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In vitro molecular analysis of ribosomal initiation complexes assembly and RNA-protein interactions during the initiation of translation of a prototype Coxsackievirus B3 and a live-attenuated Sabin 3-like RNAs

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Coxsackievirus B3 (CVB3) is an Enterovirus of the family of Picornaviridae. The Group B coxsackieviruses includes six serotypes (B1 to B6) that cause a variety of human diseases including myocarditis, meningitis and diabetes. Among group B, the B3 strain is mostly studied for its cardiovirulence and its ability to cause acute and persistent infections, yet no effective therapeutic against CVB3 is available. Translation initiation of CVB3 RNA is directed by an internal ribosome entry site (IRES) within the 5' untranslated region. It is a complex process in which initiator tRNA, 40S and 60S ribosomal subunits are assembled by eukaryotic initiation factors into an 80S ribosome. Host cell factors involved in this process include some canonical translation factors and additional RNA-binding proteins. We have, previously, described that the Sabin3-like mutation (U475 --> C) introduced in CVB3 genome led to a defective mutant with a serious reduction in translation efficiency. In this regard, we analyzed the efficiency of formation of ribosomal initiation complexes 48S and 80S through sucrose gradients using Rabbit reticulocyte lysate and stage-specific translation inhibitors. We demonstrated that formation of 48S and 80S ribosomal complexes within the mutant CVB3 RNA was abolished compared to the wild-type RNA. With the aim to identify proteins interacting with CVB3 wild-type and Sabin3-like IRESes and to study interactions between either HeLa cell or BHK-21 protein extracts and CVB3 RNAs, UV cross-linking assays were performed. We have observed a number of proteins that specifically interact with both RNAs. In particular, molecular weights of five of these proteins resemble to those of eukaryotic translation initiation factors 4G, 3b, 4B and PTB. According to cross-linking patterns obtained, we have demonstrated a better affinity of CVB3 RNA binding to BHK-21 proteins and a reduced interaction of the mutant RNA with almost cellular polypeptides compared to the wild-type IRES. On the basis of phylogeny of some initiation factors and on the knowledge of the translation initiation process, we focused on the interaction of both IRESes within eIF3, p100 (eIF4G) and 40S ribosomal subunit by Filter binding assays. We have demonstrated a better affinity of binding to the wild-type CVB3 IRES. Taken together, we can conclude that the reduction efficiency of Sabin3-like RNA to bind to cellular proteins involved in the translation initiation could be the reason behind inefficient IRES function.

Biography

Amira Souii is a Doctor in "Biological Sciences and Biotechnology" and an Associate University Assistant of Microbiology in the Higher Institute of Applied Biological Sciences of Tunis, Tunisia. She is a researcher in the laboratory of infectious diseases and biologically active substances in the Faculty of Farmacy of Monastir Tunisia. Her research work focuses on Molecular Virology and Vaccinology. She studied the RNA-protein and RNA-RNA inetractions during the initiation of translation of two Coxsackievirus B3 strains: a cardiovirulent wild type and an attenuated mutant strain. Interestingly, the mutant strain has shown very interesting results and so, it constitutes a promising vaccine candidate against Coxsackievirus B3 infections.

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