TITLE: CHARACTERIZATION OF THE DEAD-BOX RNA HELICASE CC1478 IN *Caulobacter crescentus* AND ITS REGULATION

AUTHORS: RIBEIRO, R. A.; MARQUES, M.V.

INSTITUTION: INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO, SP, (AVENIDA PROFESSOR LINEU PRESTES, 1374, ICB II, LAB 222, CEP 05508-900, SÃO PAULO – SP, BRASIL).

ABSTRACT:

DEAD-box RNA helicases are enzymes that participate in important biological processes, such as nucleic acids degradation, assembly of riboproteic complexes and ribosome biogenesis. *Caulobacter crescentus* is an oligotrophic bacterium belonging to the alpha-proteobacteria group, in whose genome three genes encoding RNA helicases of the DEAD-box family can be found. Previous work showed that one of these enzymes, encoded by CC1478, is a cold-shock RNA helicase, with an atypical carboxi-terminal tail rich in basic aminoacids probably generated by genetic alterations conserved in the alpha-proteobacteria, This work aims to characterize the function and regulation of CC1478, determining if its expression varies in response to growth phase, cold-shock and other stresses. Expression was measured using a transcriptional fusion to *lacZ*. The results showed that up to 3 hours at 15ºC there was no difference among the expression profiles, and after 24 hours, both in low and in optimal temperature (30ºC) there was a great increase in the transcriptional levels, suggesting a growth phase-regulation. In order to evaluate the phylogenetic information related to the C-terminal tail, *in silico* analyses were carried out using both the whole protein sequence, and only the C-terminal DbpA domain. A similar domain composition was searched with SMART, based in HMMER from the Uniprot database. The analysis was narrowed to 344 strains of alpha-proteobacteria, using both sequence and protein profile (domain architecture), followed by MAFFT alignment and assemble of maximum-likelihood trees using PhyMl (further Bayesian-based analysis are being processed). Regarding only the C-terminal region these early results showed a huge diversity among the sequences, related only by basic residues, and the tree from using the whole protein sequence generated a more conserved tree. This was probably caused by the DEAD-box and helicase domains, which are strongly conserved. The physiological importance of this domain was assessed by deletion of the C-terminus by site-directed mutagenesis. Complementation experiments of the growth phenotype of mutant under cold shock, carrying an expression plasmid with either the complete or the truncated protein were carried out. The results indicated that there is some fitness improvement in log-phase and general growth provided by the truncated protein when compared to the wild-type protein.

Keywords: Alpha-proteobacteria, Cold-shock, DEAD-box, Phylogenetic tree.

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