UKF project presentation: Elucidation of the physiological roles of human dipeptidyl peptidase III

Mihaela Matovina

Ruđer Bošković Institute

UNITY THROUGH KNOWLEDGE FUND

- UKF – Unity through knowledge fund - 1B Crossing Borders Grant
  - Croatian Ministry of Science, Education and Sports funding aimed at developing cooperation between scientists in Croatia and Croatian scientists abroad
- Funding: 1,409,411.00 kn (around 180,000 euro)
- Duration: December 15 2015 to December 14 2017
- Project leader: Koraljka Husnjak, Institute of Biochemistry II, Goethe University School of Medicine, Frakfurt, Germany
- Project co-leader: Mihaela Matovina, Ruđer Bošković Institute, Zagreb
- Co-workers: Marija Abramić, Marija Kozlović, Sandra Sobočanec, Zrinka Karačić, postdoc (to be hired), Ruđer Bošković Institute, Zagreb

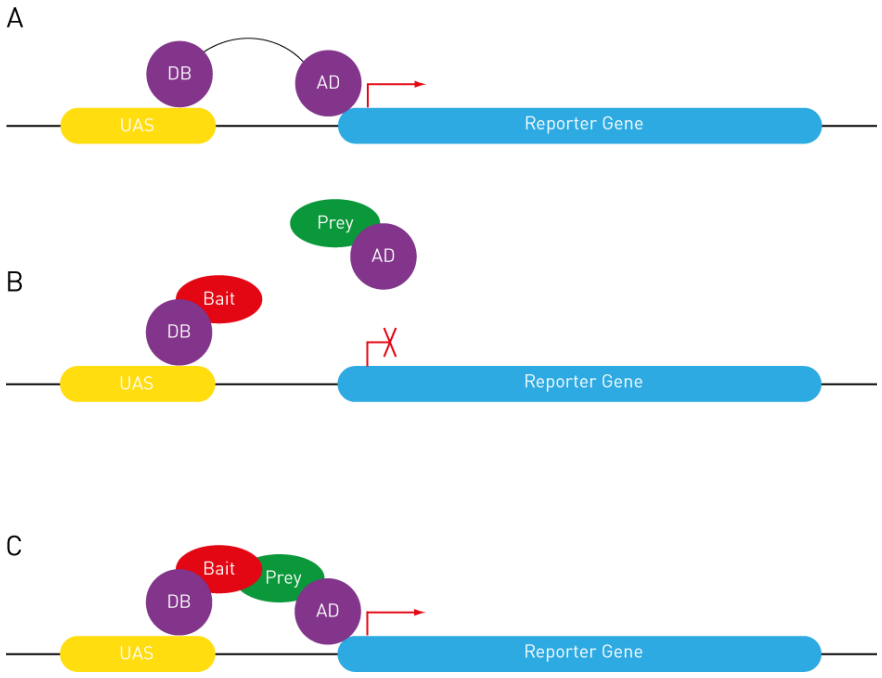
IBC II Institut für Biochemie II

Goethe-Universität Frankfurt am Main

- Koraljka Husnjak, Group leader
- Ubiquitin Signaling Group
- Selected publications:
  - Aguileta MA, Korac J, Durcan TM, Trempe JF, Haber M, Gehring K, Elsasser S, Waidmann O, Fon EA, Husnjak K**. The E3 Ubiquitin ligase parkin is recruited to the 26S proteasome via the proteasomal ubiquitin receptor Rpn13. **J Biol Chem** 2015; 290: 7492-505 (**corresponding author)
  - Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. Husnjak K, Dikic I. **Annu Rev Biochem** 2012; 81: 291-322.
  - Grabbe C, Husnjak K, Dikic I. The spatial and temporal organization of ubiquitin networks. **Nat Rev Mol Cell Biol** 2011, 12: 295-307.
  - Husnjak K*, Elsasser S*, Zhang N*, Chen X, Randles L, Shi Y, Hofmann KWalters K, Finley D, Dikic I (2008) Proteasome subunit Rpn13 is a novel ubiquitin receptor. **Nature** 2008; 453 (7194): 481-488 (*equally contributing).
  - Schreiner P*, Chen X*, Husnjak K*, Randles L, Zhang N, Elsasser S, Finley D, Dikic I, Walters K, Groll M. Ubiquitin docking at the proteasome via a novel PH domain interaction. **Nature** 2008; 453 (7194): 548-552 (*equally contributing).

Project summary

- **High-throughput methods to determine DPP III interactome**
  - Yeast-two-hybrid to determine direct interactors
    - DPP III as bait; normalized human cDNA library as prey



- SILAC-MS to determine DPP III protein complexes
  - Stable transfection of pcDNA4.HA-hDPP3, pcDNA4.HA-hDPP3-E451A, and control pcDNA4.HA in inducible eukaryotic cell lines – grown on heavy, medium, and light media, respectively
  - lysates merged in 1:1:1 ratio
  - HA affinity purification
  - eluates resolved on SDS-PAGE
  - MS analysis → MaxQuant software
    - FDR < 1 %
    - hits that are identified by the presence of at least two unique peptides
    - hits highly enriched (at least 2-fold) in DPP III interactome in comparison to empty plasmid control
    - datasets will be functionally analyzed by KEGG PATHWAY – to determinethe processes and pathways enriched in the obtained interactome

- Choose putative 5 interactors to do a more specific analysis – selection based on:
  - Y2H hits that were also deterimed as top MS-based interactome components
  - Hits that have been found in multiple copies in the Y2H screen
  - Proteins which participate in processes that have already been linked with DPP III functions
  - Proteins which participate predominantly in processes/pathway for which we have expertise as laboratories

- **Low-throughput analysis of the selected putative interactors:**
  - His and GST pulldowns from bacterial lysates
  - Co-immunoprecipitation from eukaryotic cell lysates
  - Colocalization and FRET analysis of DPP III and putative interactor by confocal microscopy
  - ITC studies with purified proteins


Thank you
for your
attention