

Short Communication

3' Noncoding sequences of the *CTA 1* gene enhance expression of the recombinant serine protease inhibitor, CPTI II, in *Saccharomyces cerevisiae*

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Expression of the gene coding for the recombinant trypsin inhibitor, CPTI II, was enhanced tenfold when yeast transcription terminating sequences were added to the expression cassette of the pJK6 yeast vector. The yield was further increased about 20% in the BJ5464 yeast strain, defective in vacuolar proteases.

We had previously reported the successful cloning of the synthetic gene coding for the trypsin inhibitor, CPTI II, originally isolated from the squash seeds (*Cucurbita pepo*) [1]. Expression of the gene was obtained in both, *E. coli* [2] and *S. cerevisiae* [3] expression and secretion systems. The yeast cells were found to be more efficient in this respect, and the yield depended on the yeast strain used for transformation, the culture medium, the stage of culture growth upon induction of expression. The most important factor was the expression vector itself [4].

In one of the yeast expression/secretion vectors previously used, pJK6-CPTI II (Fig. 1) [3], the yeast transcription termination sequences were missing. This might lower the final yield of the recombinant inhibitor.

In the present work, the 3' noncoding sequences from the *S. cerevisiae* *CTA 1* gene were inserted into the pJK6-CPTI II plasmid. The inserted sequences originated from the MSCp2

plasmid, carrying the full length catalase A gene, *CTA 1*, including its flanking regulatory sequences, kindly offered by Dr M. Skoneczny of our Institute [5].

Based on the known nucleotide sequences and the restriction map of the *CTA 1* gene, a 3' terminal *Taq* I/*Eco* RI fragment was isolated (Fig. 2). This fragment includes the terminal 17 bp of the 3' catalase A coding sequence, and the TGA stop-codon followed by an untranslated 413 bp sequence.

The bacterial pUC 19-CPTI II plasmid was constructed by ligation of the *CPTI II* gene (104 bp) [3] to the *Hind* III/*Sal* I digested pUC 19, and the resulting plasmid was characterized by the appropriate restriction analysis. Then, pUC 19-CPTI II-T (where T stands for 3' terminal sequences of *CTA 1* gene) plasmid was constructed as presented in Fig. 3. The presence and localization of T fragment within the construct was confirmed by restriction and sequence analyses. The whole expression

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Abbreviations: BAPNA, *N*α-benzoyl-arginine-*p*-nitroanilide; CPTI II, *Cucurbita pepo* trypsin inhibitor II.

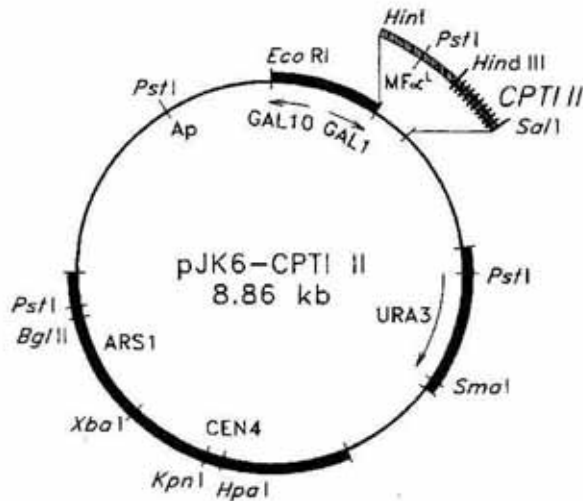


Fig. 1. Physical map of the plasmid pJK6-CPTI II [3]. Yeast sequences are shown with thick lines. Relevant restriction sites are marked. CPTI II gene is inserted between Hind III and Sal I sites, as indicated.

as shown on Fig. 4. The resulting pJK6-CPTI II-T plasmid was used for *S. cerevisiae* transformations.

Two yeast strains were used: OL1 MAT α *his3-11/15 leu2-3/112 ura3-251/328/372* from the collection of our Institute, and BJ5464 MAT α *3ura3-52 trp1 leu2 Δ 1 his3 Δ 200 pep4::HIS3 prb1 Δ 1.6R can1 GAL 1* from the Yeast Genetic Stock Center (Berkeley). OL1 strain was previously shown to give the highest CPTI II yield [4]. Strain BJ5464 carries the *PEP 4* mutation, lowering the level of vacuolar proteases which may be involved in the proteolysis of the heterologous proteins.

S. cerevisiae cells transformed with the plasmid pJK6-CPTI II-T were selected on the basis of restoration of their prototrophy to uracil, and were grown on W₀-CAA medium (0.67% yeast nitrogen base, 2% glucose, 1% casein hydrolysate). CPTI II expression was induced by replacement of glucose by galactose.

