UbiCODE Individual Projects

Ubi CODE

European Research Training to decipher the ubiquitin code
Innovative Training Networks (ITN)
Marie Skłodowska-Curie Action
GA 765445 – H2020-MSCA-ITN-2017

All projects will last 36 months, starting from September 2018, 1st.

Fellow ESR1a[1]

Title of the Project: Role of protein Ubiquitylation in the Proteasome-Autophagy Crosstalk
Host institution: Centre National de la Recherche Scientifique – Institute of Advanced Technologies for life Sciences (CNRS - ITAV), Toulouse, **France**.
Supervisor: Manuel S Rodriguez
Webpage: http://www.itav.fr/en/portfolio_page/ubicare-2/
Summary of the project: Multiple aspects regarding the biological relevance of distinct Ub chains and their role in the connection of between the Proteasome and Autophagy pathways remain unexplored. To address these questions our group has developed molecular traps (Tandem Ub Binding Entities or TUBEs) to capture and identify proteins modified by members of the Ub family. Here we propose the use of new molecular traps based in UBD from proteins implicated in autophagy (e.g. p62): i) Ubiquitylated proteins will be identified using a TUBEs-MS approach; ii) To study their specificity, captured proteins will be compared to those proteins captured with distinct Ub traps; iii) Ubiquitylated proteins-UDB interactions will also be studied using small inhibitor molecules; iv) Role of identified factors in UPS-Autophagy crosstalk under proteotoxic stress conditions will be analysed.

Fellow ESR1b

Title of the Project: Decoding the NEDD8 signal.
Host institution: Centre National de la Recherche Scientifique - Montpellier Cell Biology Research Center (CNRS - CRBM) Montpellier, **France**.
Supervisor: Dimitris Xirodimas
Webpage: http://www.crbm.cnrs.fr/en/team/ubiquitin-and-ubiquitin-like-molecules-in-cellular-stress-responses/
Summary of the project: We have recently uncovered a diverse role of protein NEDDylation in the response to genotoxic and proteotoxic stress, which is independent of the established role for NEDD8 in CRL regulation. The NEDD8 response to the above-mentioned stimuli strictly depends on the formation of either poly-NEDD8 and mixed hybrid NEDD8-Ub chains respectively. Key objectives of the project are to dissect the molecular factors that control the formation of such signals and the mechanisms of recognition and processing either through the de-conjugation or proteolytic machineries (proteasome/autophagy pathways). Our goal is to determine the role of poly-NEDD8 and NEDD8-Ub hybrid chains as potential novel molecular signals for the cellular response to stress.

[1] Early Stage Researcher (ESR)

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Fellow ESR2a

Title of the Project: Biological role and function of the ZNF451 family of SUMO E3 ligases in SUMO chain formation.
Host institution: Max Planck Institute of Immunobiology and Epigenetics (MPG), Freiburg, **Germany**.
Supervisor: Andrea Pichler
Webpage: https://www.ie-freiburg.mpg.de/pichler
Summary of the project: We identified the ZNF451 family as enzymes with SUMO2/3 paralog specific E3 ligase and E4 SUMO-chain-elongase activity that represents a model for novel SUMO conjugating enzymes. Our initial biological data indicate that the ZNF451 family functions upon stress stimulation like proteasome inhibition with MG153 or DNA damage with camptothecin (CPT), both drugs associated with SUMO chain formation. By using the SUBE and/or a combination of SUBE/TUBE pull down systems developed by the Rodriguez lab, we aim to identify ZNF451 dependent substrates specifically modified with SUMO2/3 chains and SUMO/Ub mixed chains upon stress stimulation. Identified substrates will be analysed in in vitro SUMOylation assays and diverse cell based assays by studying ZNF451 knock out cell lines and knock out cells from mice. In parallel we will design (UbiQ) and test ZNF451 family inhibitory drugs which will be analysed in comparison to ZNF451 knock out cell lines and mice.

