Description for the general public

The final stage of cellular respiration takes place at the level of membrane proteins organized into a chain. Within this chain electron transport takes place (electron transport chain, ETC). During electron transport, these proteins also transport protons across the membrane. This proton gradient thus generated is used by ATP synthase to produce ATP, a universal "energy carrier" used in cell metabolism. Depending on the organism, there may be different variants of the ETC chain.

ETC chain of animal cells is made up of only four "basic" proteins (complexes I to IV). They provide electron flow from substrates, NADH (complex I, CI) and succinate (complex II, CII) to the product, water, formed by oxygen reduction in complex IV (CIV). In this chain, the link between CI and CII and CIV is complex III (CIII, cytochrome bc1). Plant cells have a much more extensive ETC chain, which, in addition to the "basic" proteins, contains from several additional proteins. This "extended" chain contains proteins alternative to complex I and alternative oxidases (AOX, alternative proteins to the CIII-CIV complex pair). These proteins maintain the continuity of ETC work even in the event of disruption of the functioning of the basic complexes.

There is great diversity in the organization of ETC in bacteria. For example, the bacterium *Helicobacter pylori* (Fig. 1) contains CI, which uses a different substrate than the aforementioned eukaryotic CI, NADPH instead of NADH. In addition, it also contains a small protein that can be considered as an alternative to CI, and which also uses NADPH. Instead of CII, it contains the FRD protein, which carries out the reverse reaction to CII. It also has an unusual cytochrome bc_1, which has exceptional catalytic abilities. *H. pylori* uses cytochrome cbb_3 as a terminal oxidase. Interestingly, the end product of this chain may also be succinate formed in the reaction catalyzed by FRD.

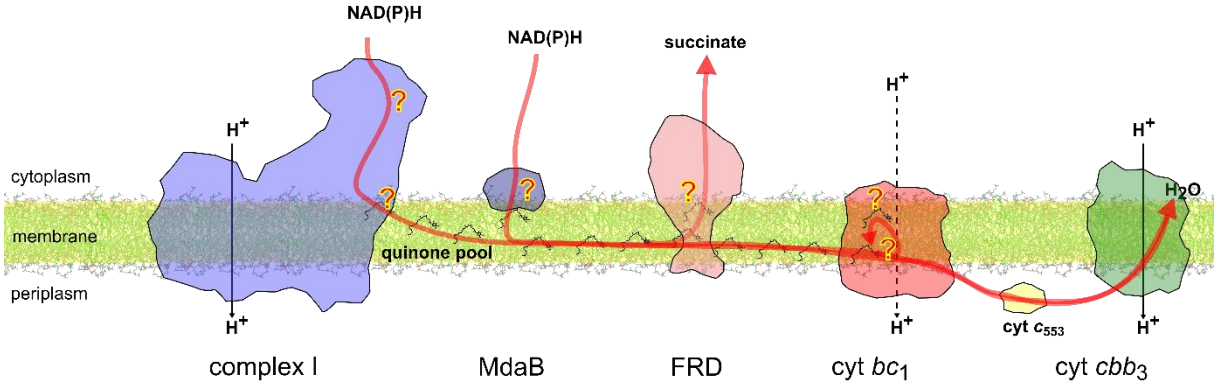

Figure 1. Schematic diagrams showing electron transfer pathways (red arrows) in the electron transport chain (ETC) of *H. pylori*. Question marks indicate locations in the ETC that could potentially be a source of reactive oxygen species. Black arrows illustrate proton transport across the membrane.

Studying the function of this unusual, essential for *H. pylori* survival, ETC chain is an interesting issue, especially in the context of the high rate of *H. pylori* infection worldwide, which is about 50%. This Gram-negative bacterium is the main cause of stomach cancer and other serious diseases. The project is of great importance, because understanding the molecular basis of the function of *H. pylori* ETC proteins may help in the future to develop specific inhibitors that impair energy acquisition by this pathogen. In this search for inhibitors, it is necessary to determine the structures of *H. pylori* ETC proteins, based on which specific inhibitors can be searched for using computational methods.

The main goal of the project is to obtain for the first time, using the cryo-EM method, the structure of *H. pylori* ETC proteins: CI, FRD, cytochrome bc_1 and to study the catalytic properties of these proteins. An important goal of the project is to describe the participation of these proteins in the production of free radicals, especially since the function of these proteins, at the level of catalytic properties and the ability to produce radicals, has not been described so far.

The studies will be conducted on purified recombinant ETC proteins of *H. pylori* expressed in *Escherichia coli*. Commercially available analogues of coenzyme Q10 (Idebenone and MitoQ) will be tested and their effect on the activity of ETC proteins and on ROS production will be checked. These studies will contribute to a better understanding of the energy conversion processes in bacteria, which in the future may help in the design of chemical compounds that uncouple/impair the activity of these proteins.