Benchmarks

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Construction of a cDNA Encoding a Repetitive Amino Acid Sequence

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PCR is a powerful method with which to generate almost any desired cDNA sequence. Construction of cDNA fragments that encode repetitive amino acid sequences by PCR has proven problematic, however, because repetitive nucleotide primers tend to amplify undesired fragments because of their mis-annealing with the template. To overcome this problem, Dombrowski and Wright (2) developed a solid-phase gene assembly protocol, but this technique requires preparation of a solid-phase reaction system and thus cannot be routinely performed in most laboratories. Here, we describe a new strategy that makes use of standard PCR protocols, yet enables assembly of cDNAs encoding repetitive amino acid sequences.

The peptide unit Ala-Thr-Pro-Ala-Pro (ATPAP) is reported to be the shortest that can still function as an acceptor for *N*-acetylgalactosaminyl-transferase-I (4). By introducing this peptide into a secretable form of fibroblast growth factor (FGF) (3) and expressing it in Chinese hamster ovary cells, we were able to produce FGF modified with a single *O*-glycan moiety (1). Subsequently, neo-*O*-glycosylated forms of FGF containing multiple *O*-glycans, similar to mucin-type glycoproteins, were created using two

cDNAs encoding FGFs with 10 repeats of the pentapeptide *O*-glycosylation unit at the N- or C-terminus. In this report, the creation of the N-terminal modified form is described in detail.

The amino acids comprising the pentapeptide *O*-glycosylation unit, alanine, proline and threonine, are encoded by codons GCX, CCX and ACX, respectively, where X is any nucleotide. We therefore designed four nucleotide cassettes that each encoded ATPAP and

differed from one another at the last nucleotide for each codon (Figure 1A). To avoid mis-annealing the cassettes, they were linked together so that they encoded more than three repeats of the ATPAP unit (Figure 1B).

Generation of clones encoding the modified FGF entailed performing two rounds of PCR. The primers for the first PCR were 5'-TTCGAATTCCCAAGG CACGCTACTGGATGCTACTCCTGC-TCCTGCGACGCCGGCGCCGGCA3'

Figure 1. Construction of a cDNA encoding a repetitive amino acid sequence. (A) Four oligonucleotide cassettes, each encoding the pentapeptide *O*-glycosylation unit ATPAP. (B) Schematic diagram of the construction of a cDNA encoding the [ATPAP]₁₀ cassette, which is to be inserted between the signal peptide and the N-terminus of FGF-1. Each *O*-glycosylation unit is indicated by a bracket containing the symbol of the last nucleotide in each codon, as shown in panel A. PCR protocols were initially carried out separately using oligonucleotides #235 and #225 or #226 and #236 without a template. A mixture of the two reaction products was then subjected to PCR using primers #237 and #238. (C) Alignment of the two reaction products with the completed sequence. The nucleotide sequences encoding the signal peptide and the FGF-1 N-terminus are underlined. The *Eco*RI recognition sites are indicated by asterisks. Primers are shown by arrows. (D) Agarose gel (4%) electrophoresis of the PCR products and the enzyme digest of the final clone. Lane 1, the first PCR product obtained using #235 and #225 (106 bp); lane 2, the first PCR product obtained using #226 and #236 (123 bp); lane 3, the second PCR product (207 bp); lane M, 25-bp ladder markers (Life Technologies, Rockville, MD, USA); lane 4, *Eco*RI digest of the clone N-[ATPAP]₁₀secFGF/pBS/#9 (195 bp). Arrowheads indicate the DNA bands having the expected sizes.

Positive clones were initially selected according to their ampicillin resistance and white color. The clones were then screened by direct PCR using vector primers to reveal the size of the inserted sequence, and several with inserts of the expected size were obtained. The size of the harbored insert was further verified in one of the clones, designated N-[ATPAP]₁₀-sec-FGF/pBS/#9, by digestion with *Eco*RI (Figure 1D, lane 4), and the correctness of its sequence was confirmed by direct sequencing (not shown). This DNA was further ligated with the signal sequence of FGF-6 at 5'-terminal and FGF-1 cDNA at 3'-terminal and constructed cDNA that encodes secretable FGF-1

REFERENCES

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