IRES elements – tools for attenuation of Mononegavirales translation and replication

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The use of non-segmented negative strand RNA viruses (NNSV) as live vectors or vaccines requires means to regulate their replication in critical cells or tissues. We are using internal ribosome entry sites (IRESs) to direct translation of essential viral proteins in order to control the replication of NNSV like rabies virus (RV). Using a newly established RV RNA-based dual luciferase assay, we identified and characterized suitable IRES elements in diverse cell lines. We found that IRESs strongly differ in their intrinsic translation initiation activities, but lack peculiar cell-type specific activities. For example, the HRV2 IRES has a universally low translational activity, while the PV IRES displays a higher activity in most cells including neurons.

Picornavirus and hepacivirus IRESs were further incorporated into recombinant rabies viruses to direct expression levels of RV phosphoprotein P, which is essential for viral replication and furthermore is the major viral IFN antagonist. P expression from IRES-containing RV was lower than from wt recombinant RV, and correlated with a decreased replication capacity. Despite the fact that the IRES-containing viruses replicated in brain slice cultures, theses viruses were strongly attenuated in vivo, indicating synergistic effects of replication and innate immune escape for virus virulence.

Although it is indicated that the contribution of several ITAFs to internal translation initiation needs to be re-examined, we are scrutinizing ITAFs as well as small interfering peptides and oligonucleotides to control and manipulate the activity of IRESs and thereby NNSV replication.

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