Affinity Purification Mass Spectrometry and label-free quantitative Protein Correlation Profiling data analysis highlight a novel interaction between FAM192A and PA28γ, a regulated regulator of proteasome complexes.

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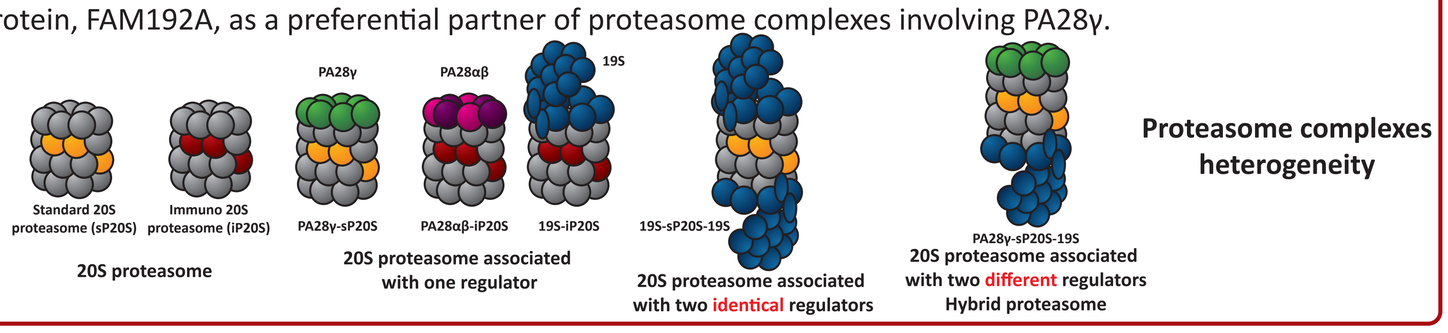
Introduction and objectives

The proteasome is a large protein complex involved in the degradation of intracellular proteins. It thus plays a crucial role in the regulation of many cellular processes. The proteasome displays a high heterogeneity in protein subunit composition: the various 20S core particles, containing 3 different catalytic activities, can be associated to one or two multimeric regulatory particles (RPs), resulting in a high diversity of different proteasome complexes (Figure) (1). One of these RPs, known as PA28γ, is overexpressed in many cancers and regulates cell proliferation and nuclear dynamics. It has been shown to control the degradation of key cell cycle regulators, to maintain a stable chromosomal segregation, and to be involved in DNA damage response.
In order to study the diversity of proteasome complexes in a large panel of human cell lines, we developed a strategy based on *in vivo* crosslinking, leading to stabilization of labile proteins interactions, coupled to proteasome complexes affinity purification, allowing the isolation of every proteasome complexes. (2,3) Quantitative mass spectrometry analysis and Protein Correlation Profiling analyses permit us to assign proteins belonging to the same proteasome complexes and to reveal new putative proteasome interacting proteins (PIP).
Through this strategy, we identified an uncharacterized protein, FAM192A, as a preferential partner of proteasome complexes involving PA28γ.

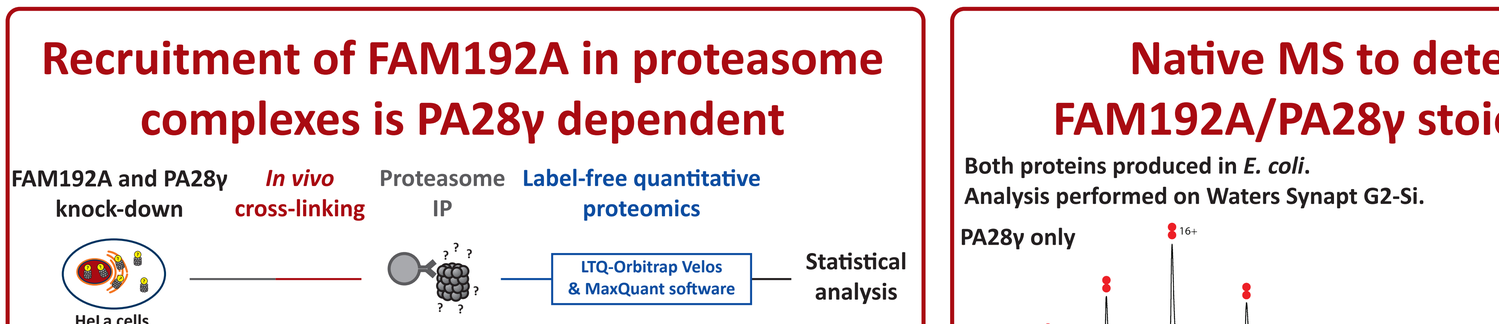
Objectives:

