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), Frank Bringold (Nanotemper), Enrique Garcia Gomez (GE Healthcare)

PROGRAMME

MONDAY 11/03

Morning *Theory*	9:00	Welcome and tour de table
	9:15	Objectives of the course
	9:30	Introduction to Ub/UbLs signalling (*Manuel Rodriguez*)
	11:00	**COFFEE BREAK offered by Werfen**
	11:30	Study of the response of Ub/UbL to proteotoxic stress (e.g. proteasome inhibitors) (*Dimitris Xirodimas*)
	12:30	How to combine different chromatographic techniques in the purification of proteins (*Enrique Garcia Gomez - GE Healthcare*)
	13:30	**LUNCH**
Afternoon *Theory*	15:00	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 (*Manuel Rodriguez, Dimitris Xirodimas*)

MONDAY 11/03

Late-afternoon *Practice*	16:00	Cell lysis and capture of proteins modified with hybrid Ub/UbLs chains using His-6 tags and binding entities to capture Ub, SUMO and NEDD8
	18:30	**FREE EVENING**

TUESDAY 12/03

Morning *Practice*	9:00	Columns wash and samples elution
	10:30	Gel electrophoresis
	11:30	Gel transfer
	13:25	Gel blocking
	13:30	**LUNCH**
Afternoon *Practice*	15:00	Preparation of antibodies *Overnight antibody incubation*
Afternoon *Theory*	16:00	Short presentation of students' individual projects (5 min.)
	17:00	**COFFEE BREAK**
	17:30	Analysis of the affinities of Ub or SUMO chains for binding peptides/proteins using various techniques
	18:30	**FREE EVENING**

WEDNESDAY 13/03

Morning *Practice*	9:00	Membrane washing and 2nd antibody incubation
	10:30	Development
	12:00	Data collection and interpretation
	13:30	**LUNCH**
Afternoon *Theory*	15:00	Microscale Thermophoresis (MST) part I (*Frank Bringold, Pierre Soule*)
	16:00	**COFFEE BREAK**
	16:30	MST part II
	18:30	**FREE EVENING**

THURSDAY 14/03

Morning *Practice*	9:00	MST experiments including affinities of binding entities for Ubiquitin, SUMO, or nanobodies for K48 and K63 chains
	13:00	**LUNCH**
Afternoon	15:00	MST data collection and analysis.
	18:30	**FREE TIME**

20:30 - FAREWELL DINNER

FRIDAY 15/03

Morning	9:00	Data analysis and interpretation
	13:00	**LUNCH**
Afternoon	15:00	Final discussion

17:00 - SCHEDULED END OF THE COURSE

PRACTICAL INFORMATION

Venue - Centro de Investigación Príncipe Felipe (CIPF)

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