Quest for rho dependent terminators in prokaryotic genomes

Bilardi A., Campagna D., Campanaro S., Cestaro A., Levorin F., Vitulo N., Vezzi A., Valle G. and Cannata N.

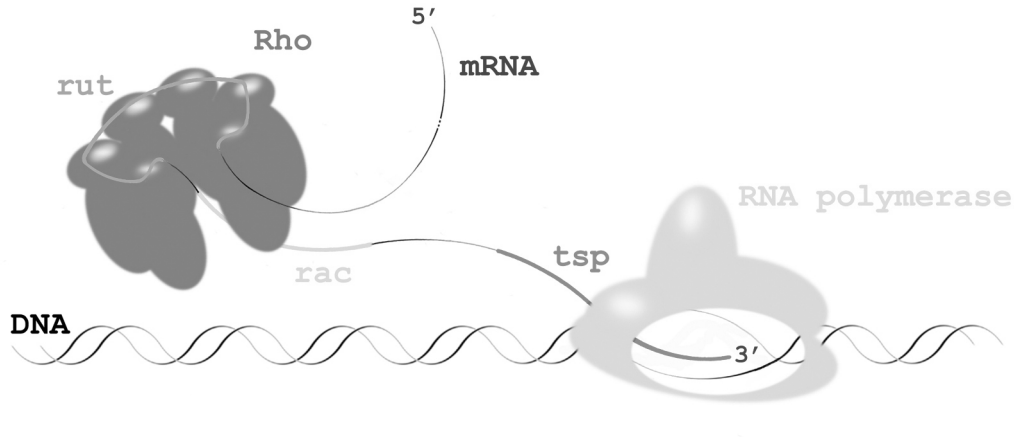
CRIBI, University of Padova, Padova

Motivation

In prokaryotes are known two kinds of transcription terminators that are distinguished by their mechanisms and DNA sequences. When RNA polymerase encounters an intrinsic terminator (RIT), it can release the nascent RNA spontaneously, but when it encounters a Rho dependent terminator (RDT), the release of the RNA depends on the action of a protein factor called Rho [1]. RDT are involved in the gene expression as attenuators in the leader or intra operon and as terminators at the end of operons. A RDT consists of three distinct parts, which together extend over 150-200bp of DNA (figure 1). The upstream part, called the Rho utilization (rut) site, encodes a segment of the nascent transcript to which Rho can bind and is essential for starting termination [2]. The central part, that we called Rho activity (rac) sequence, is the second mRNA binding site to which Rho can bind and is essential for helicase/traslocation activity [3]. The downstream part, called the transcription stop point (tsp) region, is where RNA polymerase pauses during elongation in the absence of Rho [4, 5]. In the literature is present only a small number of studies of single RDT, very little is know about their structure and sequence and is not existing any in silico predictive method.

Results

RDT are not characterized by a specific sequence pattern and we did not use a machine learning approach because the RDT collection is very limited. After a "manual" study of the nucleotidic composition and secondary structure of 29 sperimentally discovered RDT presented in the literature, we identified new details of each element (rut, rac, tsp) and their relative collocation. We implemented the first algorithm (RDTSCAN) that detects putative RDT on the basis of these new observations and applied it to the analysis of some genomes. In the sequence of PhageF1 (6408bp), RDTSCAN detects 10 RDT forward and 9 RDT reverse of which only one is described in the literature. The current version of the program is written in C++ language and uses only a part of the newly discovered features, therefore we aim to further develop the algorithm, refining selectivity of program with new parameters.



Contact email: bilardi@cribi.unipd.it

Supplementary Information: Reference: 1. E. Skordalahes and J.M. Berger. Structure of the Rho transcription terminator: mechanism of mRNA recognition and helicase loading. Cell, 114:135-146, 2003 2. C.Y. Chen and J.P. Richardson. Sequence elements essential for rho-dependent trascription termination at lambda tR1. J Biol Chem, 262:11292-11299, 1987 3. R.R. Wei and J.P. Richardson. Identification of an RNA binding site in the ATP binding domain of Escherichia coli Rho by H2O2/Fe-EDTA cleavage protection studies. J Biol Chem, 276:28380-28387, 2001 4. William D. Morgan, David G. Bear and Peter H. von Hippel. Rho-dependent termination of transcription . J Biol Chem, 258:9565-9574, 1983 5. Lester F. Lau, Jeffrey W. Roberts and Ray Wu. RNA polymerase pausing and transcript release at the lambdatR1 terminator in vitro. J Biol Chem, 258:9391-9397, 1983