- **Caracterization of FAM192A and PA28γ interaction by mass spectrometry.**
- **Effect of FAM192A on PA28γ-dependent protein degradation.**



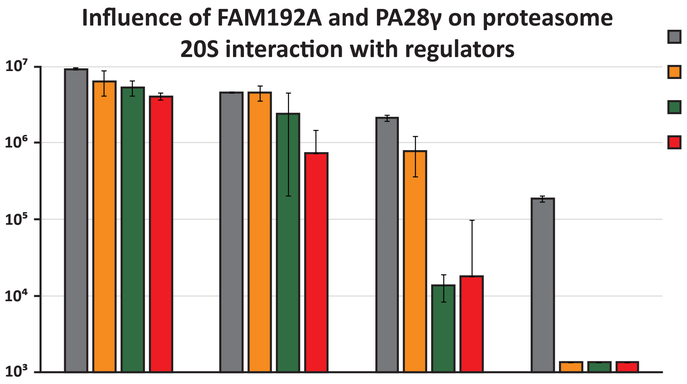
Protein Correlation Profiling (PCP) Mass Spectrometry on affinity purified proteasomes to identify new Proteasome Interacting Protein

Strategy:

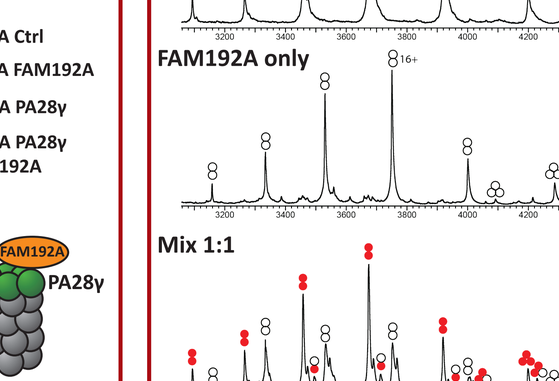


Results:

Correlation of protein abundances in proteasome IP in 9 cell lines

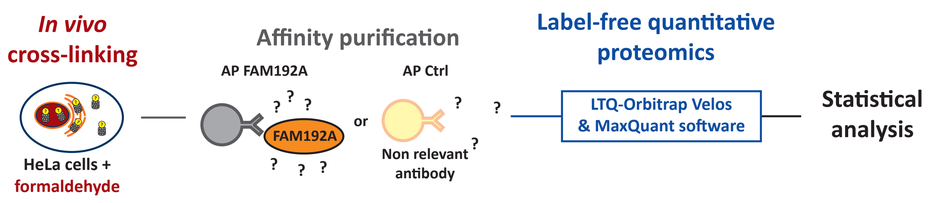


Correlation of protein abundances in proteasome IP from 3 compartments of U937 cells