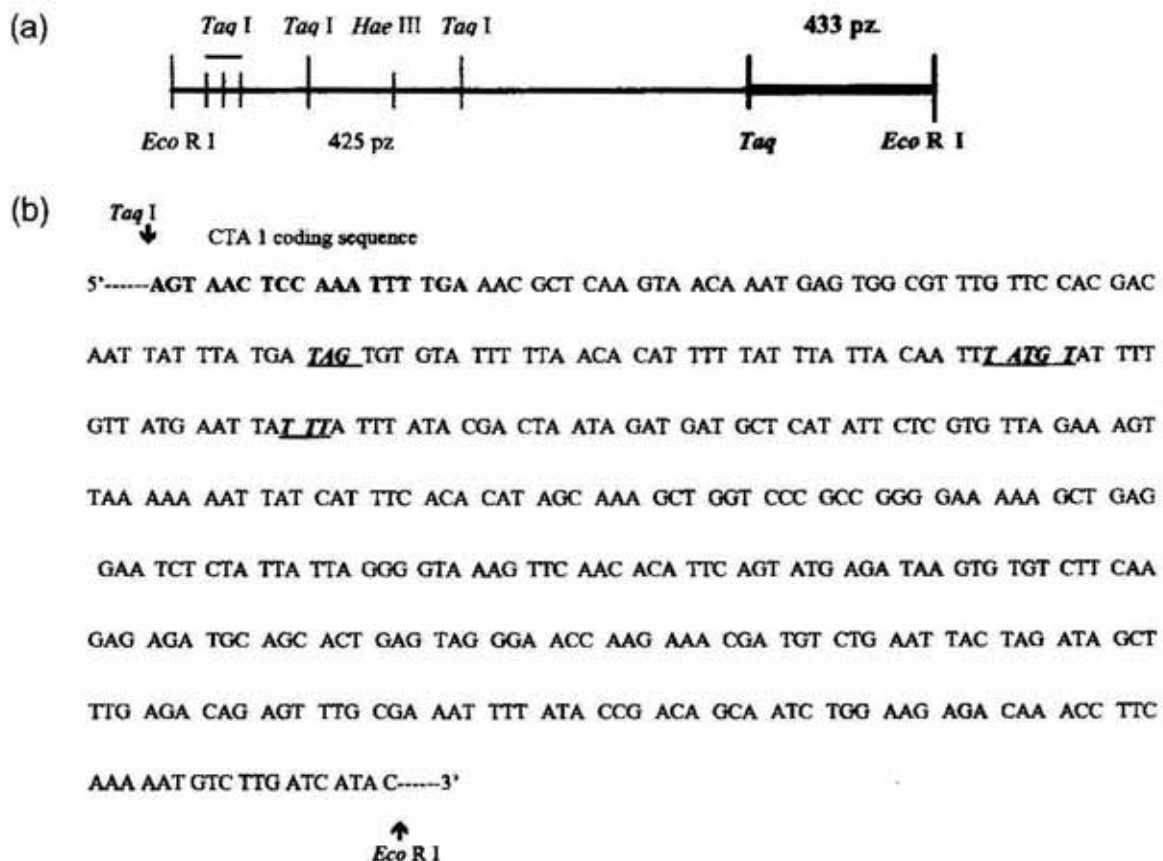


Fig. 2. (a) Physical map of the CTA 1 gene, with relevant restriction sites indicated. The 3' noncoding fragment enclosed between Taq I and Eco RI sites (T sequence) is depicted as a thick line, and its sequence is presented in (b). Putative transcription termination sequences [7, 8] are shown in bold italic.

