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Poster Presentation

Stabilization of heterologous transcripts with *hrpA*, mRNA of a type III secretion system component

Elina Hienonen*[1], Martin Romantschuk[2] and Suvi Taira[1]

Address: [1]Department of Biological and Environmental Sciences, Division of General Microbiology, University of Helsinki, Finland and [2]Department of Ecological and Environmental Sciences, University of Helsinki, Finland

* Corresponding author

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Background

Type III secretion systems are used by many Gram negative bacteria pathogenic to plants and animals to transfer virulence factors (effectors) directly into host cells, and to cause disease. The flagellar secretion systems are also classified as type III secretion systems. Both systems are well studied, but some elements still remain puzzling. No clear consensus sequence has been found for the type III secretion signal, and opinions of its nature vary from an amphipathic amino acid signal to an mRNA signal. Some type III secretion substrates use specific chaperones to aid in their secretion, but others seem to rely completely on another kind of a signal. This signal is found in the first 10–28 amino acids or codons of these proteins.

The possibility of an mRNA secretion signal led us to study the transcript of one type III secreted protein of the plant pathogenic bacterium *Pseudomonas syringae*, HrpA. Hrp-pilus, a component of the secretion apparatus, is composed of HrpA pilin subunits. HrpA is itself secreted by the type III secretion system. *hrpA* forms an operon with *hrpZ* in *P. syringae* pathovar *tomato*, but not in pv. *phaseolicola*. We have shown that the secretion signal of *hrpA* from *P. syringae* pv. *tomato* is in the first 15 codons [1]. The half-life of *hrpA* mRNA from different plant pathogenic species is exceptionally long, approximately 20–40 minutes [2]. This was true under varying temperature conditions and also when the transcript was produced in *E. coli*. Thus the stability of the mRNA is a characteristic of the transcript itself, not dependent on extra factors of the *Pseudomonads*. No physiological function has been assigned for the long half-life, but it may be related to the high abundance of HrpA protein or to the function of the mRNA as a secretion signal.

Results

We have used the unusual stability of *hrpA* mRNA to stabilize heterologous mRNAs fused to it. Heterologous transcripts originating from various sources were stabilized by *hrpA* in *E. coli* and in *P. syringae*. Their half-lives were increased from a few minutes of the control strains with no stabilizing elements to up to 25 min. The regions needed for the stabilizing effect were narrowed down. Protein production levels were also improved in this system. The amounts of heterologous proteins produced from these stabilized constructs were up to 5 times that of the control strain.

Conclusion

Naturally stable transcripts can be used to stabilize heterologous transcripts. Specific structures in mRNAs, often hairpins in the 5' or the 3' regions, have been shown to protect mRNAs against RNases. These structures are often conserved in evolution. In the case of *hrpA*, the hypothetical stabilizing secondary structures are highly conserved, and thus likely to serve an important function.

References

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