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An attempt at application of tRNA genes as promoters of protein-coding genes in transgenic plants

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During the last few years the structure, properties and functions of DNA-dependent RNA polymerase III, as well as the structure of promoters of the genes transcribed by this enzymatic complex, were one of the areas of studies on functioning of the genetic apparatus. Recently a report was published on attachment — to a typical gene transcribed by polymerase III and deprived of the transcription termination region — of other DNA sequences [1]. The authors have constructed a hybrid genes built of the soybean $tRNA^{Met}$ gene deprived of the transcription termination signal and DNA fragments coding for different RNAs, antisense with respect to the chloramphenicol acetyltransferase (CAT) mRNA. Chloramphenicol acetyltransferase gene is widely used in studies on transgenic plants.

Our aim was to check whether it would be possible to obtain expression of protein when the gene coding for that protein would be placed not after classical promoter but after the tRNA deprived of the transcription termination signal. Such a gene would be transcribed by RNA polymerase III (instead of by polymerase II). Placing of a protein-coding gene (for production of protein in transgenic plants) under control of RNA polymerase III promoter could be of advantage because this enzyme synthesizes RNA at a great velocity.

To verify our assumptions, we inserted into polylinker of the pBI101.1 plasmid [2], upstream to the β-glucuronidase (GUS) gene, the main cytoplasmic $tRNA^{Tyr}$ gene from *Nicotiana rustica* deprived of the transcription termination signal, or so-called "consensus" $tRNA^{Tyr}$ gene. The "consensus" $tRNA^{Tyr}$ gene is an artificial product constructed in our laboratory; it contains all the constant nucleotides of the $tRNA^{Tyr}$ genes but is deprived of the intron, and all its others base-pairs are replaced by A-T pairs.

The two genes mentioned above were cloned into the pBluescriptIIKS^{+} vector, and their structure were confirmed by sequencing. After determination of their orientation within the vector, the two genes together with the adjacent sequences of the pBluescriptIIKS^{+} vector were amplified using PCR and following restriction, were cloned into the pBI101.1 plasmid. The presence in the vector of the *N. rustica* $tRNA^{Tyr}$ gene was confirmed by electrophoresis of the analytical-scale PCR products (Fig. 1). We have named the pBI101.1 plasmid with cloned *N. rustica* gene — pNBI, and the same plasmid with cloned "consensus" tRNA gene — pKBI (Fig. 2).

The plasmids obtained were introduced into *Agrobacterium tumefaciens* by the triparental mating technique. Simultaneously we have introduced in the same way into *Agrobacterium tumefaciens* the plasmids pBI101.1 and pBI121 [2] which served as references with respect to the constructs studied. The bacterial strains which contained the constructed plasmids were resistant to both kanamycin and rifampicin, and gave positive result in ketolactose test. These strains were then used for transformation of leaf discs and fragments of shoots of 6-week-old *Nicotiana tabacum* plants. By pas-

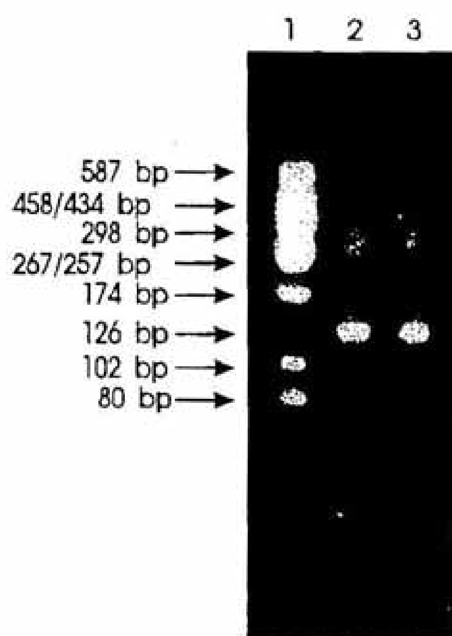

Fig. 1. Electrophoretic separation of PCR products: lane 1, DNA size marker (pUC19 digested with HaeIII); lane 2, PCR pNBI1; lane 3, PCR pNBI2.

sing the transformed discs and shoot fragments on appropriate media containing kanamycin and carbenicillin, we were able to culture calluses which were kanamycin-resistant. These calluses contained probably, in addition to the kanamycin-resistance gene, the β-glucuronidase gene. Each callus was divided into two parts; the first was placed in medium inducing the growth of shoots, and the other was subjected to histochemical staining [3, 4] to check whether the transformants showed β-glucuronidase activity. Among 12 calluses subjected to staining, we have obtained a positive result in three cases. Two of the blue-staining calluses originated from pKBI1-transformed discs, and one from pNBI2-transformed disc.

The following preliminary conclusions can be drawn from the degree of staining of individual calluses:

–1) The hybrid genes constructed by us undergo effective transcription.

–2) The transcripts formed are recognised as mRNA for protein synthesis.

–3) The "consensus" $tRNA^{Tyr}$ gene shows strong promoter activity.

Studies on this problem will be continued. To complete the experiment it is necessary to obtain regenerated plants from the calluses which are now in our possession. Such plants will be used for determination of β-glucuronidase activity by spectrofluorimetric method, and for confirmation by hybridization and reverse transcription of the presence of desired construct.

REFERENCES

1. Bourque, J.E. & Folk, W.R. (1992) *Plant Mol. Biol.* **19**, 641–647.
2. Jefferson, R.A., Kavanagh, T.A. & Bevan, M.W. (1987) *EMBO J.* **6**, 3901–3907.
3. Jefferson, R.A. (1987) *Plant Mol. Biol. Reporter* **5**, 387–405.
4. Stomp, A.M. (1992) *GUS Protocols* 103–113, Academic Press.

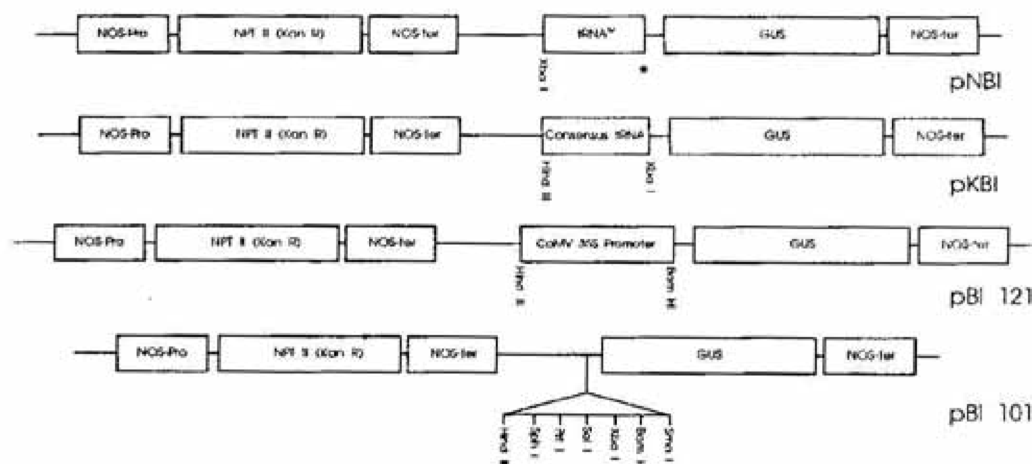

*Fig. 2. Scheme of the plasmids used for experiments; * — gene was cloned on blunt ends produced by SmaI and HindII enzymes, restriction site was lost.*