsu and Rotwein⁵⁾ in exon 5 of the rat IGF-I gene. The presence of this cDNA proved the previous suggestion to be true that the size heterogeneity of IGF-I mRNA is primarily due to the heterogeneity of the 3'-untranslated region.

Several molecular species of rat insulin-like growth factor I (IGF-I) mRNA have been reported.^{1–3)} Their size ranges from 1 to 7.4 kb. Since animal cells contain a single IGF-I gene,^{4,5)} those mRNAs are presumed to be produced by differential splicing of the gene product. Indeed, two or three kinds of IGF-I cDNA with different structures in the 5'-untranslated region have been reported.^{7,9)} However, the difference in the structure of 5'-untranslated regions has been proved not to explain the difference in the molecular size of mRNAs, which ranges from 0.8 to 7.4 kb, because every one of the different molecular size species of IGF-I mRNA was proved to be a mixture of the mRNAs with the different 5'-upstream sequences.¹⁴⁾

Some authors have reported cDNA species of rat IGF-I mRNA,^{6–9,12)} but the longest one ever known is 1.0 kb. This size is far shorter than the longest mRNA. In this paper, we report a new cDNA clone for rat IGF-I

Fig. 1. DNA Sequence of a Rat IGF-I cDNA.

The predicted amino acid sequence is shown below the DNA sequence. The region corresponding to the mature IGF-I molecule is indicated by the box. The sequence of two oligonucleotide probes used in northern blot analysis (Fig. 2) are shown below the solid and broken lines. The nucleotide sequence shown by small letters in the 5' terminal is not contained in this cDNA. This sequence was elucidated previously.^{6–9,12)} "Poly A signal" (overlined) and the possible polyadenylation site (poly A₁ and A₂) reported by Shimatsu and Rotwein⁵⁾ are also indicated.

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mRNA, which contains a new sequence as cDNA in the 3' region.

Materials and Methods

cDNA of adult rat liver mRNA was synthesized with a cDNA synthesis system plus (Amersham) and packaged into λ gt10 with a cDNA cloning system (Amersham). Three probes were used. One was rat IGF-I cDNA with a class A type 5'-untranslated region¹²⁾ and the others were the synthetic oligonucleotide probes shown in Fig. 1 below the solid and broken lines. Nucleotides were sequenced by the dideoxy chain-termination method using M13 mp18 and 19.¹⁰⁾

Results and Discussion

Figure 1 shows the whole sequence of our new cDNA of rat IGF-I mRNA. The boxed area shows the sequence that codes for the mature IGF-I. There are poly adenylate sequences ("A clusters") in this cDNA, which is from 614 to 653 bases from the 5'-terminal. The structures of cDNAs ever reported contain only the upstream of these "A clusters." This suggests that those cDNAs were produced after hybridization of oligo dT primers to these "A clusters." The sequence of our new cDNA clone contained a downstream region from the "A clusters" and the base sequence of the region was the same as a part of the sequence reported earlier for exon 5 of the IGF-I gene (Shimatsu *et al.*⁵⁾).

Figure 2A shows the result of northern blot analysis of the rat liver total RNA using a probe of rat IGF-I cDNA. Five bands, the sizes of which were 0.8–1.2, 2.0, 3.6, 4.0, and 7.4 kb, were detected. Figure 2B shows the northern blot analysis of the rat liver poly(A)⁺ RNA. In this case, a chemically synthesized complementary chain of the sequence solid-underlined in Fig. 1 was used. Three bands, 0.8–1.2, 2.0, and 7.4 kb, and an additional weak band of 6.0 kb were found. Figure 2C shows the result of the same kind of the experiment shown in Fig. 2B with another probe that was complementary to the broken-lined sequence in Fig. 1 was used. The region is downstream from one of the possible poly-

Fig. 2. A: Northern Blot Analysis of Rat Liver Total RNA with a Rat IGF-I cDNA.¹²⁾

Forty μ g of adult rat liver RNA was denatured and size-fractionated on 1.5% agarose gel and transferred to a nitrocellulose membrane. The blotted RNA was hybridized with a ³²P-labeled rat IGF-I cDNA (Y. Miura, H. Kato, and T. Noguchi, manuscript in preparation). The size of each molecular species of mRNA is indicated.

B: Northern Blot Analysis of Rat Liver Poly(A)⁺ RNA with a 5'-Region Specific Oligonucleotide Probe.

Five micrograms of poly(A)⁺ RNA was fractionated and fixed on a nylon membrane (GeneScreen) and hybridized with a 5'-labeled oligonucleotide probe (Fig. 1, below the solid line).¹²⁾

C: Northern Blot Analysis of Rat Liver Poly(A)⁺ RNA with a 3'-Region Specific Oligonucleotide Probe (Fig. 1, below the broken line).

Hybridization of B and C was done by the method of Lowe *et al.*¹³⁾

adenylation sites ("poly A₁") suggested by Shimatsu and Rotwein.⁵⁾ Our result shows that the mRNA of 0.8–1.2 kb does not contain the broken-lined sequence in Fig. 1. This result coincides with that reported by Lund *et al.*¹¹⁾ who used a genomic fragment of IGF-I exon 5 as a hybridization probe. This means that among the possible poly A sites suggested by Shimatsu and Rotwein,⁵⁾ "poly A₁" is not used for the synthesis of IGF-I mRNA species

of the molecular size larger than 2 kb in rat tissues.

From indirect evidence, the difference in the size of IGF-I mRNA has been suggested to be primarily due to differences in the 3'-untranslated region.¹¹⁾ Our new cDNA clone of IGF-I mRNA gives direct evidence for this hypothesis. Our new cDNA clone is the first one which contains a sequence downstream from the "poly A clusters" in the exon 5 of the rat IGF-I gene.

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