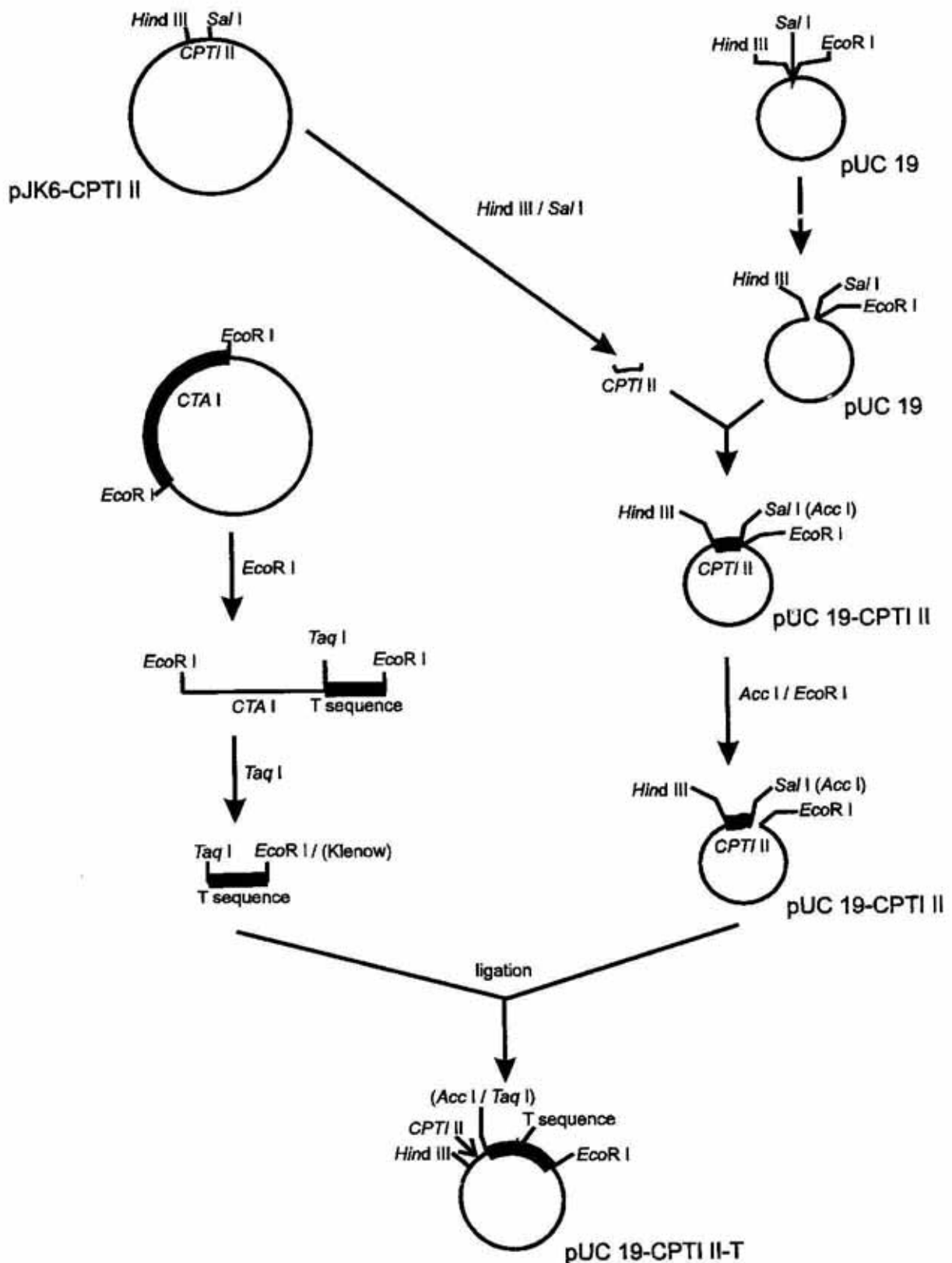


Fig. 3. Schematic presentation of the construction of pUC 19-CPTI II-T plasmid with relevant restriction sites as indicated. Plasmids are not drawn to scale.

For induction of the CPTI II production two different culture media were used: YNB-CAA-Gal (0.67% yeast nitrogen base, 1% casein hy-

drolysate, 2% galactose), and YPGal (1% yeast extract, 1% bactopectone, 2% galactose). Cells were induced for 120 h, each 24 h 100 μ l and 1.5

