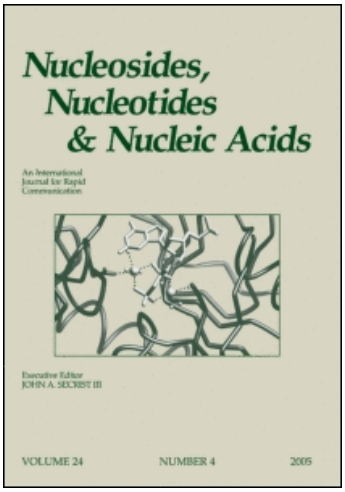

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Test System for Measurement of Translational Activity in Vivo

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TEST SYSTEM FOR MEASUREMENT OF TRANSLATIONAL ACTIVITY IN VIVO.

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ABSTRACT

A new plasmid which makes it possible to measure formation of a mature protein, as well as prematurely terminated gene products, has been constructed. It can be used for the analysis of translational efficiency of specified codon sequences in vivo.

Most knowledge concerning mechanistic aspects of the translational machinery originates from in vitro studies using purified components. Such results need to be compared with the situation in growing cells in order to minimize possible artifacts and limitations which are intrinsic to such in vitro systems. Measurements of translational misreading as well as activity of mutant forms of tRNAs (suppressor tRNAs) have been found to provide a sensitive and reliable signal for translational activity in vivo in strains with a mutationally altered translational machinery. One problem with most currently available test systems is that only the successful result of translation, i.e. the mature protein product which is formed, can be measured. Quantitative information is lacking concerning the fate of certain abortive reactions that take place on the ribosome.

Activity of the translational machinery in vivo can be measured by using suppression or mistranslation of appropriate mutant codons. Suppression efficiency of nonsense codons by mutant tRNAs provides a good insight into the contribution by individual bases to the overall activity of a particular tRNA species. Such measurements

are easy to perform since a full length protein is formed instead of a prematurely terminated peptide (Andersson et al., 1982). Alternatively, missense suppressor tRNAs, which are altered in codon recognition but maintain their aminoacid charging specificity, can be studied (Murgola 1985).

However, these systems have several limitations. Demands are put on the nature of the aminoacid which is inserted into the protein in cases when enzymatic activity of the protein is assayed for. Also, decreased affinity of a particular tRNA for its codon could lead to drop off from the ribosome in the form of peptidyl-tRNA. This effect can not easily be discriminated from the efficiency of the tRNA in the search for an empty aminoacyl-tRNA site. A potential translational frame-shifting event in connection with codon reading will be unnoticed. The location of a misincorporated amino acid is difficult to assess when translational error is used as the biological signal for translational efficiency. Taken together we wanted to design a test system which minimizes a number of complications. We also wanted to be able to study several parameters in one and the same experiment.

A test system with the following characteristics was desired; the test codon should be in a gene sequence such that the nature of the incorporated aminoacid at the corresponding position is neutral; the amount of gene product formed should be easy to determine by a direct measurement at the protein level without using enzymatic activity of the gene product formed; the protein formed should be possible to isolate in such an easy manner that many samples could be analysed at the same time; a number of aminoacids should be missing in the protein in order to be able to measure incorporation of these as the result of mistranslation (by using radioactivity tracing); gene products, which arise at low levels as the result of translation initiation downstream of the proper initiation codon, should be eliminated; gene products which arise as

the result of translational frame-shifting in connection with translation of the test codon should be possible to quantify; the peptide surrounding the aminoacid coded by the test codon should be easy to isolate and analyse with respect to aminoacid composition; degradation of the test protein should be minimal; the output of mRNA from the test gene should be possible to vary and the gene should be easy to transfer between various strains with mutationally altered components in the translational machinery.

