Open position for LMU-CSC scholarship candidates 2023

Department/Institute:

Institute of Medical Psychology
Department of Systems Chronobiology, Prof. Dr. Maria Robles
Faculty of Medicine, LMU
Goethestr. 31
80336 Munich

Subject area:

Basic research in biology/medicine
Keywords: Lifespan of proteins, protein quality control, N-terminal processing, N-terminal acetylation, ubiquitination, mass-spectrometry based proteomics

Name of supervisor:

Dr. Tanja Bange, Habilitant, Institute of Medical Psychology, LMU, Munich
tanja.bange@med.uni-muenchen.de
Tel: +49-176-63138637
Tel: +49-89-218075-650

Number of open position: 1

Project title:

Identification and characterization of N-degron pathways using quantitative mass spectrometry

Project description:

Background and preliminary work: The lifespan of cellular proteins ranges from less than a minute to several days. Regulated protein degradation controls levels of all short-lived proteins to ensure cellular homeostasis and protects cells from accumulating abnormal proteins(*1*). Dysfunctional degradation causes multiple pathological processes, spanning from neurodegenerative disorders to cancer(*2*). Therefore, the identification and understanding of degradation pathways is of pivotal importance. Regulated protein degradation is mostly mediated by the ubiquitin proteasome system (UPS)(*3*). The most important players in this system are E3 ligases, so called recognins, which recognize exposed sequence motifs, so-called degrons, of target proteins and conjugate ubiquitin (Ub; 8kDa protein) to nearby lysine residues on them(*4*).

Ubiquitinated proteins are subsequently destroyed by a multi-subunit protease, the 26S proteasome. The aim of the project is to investigate a specific subset of protein degradation pathways, so called N-degron pathways, in which N-termini of proteins are recognized as degradation signals. So far four N-degron pathways have been described. We established N-terminal peptide pull-downs combined with quantitative mass spectrometry to identify additional N-degrons with their corresponding E3 ligases, so called N-recognins. With this approach, we were able to add a fifth N-degron pathway targeting non-acetylated N-termini starting with alanine, and possibly serine and threonine (nonAc/Ala N-degron pathway)(*5*). This pathway targets proteins which harbor cryptic N-degrons masked by their natural N-terminal (Nt-) acetylation (ac). After reduction of Nt-ac by RNAi of NatA, the responsible Nt-acetyltransferase, the N-degron is exposed and recognized by the E3-ligase family inhibitor of apoptosis proteins (IAPs) that are key regulators of programmed cell death in development, tissue homeostasis, and tumorigenesis identifying IAPs as new N-recognins. Target proteins are subsequently degraded and the IAPs apoptotic potential unleashed. Starting from these data and our established workflows, this project aims to identify and characterize N-degron pathways.

During our investigation of the role of IAPs as N-recognins, it came as a surprise that we identified additional E3 ligases interacting stably with short (1-4 amino acid) specific acetylated or non-acetylated N-terminal sequences. We identified three additional E3 ligases (CUKL3-KLHL13/22, CUL4-DDB1-DCAF10, SKP1-MYCBP2-FBXO4) as potential new N-recognins and confirmed two E3 ligases with an established role (CLU2-ZYG11 and GID/CLTH complex) validating the feasibility of our approach. Our hypothesis is that many more E3 ligases act in N-degron pathways than hitherto believed and improperly modified or unexpectedly unmodified proteins become rapidly removed through Ub-conjugation after synthesis.

Key objectives and overview work plan: This project aims to unravel the identity and function of N-degron pathways. We will reach our goal through two objectives:

Objective 1: Identification of N-recognins recognizing N-degrons in a medium-throughput manner including all 20 aa by using peptide pull-downs combined with quantitative mass spectrometry (MS).

- Performance of peptide pull-downs and preparation of the samples for MS.
- MS measurements.
- Data analysis and selection of candidates.

Objective 2: Validation and characterization of selected (e.g. relevant for human cancers) N-recognin candidates/ new pathways identified in objective 1 in normal and cancer cell lines by using biochemical and cellular assays.

- Confirmation of a direct interaction between N-degron and N-recognin by *in vitro* binding experiments.
- Test candidates for E3 ligase-mediated ubiquitination of N-degron containing proteins using *in vitro* ubiquitination experiments and cellular assays.

- Characterization of N-degron pathways in normal and cancer cell lines (choice of cell lines will depend on role and knowledge available about the selected E3 ligase) using overexpression and RNAi/ CRISPR-Cas9 techniques.

