Organization, activities and inhibitors of viral RNA synthesis apparatus

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RNA viruses (realm Ribovira) are the only biological objects capable for efficient synthesis of RNAs on the RNA templates. This is carried out by RNA-dependent RNA polymerase assisted by other virus- and host derived factors which together form replicase complex (RC). In eukaryotic cells the RC is anchored to cellular membrane and is capable of multiple enzymatic reactions including methylation, polymerization and ATP hydrolysis. As these activities must occur in coordinated manner the RC has defined structure. RC of alphaviruses consist from four viral subunits while RC of coronaviruses has sixteen subunits. Using reverse genetics, synthetic and structural biology methods we have demonstrated that: i) binding of RC to the cellular membranes is triggered by palmitoylation of viral methyltransferase and its subsequent interaction with cholesterol; ii) binding of specific RNAs by RC of alphaviruses is based on recognition of RNA secondary structures by viral polymerase; iii) RNA binding is assisted by viral helicase/protease. The interaction involves hydrophobic stacking between RNA bases and aromatic amino acid residues and regulates protease activity of the enzyme; iv) virus encoded enzyme with mono(ADP-ribosyl)hydrolase activity is covalently modified by phosphorylation of serine/threonine residues; v) viral polymerase can form catalytically active RCs with other RC subunits originating form heterologous viruses. All these activities and interactions can be specifically inhibited by chemical compounds. In some cases, the inhibition of viral activities is straight forwarded: binding of RC to membranes is sensitive to drugs inducing cholesterol sequestration, protease activity can be inhibited by compounds that interact with active site of the enzyme, and viral polymerase can be inhibited by nucleotide analogues. However, due to plasticity of viral genomes resistance to these inhibitors is rapidly developed, though often this adaptation is associated with fitness cost. Other type of inhibitors, such as hyper-mutagenic nucleoside analogues, are active against numerous viruses and have high barriers for development of resistance; however, they suffer from potential side effects. Therefore, the compounds targeting higher-order processes such as specific interactions between viral and host components would be valuable as novel drug leads as well as tools for studies of molecular biology of RNA viruses