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Soutenance de thèse

Methodological development in surface plasmon resonance and applications to the study of virological regulatory mechanisms.

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We are interested in understanding how interactions involving nucleic acids regulate the life cycle of the Hepatitis C virus (HCV). The HCV genomic RNA is highly structured at the 5' and 3' ends. The stem-loop 5BSL3.2 was described to interact with the SLIIId, the Seq9110 and the SL2 by reverse genetics and complementation mutation experiments. Surface plasmon resonance (SPR) was used to characterize these interactions. This led us to develop methods to expand the use of this technique. We described *in vitro* evidences for an interaction between 5BSL3.2 and miR-122, a microRNA highly expressed in hepatocytes. As shown by our results, SL2 is a highly dynamic RNA motif that fluctuates between at least two conformations: one is able to hybridize with 5BSL3.2 and the other one is capable of self-associating. The study of this dimerization in living cells has shown an implication of this phenomenon in viral replication processes. The development of a ternary complex analysis method allowed the characterisation of the Seq9110 (or SLIIId) and SL2 (or miR-122) interacting with 5BSL3.2. Our results shown that the two binding sites of 5BSL3.2, the apical and internal loops, are structurally independent, suggesting that these interactions may coexist in a physiological context. SLIIId and Seq9110 were shown to compete to interact with 5BSL3.2 internal loop while SL2 and miR-122 were shown to compete to interact with 5BSL3.2 apical loop. In conclusion, 5BSL3.2 is a structured RNA motif that could act as a molecular hub capable of interacting with other genomic RNA regions while the two interactions of the SL2, mutually exclusives, were shown to be crucial for viral proliferation. The competition mechanisms observed could be involved in the commutation between viral cycle steps.

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