At low temperature, the formation of secondary structures in the RNA molecule is energetically favorable; this process can, however, jeopardize its function and consequently the cellular processes in which they participate. At this point, it is essential the expression of specialized proteins, such as RNA helicases and RNA chaperones, in order to help the cell to adjust to the new environmental condition. The DEAD-box RNA helicase RhIA has been proposed to participate in adaptation to cold-shock stress in Caulobacter crescentus, since mutants have shown reduced fitness at 30°C and high sensitivity to cold stress. Here, we constructed a strain in which the RhIA protein was fusioned to an epitope (FLAG) that allowed the investigation of protein expression during the cell cycle and also under low temperature conditions. Western blotting was performed using an anti-FLAG commercial antibody, under cold-shock stress and after synchronization of the cell cycle. Culture aliquots were taken before and at time intervals after exposure to cold stress (10°C for 4 hours) in order to verify whether the expression profile could be altered; interestingly, the results indicate an increase in protein concentration after stress. In the synchronized cell culture, in turn, a constitutive pattern of protein expression was observed during each phase of cell cycle. Moreover, gene expression analysis was carried out using rhlA regulatory region fusioned to the lacZ reporter gene and introduced into wild type strain and null mutants for rhIA and rhIB. Mutant strains showed a higher rhlA expression when compared to wild type at low temperature. Finally, deletion mapping of the long 5’-untranslated region (5’UTR) was also studied, indicating an important region for gene expression.

Key Words: DEAD-box RNA helicases; cold-shock stress; Caulobacter crescentus.

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