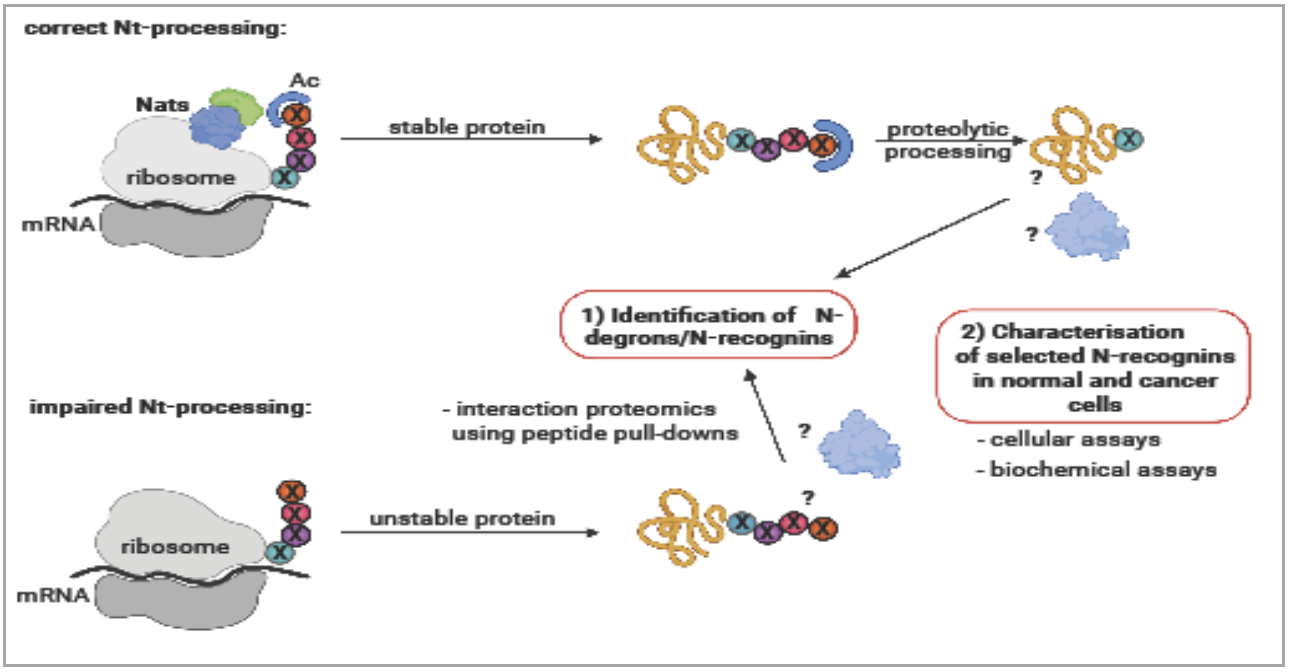

Figure 1. Graphical scheme of proposed activities. Nata are N-terminal acetyltransferases. Ac is N-terminal acetylation (presented as a protective cap on the N-terminus of proteins). X represents any amino acid of interest.

Risk assessment and impact of the project: N-degron pathways and Nt-acetylation are basic biological concepts studied already for decades but nevertheless there are still many unexplored aspects. A systematic screening for N-recognins and N-degrons to establish the pervasiveness of Nt-degradation signals and their roles has not been done so far. The workflow has been already applied successfully and is set up in the lab. We have several possibilities to adopt pull-downs in case the first round will not lead to a satisfactory number of E3 ligase candidates. Thus, I expect that objective 1 will lead to a comprehensive list of potential candidates for N-degron pathways in a short period of time opening up several lines of research. Within this proposal, I consider it reasonable for a PhD student to be able to characterize (objective 2) two or three candidate E3 ligases and N-degron pathways depending on the complexity of the pathways involved. In sum, the objectives are highly promising to be successful in identifying unknown N-degron pathways within three years. The results will be novel and of high impact for various fields of basic research (Nt- processing, Nt-acetylation, protein quality control and stability, assignment of new roles to E3 ligases) and publishable. Additional, it might give new insights for unknown human pathological mechanisms regarding these two E3 ligases.

Bibliography

1. A. Varshavsky, N-degron and C-degron pathways of protein degradation. Proc Natl Acad Sci U S A 116, 358-366 2019).
2. D. Popovic, D. Vucic, I. Dikic, Ubiquitination in disease pathogenesis and treatment. Nat Med 20, 1242-1253 (2014).
3. A. Hershko, A. Ciechanover, The ubiquitin system. Annu Rev Biochem 67, 425-479 (1998).
4. T. Ravid, M. Hochstrasser, Diversity of degradation signals in the ubiquitin-proteasome system. Nat Rev Mol Cell Biol 9, 679-690 (2008).
5. F. Mueller et al., Overlap of NatA and IAP substrates implicates N-terminal acetylation in protein stabilization. Sci Adv 7, (2021).

Project time plan:

➢ Full Doctoral Study-Model: 36 or 48 months

This project is intended to run for 36 months to fulfill the described objectives.

The project time line is given in month.

Objective	6	12	18	24	30	36
Objective 1						
○ Performing peptide pull-downs ○ MS measurement ○ Data analysis and selection of candidates						
Objective 2						
○ Confirmation of a direct interaction between N-degron and N-recognin by in vitro binding experiments.						
○ Test candidates for E3 ligase-mediated ubiquitination of N-degron containing proteins using *in vitro* ubiquitination experiments and cellular assays.						
○ Characterization of N-degron pathways in normal and cancer cell lines.						
Objective 3						
○ Wrap up results, write publication and doctoral thesis						

Language requirements:

Very good English skills, both written and spoken.

Academic requirements:

The applicant requires a master´s degree (or equivalent) in one of the following areas: biology, biochemistry, biotechnology, biomedicine or related life sciences.

The project includes wet lab work (mammalian cell culture, biochemistry), mass-spectrometry-based proteomics and bioinformatics/data analysis. The optimal applicant has first experiences in one of the three areas and enthusiasm/interest to learn the other two.

To applicants: Please send following initial application documents to LMU-CSC Office before 15th December:

➢ Resume and Research Motivation Letter

➢ Certificate of Proficiency in English, equivalent to IELTS Test Academic 6.5 (no module below 6) or TOEFL IBT 95, is required

➢ Two letters of recommendation directly sent from your current Supervisors/ Professors to LMU-CSC Office

Contact LMU-CSC Office: csc.international@lmu