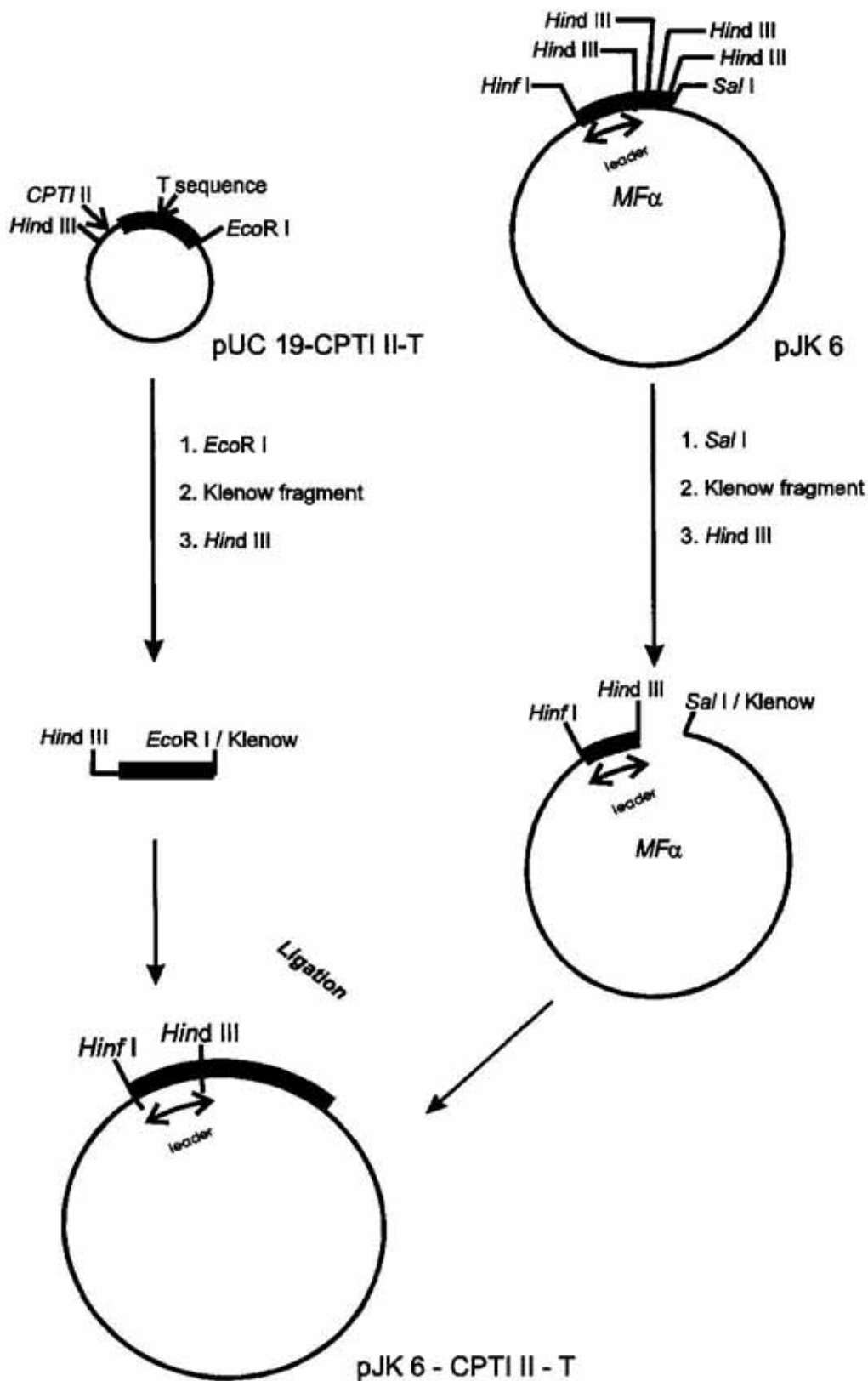


Fig. 4. Schematic presentation of the construction of the expression/secretion plasmid, pJK6 - CPTI II-T, with relevant restriction sites as indicated. Plasmids are not drawn to scale.

ml samples were analyzed for antitrypsin activity by an electrophoretic test on gels containing edestin [3]. Plasmid stability and the rate of the

cellular growth were determined for both strains on the solid W₀ media (0.67% yeast nitrogen base, 2% glucose) and YPG (1% yeast

Table 1
Yield of CPTI II obtained from recombinant yeast clones grown on YPGal medium

Yeast strain	Initial cell density	Final cell density	CPTI II concentration (mg/l)
OL-1/pJK6-CPTI II	5.24×10^7	4.86×10^8	0.123
OL-1/pJK6-CPTI II-T	6.11×10^7	4.98×10^8	1.14
BJ5464/pJK6-CPTI II	5.24×10^7	4.93×10^8	0.15
BJ5464/pJK6-CPTI II-T	5.24×10^7	4.98×10^8	1.36

extract, 1% bactopectone, 2% glucose). Plasmids were stable, independently of the yeast strain and the culture medium.

For all systems studied the yield was markedly higher for CPTI II-T constructs than for the corresponding CPTI II ones. The active product was always electrophoretically heterogeneous [3], which probably resulted from the inefficient posttranslational processing of the signal peptide [6].

CPTI II secreted to the medium was partly purified by the affinity chromatography, and quantitatively determined by BAPNA test [3] (Table 1).

Although highly conserved consensus sequences regulating transcription termination in *S. cerevisiae* were not found, many investigations point to the role of 3' gene flanking sequences in determining the level of gene expression [7–10]. Skoneczny had shown previously, that the expression of the *CTA 1* gene cloned on the MSCp2 plasmid equals that from the chromosomal gene [11]. This suggested that all sequences needed for the native expression were transferred to the above plasmid. Those putative transcription terminating sequences increased CPTI II production about tenfold. The BJ5464 strain was found to be more efficient, by 20%, in CPTI II production than the OL1 strain. The maximal yield of the recombinant CPTI II was observed in 120 h cultures of BJ5464/pJK6-CPTI II-T cells in YPGal medium at 28°C. This yield was, however, three times lower than the yield obtained with the pYET expression/secretion vector constructed in our laboratory [4].

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