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Transcript stabilization by mRNA sequences from *hrpA* of *Pseudomonas syringae*

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Abstract

Production of heterologous proteins in bacteria is one of the main applications of biotechnology. Although several high-efficiency expression systems have been developed, different steps in protein production may become rate-limiting depending on the production system and the protein being produced. One bottle neck can be the instability of the mRNA. We have used fragments of the unusually long-living mRNA *hrpA* from the plant pathogenic bacteria *Pseudomonas syringae* pathovars *tomato* and *phaseolicola* to increase the half-lives of heterologous transcripts. The stabilizing effect was extended to *Escherichia coli*, as half-lives of several heterologous transcripts were increased from a few minutes to up to 19 min. Production of heterologous proteins was also increased manifold by the addition of the stabilizing *hrpA* elements. We have mapped the regions of the *hrpA* transcript necessary and sufficient for the stabilization process.

Keywords: mRNA stability; Protein production; *hrpA*

1. Introduction

Microbes are used for the production of foreign proteins for medical and industrial use. Problems still exist in the optimization of the production processes. The main factors affecting the yield of the protein product are promoter activity, transcript stability, translational efficacy and protein stability, compartmentalization and conformation. Overproduction of heterologous proteins can also lead to destabilization of the transcripts coding for them (Carrier and Keasling, 1997a,b). Cell growth can be slowed down when the protein is being overproduced, and gene expression can be reduced. Improving the production of heterologous proteins by removing rare codons can also have the opposite results to those intended. mRNA stability can be reduced if the ribosome protection effect offered by the rare codons in the transcript is lost

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or the secondary structures forming in the transcript are changed. The amount of heterologous proteins produced may therefore not increase at all (Carrier and Keasling, 1997a,b; Lammertyn et al., 1996; Wu et al., 2004). Hence all of the steps described above affect one another and the outcome of changing one parameter is often unpredictable.

Transcript stability is often ignored in attempts to produce large amounts of heterologous protein. Heterologous transcripts can be stabilized by adding natural (Agaisse and Lereclus, 1996; Chen et al., 1991; Wong and Chang, 1986) or man-made structures (Carrier and Keasling, 1997a,b, 1999; Smolke et al., 2000; Smolke and Keasling, 2002a,b), often hairpin structures, to the ends of the transcript. Typically, three- to five-fold elevations in half-lives are seen with the use of stabilizing elements. For example, fusion with the 5′ stabilizing region of *ompA* elevated the half-life of a fragment of *bla* transcript from 3 to about 15 min (Chen et al., 1991) and the half-life of the penicillinase gene from *Bacillus licheniformis* was elevated from 2 to 6 min with the 3′ region of *B. thuringiensis cry* gene (Wong and Chang, 1986) By varying transcript stabilities, expression of genes coding for multi-subunit complexes or components in metabolic pathways can be controlled (Carrier and Keasling, 1997a,b; Smolke and Keasling, 2002a,b). Stable transcripts are beneficial in large scale production in industry with respect to the cost of the inducer, as well as in applications where the production of the heterologous proteins occurs in conditions where the addition of the inducer is difficult, e.g. when the inducer has to be ingested by an animal or by man, or applied directly to the environment.

We have earlier shown that the transcript coding for a virulence-associated pilin HrpA of the plant pathogenic *Pseudomonas syringae* has an extremely long half-life, approximately 20–40 min (Hienonen et al., 2004). The average half-life of bacterial transcripts is 3–8 min (Bernstein et al., 2002). HrpA is an essential component of the type III secretion system (TTSS) (Roine et al., 1997). Pathogens use these multi-protein complexes to inject virulence factors directly into their host cells, and to cause disease. The half-life of the *hrpA* transcript is extremely long in unrelated species including *Erwinia carotovora* and *P. syringae* pv. *phaseolicola*, and the mRNA is stable even in the heterologous production host, *Escherichia coli* (Hienonen et al., 2004). The physiological meaning of the stability of the *hrpA* transcript is unknown, but might be linked to its proposed role in selection of substrates for TTSS (Anderson and Schneewind, 1997). Studying such unusually stable transcripts could help in elucidating the role of control of transcript degradation in general and thereby lead to important applications in industrial protein production.

From our previous studies we know that the 5′ UTR and the first 15 codons of the *hrpA* sequence, as well as the hypothetical GC-rich stem loop in the 3′ UTR are essential for accumulation of the transcript (Hienonen et al., 2002, 2004; Taira et al., 1999). The sequences of the *hrpA* transcripts are divergent even between otherwise closely related pathovars of *P. syringae*, yet their 5′ UTRs and 3′ UTRs are highly similar, suggesting that the UTRs form similar secondary structures. The same functional double-helical regions have been shown to exist on sequentially divergent transcripts coding for polypeptides with the same function in different species (Chen et al., 1991). Hypothetical double-helical regions are likely to exist in vivo only if they are present in homologous sequences (James et al., 1989). We studied whether the same elements that are important in the accumulation of the transcript, the 5′ region and the 3′ UTR, are needed for the extreme stability of *hrpA* mRNA. Our results show that the stabilizing 5′ and 3′ elements can confer stability to mRNAs of heterologous polypeptides, and also increase the production of the heterologous protein.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains used in this study were *E. coli* DH5α, MC1061 and the original rifampicin sensitive *P. syringae* pv. *tomato* (*P. syringae*) DC3000 isolate (D. Cuppels, London, Ontario, Canada). Plasmids were introduced to *P. syringae* by electroporation. Hrp-inducing medium (Huynh et al., 1989) or Kings B (King et al., 1954) were used as culture media for *P. syringae* and LB for *E. coli*. *Pseudomonas* strains were grown at 28 °C, and for Hrp-induction at 18 °C, and *E. coli* strains at 37 °C.

2.2. Vectors

The shuttle plasmid pDN18-N (Taira et al., 1999) with *hrpA* containing a *Not*I site in codon 57 (hereafter