Fellow ESR3a

Title of the Project: Investigation of crosstalk between SUMOylation and Ubiquitylation via novel chimeric SUMO-Ub chains.
Host institution: Leiden University Medical Center (LUMC), Leiden, **Netherlands**.
Supervisor: Alfred Vertegaal
Webpage: https://www.lumc.nl/org/moleculaire-celbiologie/medewerkers/AlfredVertegaal?setlanguage=English&setcountry=en#introductie
Summary of the project: Recently, we have identified unexpected chimeric chain formation between SUMO-2 and Ub. We found that Ub can be SUMOylated at lysines 6, 11, 27, 48 and 63. The functions of these chimeric SUMO-Ub chains are currently unknown. To shed light on these chimeric chains, we aim to identify receptors with a preference for these chimeric chains. To this end, biotinylated chimeric chains will be generated, incubated with cell lysates, purified and binding proteins will be identified using our Q Exactive Orbitrap. These receptors are expected to have closely spaced SUMO Interaction Motifs and Ub binding domains to enable preferential binding to the chimeric SUMO-Ub chains. Mutagenesis studies will be performed to study the functional relevance of these binding domains for the identified receptors.

Fellow ESR3b

Title of the Project: Expanding chemical technologies towards SUMO and NEDD8-based reagents. This project relates to all WPs, allowing the generation of a broadly applicable scale of reagents.
Host institution: Leiden University Medical Center (LUMC), Leiden, **Netherlands**.
Supervisor: Huib Ovaa
Webpage: http://www.ovaalab.nl/index.html
Summary of the project: The Ub/UbL code is translated into biochemical action through interaction with specific binding domains. These domains specifically bind to Ub or UbLs that can be present in larger chains. In order to interfere with chain signalling, modulation of these interactions is an interesting but so far unexplored option. It is important to have good methods available that help determine where and when such domains interact. Currently we can efficiently generate a board scale of Ub-based research reagents based on chemical synthesis. This project aims to translate synthetic methods that allow the generation of Ub-based reagents, probes and chains towards chemical methods to do the same for UbL proteins, starting with SUMO-1, -2 and -3 as well as NEDD8. The resulting UbL reagents will then be used in collaboration with other members of the network to study Ub, SUMO and NEDD8 biology.

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Fellow ESR4

Title of the Project: Molecular basis of thalidomide action.
Host institution: Friedrich Miescher Institute for Biomedical Research (FMI), Basel, **Switzerland**.
Supervisor: Nicolas Thoma
Webpage: http://www.fmi.ch/research/groupleader/?group=118
Summary of the project: Thalidomide (Contergan) was introduced to the European market in 1957 as a sedative. Unfortunately it took more than two years before its teratogenicity was discovered. Thalidomide and its second-generation derivatives (together known as IMiDs) have since been recognized as potent anticancer drugs, and are now widely used in treating multiple myeloma and 5q-dysplasia. The primary cellular target of thalidomide is CRBN (CeRClon) as integrated into the CRL4CRBN complex, an E3 Ub ligase. IMiDs prevent endogenous substrate, such as the transcription factor MEIS2 from binding, and also induce degradation of neo-substrates such as IKZF1/2 and Ck1a. The underlying molecular mechanism is unknown. We will use MS based half-life measurement to identify novel endogenous and neo-substrates, and resolve their mode of binding to CRL4CRB by X-ray crystallography and cryo-EM, as a model for E3 ligases regulation.

