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Review of the doctoral thesis of Ms Nikol Dibus "Functional Characterization of SCFFBXO38 Ubiquitin Ligase-dependent Protein Degradation

The doctoral thesis by Ms. Nikol Dibus deal with one of the core problems of current molecular biology: what are the hallmarks of protein stability in the cells? What is it that determines their halflife in the biological system, and what are the mechanisms by which they are degraded?

These questions are relatively new. Until quite recently, biologist were much more interested in the way how proteins are synthesized, processed, modified and translocated to their final compartment than in the mechanisms by which they are degraded. Few decades ago, students would found only several paragraphs on the protein degradation in the voluminous biochemistry textbooks, mostly describing the mechanism of action of trypsin. Now we only begin to understand that the mechanisms by which proteins are sorted to degradation, transported to the degradation sites and finally hydrolysed (not to speak about very interesting ways in which the degradation products are being used by the cells) are at least as complex as the mechamisms by which the proteins are synthesized. The degradation of proteins is not just simple "catabolism", a way of degrading proteins to yield amino acids, simple sugers and metal ions, but highly organized, tightly controlled (because deadly dangerous) operation using specific organelles, huge multienzyme complexes and very complex aray of signals with multiple feedback controls. It must be mentioned that the

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proteolysis is a very rare example of almost irreversible secondary modification: once the peptide bond is cleaved, is almost impossible to restore it in the cell.

One of the most important mechanisms of controlled intracellular degradation is the proteasome-ubiquitin system. The crucial role in the molecular recognition of proteins deemed for degradation is executed by ubiqitin ligases: highly specific enzymes that attach the ubiquitin moiety to that particular protein. Any mutation leading to the loss of function of these ligases could (and often does) have significant impact and often leads to pathological condition.

The dissertant chose to study one such an important ubiquitin ligase designated SCF^{FBXO38}. This enzyme has not been much studied before, although its mutation causes one form of spinal muscular distrophy.

Two papers, published very recently in the Frontiers in Cell and Developmental Biology (of which dissertant she is the first author)form the core of the presented doctoral thesis. In the first manuscript, she report the identification of the substrates for SCF^{FBXO38}, the less known nuclear proteins ZXDA and ZXDB. In the paper, she shows that these substrates function as positive regulators of centromeric chromatin integrity. Furthermore, the inactivation of the ligase leads to the stabilization of CENP-A and CENP-B proteins in the centromeric regions.

In the second paper, the dissertant analyzed the Fbxo38 gene deficiency using mouse model. Somewhat surprisingly, she shows that the loss of Fbxo38 leads to growth retardation affecting mostly the male reproductive system, leading to lower number of spermatozoa and decreased fertility. Interestingly, the FBXO38 protein is functionally expressed in Sertoli cells that are crucial for the spermatogenesis. The knock-down of FBXO38 resulted in stabilization of ZXDB protein, delayed maturation of Sertoli cells and ultimately in defect in spermatogenesis.

The author used a variety of molecular biology, cell biology, immunochemistry and advanced analytical chemistry methods, including advanced mass spectrometry, DNA sequencing and gene silencing to reach these conclusions. I find the methodological breadth of the study unusual and impressive.

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The thesis are written clearly, the theoretical instroduction is very informative (I learned a lot about centromere organization and spermatogenesis!), the resuts are well documented, methods described completely in the manuscripts that are in extenso part of the thesis, and the outcomes of the study are clearly postulated and adequately discussed.

I have few question to be answered by the dissertant:

The interaction of Fboxo38 with ZXDA/B was determined by MS analysis of corresponding tagged proteins in cell lysates. Did the dissertant attempt to show or even quantify the direct protein/protein interaction of Fboxo38 with ZXDA/B or their individual domains?

The disertant performed SwissProt – Alpha Fold modelling of the potential protein partners but I have not seen any description of the structural mode of their interaction. Are there any plans to establish the structure of the protein/protein interaction experimentally (X-ray or cryoEM structure analysis) or using molecular modelling?

Little speculation to the end. The thesis show very clearly that FBXO38 is involved in a novel biochemical pathway controlling centromere integrity. I find quite probable that there are other pathways that can be regulated by this ligase as well. Could the dissertant speculate which one it could be and how to identify it?

Finally, I am very pleased to conclude that the work submitted by Mgr. Nikol Dibusová completely fulfils even the most demanding requirements for a dissertation in the field of molecular and cell biology and therefore I fully recommend it for defence and as a basis for the award of the PhD title.

Prague, August 31, 2022 Jan Konvalinka

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