Among animal cells, neuronal cells (neurons) have the most complicated morphology and functions. The complex morphology of neurons reflects the role they play in analyzing information by the nervous system. These cells with help of their extensive cytoplasmic protrusions (dendrites and axons) form a complex connection network, in which dendrites receive signals from other members of the network while axon transmits information to the subsequent circuit elements. The generation of nerve cells, including an acquisition of the correct neuronal shape and formation of a functional neuronal network, is known as neurogenesis. This process is regulated by a number of molecular mechanisms, some of which engage protein called mTOR. mTOR integrates information about resources cell has at its disposal (e.g., availability of amino acids, or energy level) with extracellular growth stimulating signals. If, mTOR "decides" that the internal resources allow the cell growth and increased synaptic communication, by means of its target proteins, initiates necessary molecular changes needed for example for neuroprogenitor migration and differentiation, axonal and dendritic growth and formation of active synapses.

Communication between extracellular and intracellular environment as well as between different compartments inside the cell is very important for movement, growth, and maturation of neurons. This requires proper sorting of cellular proteins (cargo) through the complicated network of intracellular membranes. The proper sorting involves specialized ensembles of proteins, which take care of cargo recognition and partitioning to the correct compartment depending on cellular needs (e.g., some cargo proteins will be reused by the cells while the others will be sent to the cellular trash can - lysosome). One of the protein complexes needed for cargo sorting is retromer. Our preliminary data show that mTOR is involved in the retromer regulation by changing the activity of a protein named TBC1D5. Yet, exact stages of neuronal development which would require such regulation remain unknown. Also, it has not been investigated thus far whether dysregulation of retromer functions takes place in neurodevelopmental disorders related to mTOR hyperactivation.

The main goal of this project is to test the hypothesis that during neuronal development mTOR modulates the protein sorting, which depends on the activity of TBC1D5 and retromer. At the same time, we plan to check whether disturbance of the retromer-dependent protein sorting may be involved in the development of tuberous sclerosis, a disease arising from excessive activity of mTOR, resulting in several neurodevelopmental symptoms.

The proposed research will verify if mTOR-dependent regulation TBC1D5 and retromer is important for neuronal development and is disturbed in tuberous sclerosis complex, a prototypical mTORophaty. Using *in vitro* cultured neural stem cells and maturating neurons as well as *in vivo* models of brain cortex formation we plan to verify if disturbance of TBC1D5 regulation by mTOR affects neural stem cells migration and differentiation, axonogenesis and formation of functional neuronal circuits, processes that are disturbed in TSC. Using similar approaches we will test if TBC1D5 regulation by mTOR is disturbed in cellular models of TSC. Finally, we will also attempt using mass spectrometry analysis to define retromer cargo relevant for neurodevelopment.

As a result of this research project, we expect to describe a new mechanism of regulation of intracellular protein sorting in nerve cells undergoing intensive growth during neuronal network formation. In addition, we will provide information on the potential contribution of retromer and its cargo to the pathogenesis of tuberous sclerosis complex, which will contribute to a better understanding of the molecular basis of this still mysterious disease.