Fellow ESR5

Title of the Project: Dynamic Ub modification regulated and interpreted by endosomal sorting complexes.
Host institution: University of Liverpool (UNILIV), Liverpool, **United Kingdom**.
Supervisor: Sylvie Urbé
Webpage: http://pcwww.liv.ac.uk/~urbe/
Summary of the project: We seek to understand the modes of Ub chain recognition and editing associated with endo-lysosomal degradative pathways. The ESCRT-0 complex (HRS and STAM) engages ubiquitylated growth factor receptors for lysosomal sorting via five Ub binding domains, which combine to provide a unique topology for Ub chain interactions. We propose to leverage stable cell lines we either have already made or will generate to identify ubiquitylated proteins interacting with GFP or APEX-tagged HRS/STAM and profile the preferred chain types that associate with the ESCRT-0 complex ± growth factors. We will also immunoisolate intact endosomes or use APEX mediated biotinylation to profile the endosomal ubiquitylation landscape. Finally we will examine the influence of endosome associated de-ubiquitylases (DUBs) with differing chain specificities for their influence upon ESCRT capture of receptors, ESCRT-0 associated chain types and the endosome associated Ub profile.

Fellow ESR6

Title of the Project: Development and application of novel tools to detect protein interactions in vivo.
Host institution: Center of Cooperative Research in Biosciences (CIC bioGUNE), Bilbao, **Spain**.
Supervisor: Rosa Barrio
Webpage: http://personal.cicbiogune.es/rbarrio/
Summary of the project: As the gatekeepers of protein homeostasis, Ub and SUMO are implicated in almost every cellular pathway described, and it is no surprise that the complexity of their regulation seems to be increasing weekly in the literature. The fact that Ub can make chain-linkages through every one of its lysines raises questions about the lysines of all other UbL proteins. Mixed-chains are seemingly less abundant, but this may be due to lack of good tools to capture and analyse them. We hypothesize that Ub-SUMO mixed chains have important homeostatic and stress-responsive roles, and that novel regulators oversee their formation and destruction. We will design split-BirA for recognising Ub-SUMO mixed chains and to identify the proteins modified by these mixed chains by MS. The application of split-BirA to this problem will hopefully confirm this hypothesis, and also be applicable to other protein complexes in the future.

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Fellow ESR7

Title of the project: Understanding the complexity of polyUb signaling in mitophagy and proteostasis.
Host institution: MRC Protein Phosphorylation and Ubiquitylation Unit, University of Dundee, Dundee, **United Kingdom**.
Supervisor: Yogesh Kulathu
Webpage: https://www.ppu.mrc.ac.uk/research/principal-investigator/yogesh-kulathu
Summary of the project: Polyubiquitin chains have important roles in targeting proteins for degradation and also have important signalling roles in processes such as mitophagy. Different types of polyubiquitin signals are assembled in different settings and produce distinct outcomes. Recent research has revealed that the ubiquitin signal is more complicated than previously anticipated with polyubiquitin chains of complex architectures being used. It is therefore an immense task for the cellular machinery to tell the difference between all the different types of polyubiquitin modifications, and the underlying mechanisms are not well understood. The goal of this project is to address how ubiquitin signals of complex topologies are assembled and decoded to produce distinct outcomes. A second aim is to define the topology of ubiquitin signals of complex architectures using recently developed tools and methods to study polyubiquitin. By employing a combination of biochemical, proteomic and genetic approaches, this research will reveal fundamental insights not only into ubiquitin signalling, but also how proteins and defective mitochondria are targeted for degradation.
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Fellow ESR8

Title of the Project: Linear chain ubiquitinome and its pharmacological targeting in inflammation and bacterial infection.
Host institution: Johann Wolfgang Goethe University Frankfurt am Main - Institute of Biochemistry II (GUF), Frankfurt, **Germany**.
Supervisor: Ivan Dikic
Webpage: https://www.biochem2.com/index.php/component/contact/contact/21-group-leaders/1-prof-ivan-dikic
Summary of the project: By applying our recently developed MS method we identified global ubiquitinome of pathogenic Salmonella. We further demonstrated that linear ubiquitylation is required for activation of NF-kB at the surface of bacteria. Therefore, the first objective of this proposal is to investigate now the global linear Ub changes during inflammation responses and bacterial infection using cell culture models and primary cells. The second objective is to perform detailed functional characterization of newly identified pathways mediated by linear ubiquitylation. The third objective is to develop activity-based probes and a robust high-throughput assay platform for pharmacological inhibition of linear Ub ligases.

