complete elimination of the SD sequence, as it had in the construct L_{27}-CAT.

The role of the constructs L_{PolyA}-CAT and L_{PolyT}-CAT in this study was to assess the effect on translation of two homopolymeric tracks (poly-A and poly-U) in the non-translated regions of mRNAs. As seen in Table 1, they had a different effect on the efficiency of translation. Whereas the yield of CAT obtained with the construct L_{PolyT}-CAT corresponded to that of SD_n-CAT, the poly-A track resulted in a 10-time decrease in the protein yield in comparison with the latter construct and a 50-fold decrease as compared with R_9-CAT (containing the SD consensus sequence). Taking into consideration that the S1 ribosomal protein is endowed with high binding affinity to U-rich sequences (Tzareva et al., 1994), one can assume that the higher protein yield obtained with the L_{PolyT}-CAT construct was due to the interaction of the poly-U track in mRNA with the S1 protein.

The last three constructs, L_8-CAT, L_6-CAT and L_1-CAT, were designed to evaluate the effect of the leader sequence length on translation. As seen in Table 1, the yield of CAT obtained with the L_8-CAT was 80 times lower than that of the referent construct R_9-CAT and very close to that of L_{PolyA}-CAT. When the non-translated leader sequence was further shortened to six (in L_6-CAT) and even one nucleotide (in L_1-CAT), the yield of CAT decreased negligibly but never reached the zero level.

The antibiotic resistance assay showed that *E. coli* cells transformed with R_9-CAT and SD_n-CAT (both overexpressing the *cat* gene) were capable of surviving at extremely high concentrations of chloramphenicol (exceeding 1 mg/ml). Although the decrease in antibiotic resistance correlated with that of the yield of CAT, Table 1 shows that bacteria transformed with the leaderless construct L_1-CAT did survive at concentrations of chloramphenicol as high as 50 μg/ml.

Expression of tetR-derived genes

The *tetR* gene was modified and expressed in *E. coli* LE392 following the same strategy as applied to the *cat* gene. However, because of the lack of specific assay for quantification of the yield of protein, the efficiency of *tetR* gene expression was evaluated indirectly, measuring the resistance of transformed *E. coli* cells to tetracycline only. Our results (Table 1) imply a similar mode of dependence of antibiotic resistance on the leader sequence length and nucleotide composition as observed with the *cat* gene. As shown in Table 1, *E. coli* cells bearing L_6-Tc^R (containing a six-nucleotide-long neutral leader) grew at tetracycline concentrations as high as 12 μg/ml.

Discussion

The results presented in this study definitely show that CAT and tetR mRNAs devoid of SD sequences or any other specific translational initiators, or even devoid of leader sequences, are translated in *E. coli* cells. Although the level of translation in the latter case is too low, the content of CAT and tetR is enough for bacteria to survive at chloramphenicol and tetracycline concentrations of 50 μg/ml and 12 μg/ml, respectively. Since such leaky expression has not been previously observed with other reporter (eukaryotic) genes tested in our laboratory (Ivanov et. al., 1992a), we are tempted to speculate that this could be a priority for mRNAs coding for proteins of vital importance for bacteria growing under selective conditions.

A question raised from these results is related with the mechanism of initiation of translation of mRNAs devoid of SD or leader sequences *in vivo*. It is known that highly expressed genes carry less randomly organized domains located 5 to 20 nucleotides downstream from the initiation codon (Petersen at al., 1988). This "downstream box" region is shown to be important for the efficient translation of some prokaryotic mRNAs in *E. coli* (Sprengart at al., 1990; Sprengart at al., 1996). In few cases mRNAs naturally devoid of leader sequences are thought to interact with the ribosome solely by their initiation codon and downstream box(es) (Shean at al., 1992; Winzeler at al., 1997). Translation of natural leaderless mRNAs has also been observed with the *Streptomyces cat* gene (Wu and Janssen 1997). Although the latter is poorly expressed in *E. coli*, it supplies the cells with substantial chloramphenicol resistance. These results undoubtedly show that the conventional SD-anti-SD mode of mRNA-ribosome interaction is dispensable (at least in some cases). If so, the conclusion follows that other areas on bacterial ribosome rather than the 3' end of 16S rRNA and/or ribosomal proteins (such as S1) are also capable of binding mRNA in a way allowing its efficient translation. The exact mechanism of the non-SD translation initiation, however, is still obscure.

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