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Undergraduate Research Program

Project Name	Characterization of the nsp14-nsp10 complex of SARS-CoV-2
Campus & Location in Mexico	Toluca
Faculty	Engineering and Sciences
Research Area	Biology, virology, biochemistry, molecular biology
Research Responsible	Aarón Hernández Cid
Description of the Project	The nsp14 is a bifunctional protein bearing 3Â´-to- 5Â´ ExoN (Minskaia et al., 2006) and N7-MTase (Jin et al., 2013) activities. This striking protein is considered an evolutionary hallmark of the nidovirales order and hence the CoV family; as it is thought to have played a key role in the replication competency, fitness, pathogenesis, quasispecies diversity, genome size and fidelity (Eckerle et al., 2010; Snijder et al., 2003). Nsp14 among the CoV viruses share common signature sequences that compose conserved structural features across the Nidovirales (Huang et al., 2016). Nsp14 amino acid sequence can be broken down into two parts, the N-terminal ExoN and the C-terminal guanine N7-Mtase. The CoV nsp14 ExoN is a member of the DEDDh superfamily (Huang et al., 2016), where the second aspartate was replaced by a glutamate, and two zinc fingers (ZF) with conserved cysteines and histidines (ZF1: Cys208, Cys211, Cys227, His230; ZF2: His257, Cys261, His264, Cys279). Mutants of ExoN activity of SARS have shown impaired growth in VeroE6 cell cultures compared to wild types, and after many passages continue to carry the same ExoN mutations (Eckerle et al., 2010). Surprisingly, mutations in the ExoN domain III reversed from Ala to Asp, enforcing the hypothesis of the balance of genome size/fitness of nidoviruses (Eckerle et al., 2010). N7-Mtase activity in nsp14 in conjunction with nsp16 comprises the two methyltransferases in CoV viruses (Wang et al., 2015). N7-Mtase depends on the complex formation with nsp10 for its activation (Wang et al., 2015). The crystal structure of nsp14 for SARS-CoV reveals that the N7-Mtase does not follow the typical Rossman fold for methyltransferases, being a unique fold that should nucleate a new structural family (Y. Chen et al., 2013). The key residues for the N7-Mtase comprises the canonical sequence in the S-adenosyl methionine (SAM) pocket DXGXPXG/A, and a third zinc finger with the typical conserved cysteines and histidines (Cys453, Cys477, Cys484, His487) (Ferron et al., 2017). Mutants of N7-Mtase in key residues of the SAM pocket domain have also shown diminished growth compared to the wild type (Yu Chen et al., 2009). N7-Mtase activity depends on residues in the N-terminal that comprises the interface for the interaction with nsp10 (Wang et al., 2015). In this project, the student will develop an in vitro test to characterize the exonuclease and methylation activity simultaneously. The project will be broken down in two parts: overexpression in cells and purification, and development of a spectroscopic test with SAM and pyrophosphate as main indicators.
Training Provided	The student will have acces to bioreactors and collaboration with the National Instituto of Medicine in Mexico city to work in the progress to study this protein. Purification, overexpression techniques will be primarily to understand.

Offered during:

SUMMER	X	**WINTER**		**SEMESTER**	

Student

Tasks/Responsibilities	Overexpression, purification and activity test.
Required Language Proficiency	English and Spanish (not essential but recommended for life in the city)
Required Skills and Abilities	Knowledge of protein purification and techniques to characterize proteins.
Other Documents	2) Accumulative grade point average (GPA) 2.5 3) Official Transcript 4) 2 letters of recommendation of faculty members 5) Resume 6) Letter of intention explaining the reason why you would like to participate in the research program