Fellow ESR9

Title of the Project: Investigation of linkage-selective Ub chain assembly and Ub binding in a yeast Ub ligase and its human homologue.
Host institution: Institute of Molecular Biology Mainz (IMB), Mainz, **Germany**.
Supervisor: Helle Ulrich
Webpage: https://www.imb.de/research/ulrich/research/
Summary of the project: As a model for polyUb interactors in yeast we will use a poorly characterised protein, Etp1, whose C-terminus binds K63-linked chains with high preference. The protein also harbours a RING domain characteristic of Ub ligases, and an additional ZnF-UBP Ub-binding domain, which usually requires Ub's free C-terminus for interaction. Its human homologue, BRAP2, which shares this unusual domain arrangement, was initially isolated as a BRCA1 interactor and associates with two closely related de-ubiquitylation enzymes. Deregulation of BRAP2 has been linked to cancer and inflammatory disorders.

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However, the relevance of its Ub-interacting domains is completely unknown. In a combination of biochemical, cell biological and genetic approaches we will characterise the linkage selectivity of Etp1's and BRAP2's UBDs, elucidate their significance for the proteins' Ub ligase activities and gain insight into their biological functions.

Fellow ESR10

Title of the Project: Establishing the role of SUMO protease SENP6 in regulating SUMO chain length.
Host institution: University of Dundee (UNIVDUN), Dundee, **United Kingdom**.
Supervisor: Ron T. Hay
Webpage: http://www.lifesci.dundee.ac.uk/groups/ron_hay/
Summary of the project: Loss of function studies with essential genes such as SENP6 are limited to conditional knock out of the gene or mRNA depletion with siRNA. The drawback with both of these approaches is that substantial depletion of the gene product takes at least 48 hours and with a very stable protein even longer. To circumvent these problems we have recently employed AID technology in live worms to deplete SUMO E3 ligase GEI17 in less than 60 minutes. Our first objective is therefore to use CRISPR/cas9 technology to engineer cell lines expressing the auxin responsive plant protein TIR1 and endogenous SENP6 tagged with GFP-AID. Using these cell lines we will determine the critical phases of the cell cycle where SENP6 depletion has deleterious consequences for passage through mitosis. Similar cell lines also expressing 6His T90K SUMO2 will allow us to realise the objective of isolating proteins containing extended SUMO chains and mapping sites of SUMO modification and thus define physiological substrates of SENP6. The final objective is to determine the basis for selective cleavage of SUMO2 chains by SENP6. In collaboration with the lab of Huib Ovaa, this will be accomplished by chemically linking SENP6 to diSUMO and determining the structure of the complex by X-ray crystallography.

Fellow ESR11

Title of the Project: Peptide inhibitors of Ub ligases to treat diabetes and diabetic complications.
Host institution: Novo Nordisk A/S, Copenhagen, **Denmark**.
Supervisor: Thomas Eiland Nielsen
Webpage: http://orbit.dtu.dk/en/persons/thomas-eiland-nielsen(9466e89f-e3a3-4fbe-9e05-289851fa2e9f).html
Summary of the project: The essential proteins that regulate cell metabolism are controlled at the level of protein stability by ubiquitylation, and this regulation can therefore contribute to a wide range of diseases. The overall process of ubiquitylation thus provides attractive targets for drug development. In this project we specifically wish to explore the E3 Ub ligases, which direct substrate proteins to the Ub–proteasome system. These ligases are virtually unexplored as drug targets and fall into two main groups: the HECT domain proteins and RING domain proteins. We will design and synthesize peptide-based inhibitors of these proteins and evaluate their potential as new drugs for the treatment of diabetes and diabetic complications. Integrated microfluidic and bead-based technologies that integrate combinatorial library synthesis and screening represent key technologies to be developed to support the efforts.

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