ABSTRACT

Ubiquitination is one of the cellular processes causing post-translational modifications of proteins. The main purpose of this enzymatic cascade is to attach a small protein - ubiquitin to modified proteins (so-called substrates). This is an extremely important cascade due to the fact that ubiquitin can mark proteins, e.g. damaged, incorrectly folded or malfunctioning, which may lead to their degradation. Importantly, disorders of at least one of the stages of this process are the cause of many diseases, including cancer, neurodegenerative or immunological diseases.

In addition to ubiquitin, a number of other structurally related enzymes are involved in the ubiquitination process. The most important of them are: ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2) and ubiquitin ligases (E3). In humans, over 30 ubiquitin-conjugating enzymes and over 600 ubiquitin ligases have been described that can ubiquitinate various substrates. The diversity of the E3 ligases therefore results in the specificity of the ubiquitination processes. It is worth noting that an increased amount of ubiquitin ligases is observed in different types of cancer, which makes ubiquitin ligases particularly interesting from the point of view of developing new therapies. The main obstacle to progress in the development of drugs, including drugs that inhibit the activity of selected ubiquitin ligases, is the complexity of the mechanism of ubiquitination processes. To date, the exact biological role of many ubiquitin ligases has not been understood and their substrates have not yet been identified.

In the presented manuscript, we characterized the interaction network of selected ubiquitin ligases using modern *in vivo* assays and standard biochemical and genetic tools. Many interactions and protein substrates have been discovered mainly through *in vitro* biochemical techniques, which may not accurately reflect the real cellular environment. Thus, studying interactions in living cells in real time enables us to characterize the actual biological processes that occur in cells. We tested the Nam7 (Upf1) and Irc20 E3 ligases using the baker's yeast *Saccharomyces cerevisiae* as a model organism. These proteins have not yet been characterized in detail for their role in the ubiquitination process. In humans, impaired function of the Nam7 protein is associated with the occurrence of certain genetic diseases and certain types of cancer.

Irc20 is an uncharacterized protein known to be involved in homologous recombination, but its direct mechanism of action has yet to be elucidated. The manuscript focuses on an in-depth understanding of the mechanisms of action of Nam7 and Irc20 as E3 ligases and their potential implications for both ubiquitination and other biological processes. We also present modern techniques in the field of biochemistry based on the complementation of NanoBiT® and NUbiCA protein fragments as well as standard techniques of molecular biology and genetics. The optimized NanoBiT® system proposed in our research will enable the study of protein interactions in the ubiquitination process in living cells on a large scale. The project will therefore contribute to a better understanding of the network of protein interactions and the consequences of these interactions in the cell.