Photo by Juan Gomez on Unsplash

Crosstalk between Ub family members under stress conditions

UBICODE & UBIRED TRAINING SESSION

MARCH 11-15, 2019

CENTRO DE INVESTIGACIÓN PRÍNCIPE FELIPE - VALENCIA, ES

GENERAL INFORMATION

Protein post-translational modification by ubiquitin (Ub) and Ubquitin-like proteins (UBls) is versatile, highly dynamic and involved in nearly all aspects of biological functions in eukaryotes. The reversibility and heterogeneity of Ub and UbL chains attached to protein substrates have complicated their isolation, quantification and characterization. Most approaches used to identify and characterise modified proteins are based in tagged versions of Ub and UbLs. Strategies have emerged to isolate endogenous ubiquitylated targets, including molecular traps based on the use of Ub-binding peptides, such as TUBEs (Tandem-repeated Ubiquitin-Binding Entities), peptide aptamers and affimers. Altogether these tools will contribute to unravel the secrets of the Ub-code in cell physiology and pathology. Knowing the benefits and limits of each of these approaches will give a better picture of the available technologies and help our fellows to choose the most appropriate tools to determine the modification of substrates with Ub/UbLs.

CONTENT

In this course we will use some of these tools and technologies to learn about their specificity, affinity and their applications. The capacity to capture and identify distinct chains with these tools will be analysed and compared. Strategies include His6 purification, pan or chain-selective molecular traps (to capture ubiquitin, SUMO and NEDD8) and chain specific nanobodies and/or minibodies.

TECHNOLOGY

Modified proteins will be captured using affinity chromatography. Captured material will be analysed by Western blot. Affinities of distinct tools for specific chains will be tested by thermophoresis (Nanotemper).

OBJECTIVES

To train students on techniques for the detection of total and individual proteins simultaneously modified by Ub and UbLs.

TRAINERS

Dimitris Xirodimas (CNRS-UbiCODE), Manuel S. Rodriguez (CNRS-UbiCODE), Laurie Bonnafe (CNRS), Rosa Farras (CIPF), Pierre Soule (Nanotemper)

PROGRAMME

MONDAY 11/03

Morning
Theory

Introduction to Ub/UbLs signalling - Cross-talk between Ub/UbLs.

Afternoon
Theory

Study of the response of Ub/UbLs to proteotoxic stress (e.g. heat shock, proteasome inhibitors);

Methods to isolate and identify Ub/UbLs substrates;

Detection of hybrid Ub/UbL chains;

Analysis of interactions Ub/UbL and specific receptors.

Late-afternoon
Practice

Capture of proteins modified with hybrid Ub/UbLs chains using His6-taggs, Ub, SUMO and NEDD8 traps.

WELCOME DINNER

TUESDAY 12/03

Morning
Practice

Gel electrophoresis for Western-blot analysis.

Afternoon
Theory

Theory of the analysis of determining affinities of Ub, NEDD8, SUMO receptors using various techniques.

WEDNESDAY 13/03

Morning
Practice

Western-blot analysis with various antibodies.

Afternoon
Theory

Analysis of Western blot results;

MicroScale Thermophoresis (MST) technology theory (Nanotemper).

THURSDAY 14/03

Morning
Practice

MST experiments including affinities of SUMO-traps, NEDD8 traps, Ubiquitin traps p62 based (tested for K63 and K48) and Nanobodies recognizing ubiquitin K63 and K48 chains.

Afternoon
Theory

MST data collection and analysis.

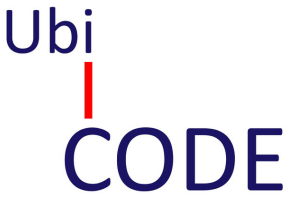
FRIDAY 15/03

Morning
Theory

Data analysis and interpretation;

Final discussion.

PARTNER ORGANISATIONS



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