The planned project aims to expand the existing knowledge on the evolution of starch biosynthesis. Starch, a polymer of glucose residues, plays a crucial role in plant functioning as a storage substance. It is a dietary staple for most of humanity and has numerous industrial applications. Chemically, starch is similar to another storage material, glycogen, but it has a more complex and regular structure. The biosynthesis and degradation of starch involve similar enzymes as in the case of glycogen. However, starch metabolism requires additional enzymes from the isoamylase group, which ensure the regularity of starch structure, and glucan- and phosphoglucan-water dikinases (GWDs and PWDs), which are essential for its degradation.

Unlike glycogen, which is synthesized by a wide variety of organisms, starch has been found only in certain cyanobacteria and a few groups of eukaryotes. All eukaryotes known to be capable of storing starch possess plastids, i.e. cellular organelles responsible for photosynthesis, which evolved through a process known as endosymbiosis from engulfed cells of cyanobacteria or eukaryotic algae. It is therefore believed that the evolution of starch biosynthesis in eukaryotes was linked to this process. Since starch can be stored in larger quantities than glycogen, its emergence during evolution may have improved the host's ability to store excess photosynthetic products supplied by the endosymbiont transforming into a plastid.

For many years, however, there have been reports about the potential presence of starch in ciliates, heterotrophic eukaryotes that do not have plastids and likely never had them in the past. Observations using transmission electron microscopy have detected starch-like granules in their cells. In one group of ciliates, more detailed biochemical analyses have also suggested the presence of starch. Recently, we have identified GWD/PWD sequences in various ciliates using bioinformatics methods. Our phylogenetic analysis of these proteins suggests that the ability to synthesize starch in ciliates was already present in their common ancestor.

We have thus decided that it is worthwhile to definitively verify whether ciliates are capable of starch synthesis, which is the direct goal of our project. We intend to check if selected strains have starch-like grains, observable with various microscopic techniques. We plan to isolate these granules from ciliate cells and determine if their structure is indeed typical of starch. Since previous studies have not detected isoamylase genes in ciliate genomes, we also aim to identify which other proteins might determine the regular structure of starch in these organisms. We will search for such proteins among those associated with the purified granules. The detected proteins and identified GWD/PWD enzymes from ciliates will be functionally analyzed to determine if they play the expected roles in starch metabolism.

The conducted experiments will allow us to verify whether the evolution of starch biosynthesis in eukaryotes indeed always had to be linked to endosymbiosis and to better characterize the diversity of starch metabolism. There is a possibility that the mechanisms determining starch structure, which we plan to identify in ciliates, likewise exist in related parasitic apicomplexans, which also accumulate starch without the involvement of isoamylases. Our research could thus facilitate the development of new drugs for diseases caused by these protists, such as toxoplasmosis. In the future, the discovered starch in ciliates and the proteins involved in its biosynthesis and degradation could have various biotechnological applications.