In order to design such a test system we have taken advantage of an engineered gene which is derived from the IgG binding domain of protein A from S. aureus (Abrahamsén et al., 1986; Nilsson et al., 1897). The corresponding gene sequence is repeated three times and the repeat is preceded by a signal peptide which gives export of the protein into the growth medium in connection with cleavage of the signal peptide. A DNA linker is inserted between the second and third domain. Into this linker appropriate deoxyoligonucleotides can be inserted. Various codons of interest, for instance nonsense codons in different codon contexts, can thus be inserted and tested. The linker region is flanked by methionine codons which makes it possible to cleave out the corresponding peptide using cyanogenbromide. Such cleavage sites are missing in the rest of the construct. Out-of-frame nonsense codons are located downstream of the test region. Thus, a two domain product is formed as the result of translational termination, peptidyl-tRNA drop off or translational frame shifting in the test region. Complete translation gives rise to a three domain product. The products, which are excreted into the growth medium, can be isolated in a single step using an IgG affinity column and can then be analysed on a polyacrylamid gel.

The principle vector construct which is used for further constructions is shown in Fig.I. Some results are also indicated which illustrate the potential of the analysis. Both incomplete product(s) as well as the mature

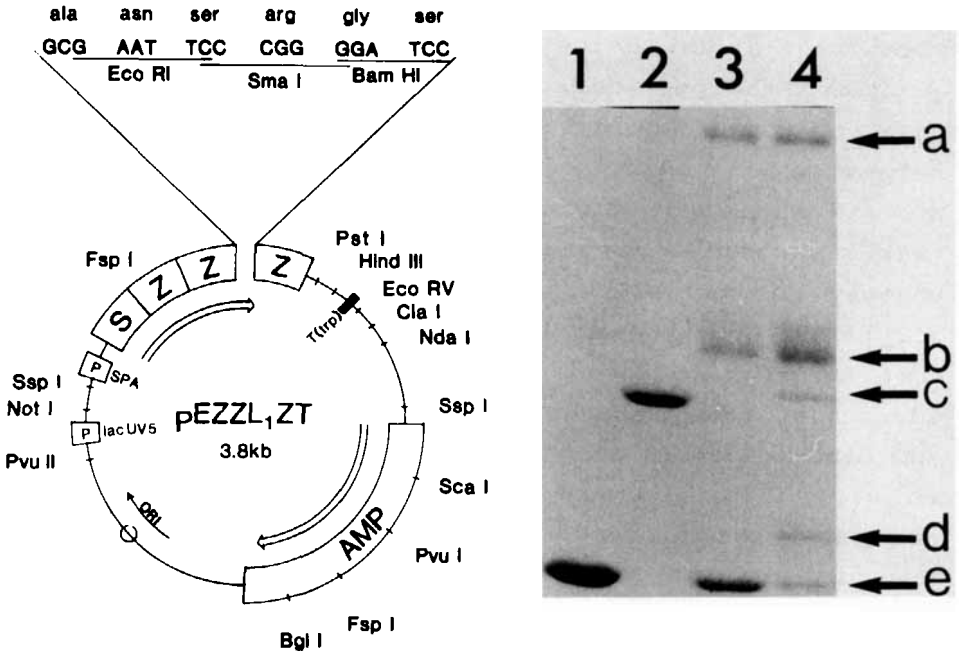

FIG.I Left; A vector with a gene coding for S (signal-peptide) and 3 units of the Z-domain derived from the IgG binding protein A from S. aureus. A multi-site linker is shown. Right; Expression of products from plasmid derivatives in different strains; The Z2 product (from a plasmid with only two Z units) in a normal strain (lane 1); The Z3 product (from a plasmid with three Z units, no insert) (lane 2); Products made from a Z3 construct with a UAG nonsense mutation in a strain lacking a nonsense suppressor (lane 3) or which carries a nonsense suppressor gene (lane 4).
The bands indicated by arrows are; IgG heavy and light chain (as a contaminant from the affinity column) (a and b); the Z3 (suppressed) product (c); a product of unknown nature (d); the (unsuppressed) Z2 product (e).

processed three domain product can be seen. The system is now used for further analysis of a number of bacterial strains with mutationally altered components in the ribosome, in tRNA or in some of the translation factors. Also, effects of various physiological conditions, including antibiotic treatment, are under study.

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