The mechanism of SARS-CoV2 coronavirus nucleocapsid protein interaction with human 14-3-3 proteins

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The coronavirus nucleocapsid protein (N) is a structural protein that regulates the transcription and replication of the viral genome and promotes the packaging of RNA into viral particles. The N protein has a complex domain structure and undergoes multipoint phosphorylation in the central unstructured region after entering the cell. According to the literature data, phosphorylation of homologous N protein from the SARS-CoV coronavirus leads to its retention in the cytoplasm due to direct binding with cellular regulatory proteins of the 14-3-3 family. Human 14-3-3 proteins are represented by seven isoforms that are highly expressed in tissues susceptible to SARS-CoV2 infection. Through interaction with N 14-3-3 proteins may play an important role in the life cycle of the coronavirus. In this work, we dissected the molecular mechanism of the interaction between SARS-CoV2 N and human 14-3-3 proteins. The phosphorylated form of N (pN) was obtained in a special system of co-expression with protein kinase A in *E. coli*, which led to phosphorylation of more than 20 sites in N. We have shown that only pN interacts with all isoforms of human 14-3-3 with micromolar affinity and the stoichiometry 2:2. Through the series of truncated mutants of N, it was shown that the presence of pS197 residue is *necessary* for the binding of 14-3-3 proteins. Utilizing the genetic code expansion allowing for the site-specific, translational incorporation of phosphoserine residues into proteins of interest we confirmed that S197 phosphorylation is *sufficient* for binding to 14-3-3 proteins. It is noteworthy that this 14-3-3-binding site is quite conserved among N proteins from various coronaviruses. So the proposed molecular mechanism for the formation of the 14-3-3/pN complex could be universal and useful in the development of new therapeutic approaches for fighting coronavirus infections. Partially supported by RSF № 19-74-10031.

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