Investigating the ubiquitylation network of Irc20 and Tfb3 ubiquitin ligases needed upon DNA damage

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DESCRIPTION FOR GENERAL PUBLIC

In 2004 three researchers Aaron Ciechanover, Avram Hershko and Irwin Rose were jointly awarded the Nobel Prize in Chemistry for "the discovery of ubiquitin-mediated protein degradation", a process, in which unneeded or damaged cellular proteins are marked for destruction by the addition of a small protein called ubiquitin. Primarily, it was thought that ubiquitin is only a tag which marks unwanted proteins for degradation. Now, it is known that adding this tag, in a process termed ubiquitylation, to a given protein may be involved in different cellular processes and have distinct functional consequences for the cell. Aberrations in protein ubiquitylation have been associated with various diseases such as cancer, neurodegenerative diseases and certain viral infections.

Apart from ubiquitin there are a variety of other proteins that are involved in the process of ubiquitylation, such as ubiquitin activating (E1), ubiquitin conjugating (E2) and ligating enzymes (ubiquitin ligases, E3). These enzymes interact with each other and have the ability to regulate the stability and function of other proteins. Humans contain more than 30 E2s and 600 E3s. Ubiquitin ligases are particularly interesting potential targets in the development of new drugs, especially for cancer treatment. So far, there are approximately ten ubiquitin ligases inhibitors in clinical trials but none of them are used for cancer treatment yet. There is an obstacle to the progress in the development of E3 ligase inhibitors that lies within the mechanism of the ubiquitylation process. This obstacle is that two types of ubiquitin enzymes, E2s and E3s, function in a complex network with one E2 being able to interact with several E3s and vice versa. There is a possibility to inhibit ubiquitylation in a cell by using small molecules inhibiting the E2/E3 interface. It is therefore important to know, which E2-E3 pair function in the cell under physiological and pathological conditions.

Although the mechanism of ubiquitylation process is very well described, little is still known on physiological E2-E3 pairs and their function in the cell because of a transient nature of the ubiquitylation process. The objective of this project is to functionally characterize some potential E2-E3 pairs in living cells, using budding yeast as a model organism. We would like to decipher E2-E3 enzymes' pairs as well as substrates of two poorly characterized ubiquitin ligases needed upon DNA damage, namely Irc20 and Tb3. For the purpose of this research we will use techniques that we have already established to assay weak and transient E2-E3 interactions in living cells, called bimolecular fluorescence complementation (BiFC) as well as an alternative approach based on luminescence (BiLC). Moreover, we will use other biochemical and quantitative proteomic approaches to look for Irc20 and Tfb3 substrates. Deciphering the molecular mechanisms of these enzymes' function will impact the current knowledge on ubiquitylation process biology but it also will aid in the development of anti-cancer treatment as well as in finding new medications for other diseases in the future. Methodology developed in this project will also serve the scientific community to conduct the research on the other important protein-protein interactions in the cell.