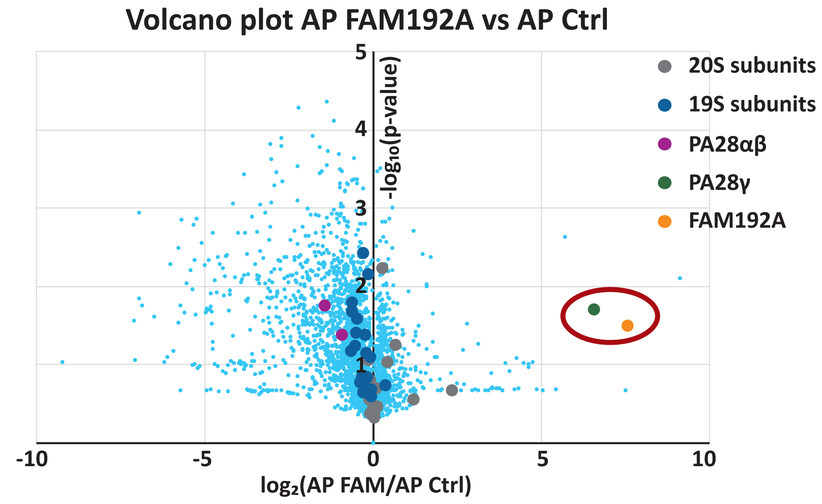


- **FAM192A preferentially interacts with PA28γ and 19S regulators in proteasome complexes.**
- **Cellular fractionation and PCP show the colocalization of FAM192A and PA28γ in the nucleus.**

Affinity Purification Mass Spectrometry to determine FAM192A partners



Volcano plot AP FAM192A vs AP Ctrl



- **PA28γ and FAM192A tightly interact together.**

Recruitment of FAM192A in proteasome complexes is PA28γ dependent

Influence of FAM192A and PA28γ on proteasome 20S interaction with regulators

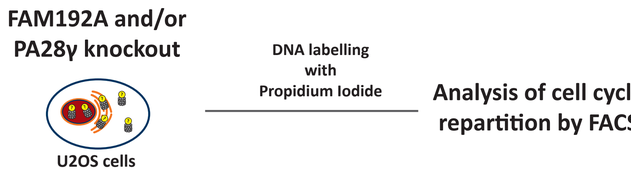
- **FAM192A can be found in proteasome complexes only if PA28γ interact with 20S proteasome.**

Native MS to determine FAM192A/PA28γ stoichiometry

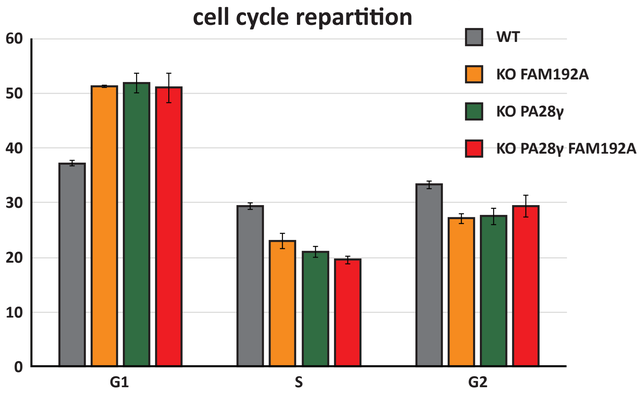
Both proteins produced in *E. coli*.
Analysis performed on Waters Synapt G2-Si.

- **FAM192A and PA28γ seem to interact *in vitro* and can form oligomeric complexes.**

PA28γ and FAM192A in cell cycle repartition



Influence of FAM192A and PA28γ on cell cycle repartition



- **FAM192A and PA28γ have an effect on cell cycle of U2OS cells.**

Conclusions and Perspectives

- **FAM192A colocalize with PA28γ in proteasome complexes across the different cellular compartments.**
- **FAM192A and PA28γ interact directly.**
- **PA28γ is required for FAM192A recruitment in proteasome complexes.**

- **The stoichiometry between these two proteins has to be further studied by optimizing Native MS conditions and mixing ratios.**
- **The potential effect of FAM192A and PA28γ on cell cycle regulators has to be further investigated (4).**

References

(1) **Finley D.** Annu Rev Biochem, 2009, 78:477.
(2) **Fabre *et al.*** J Proteome Res, 2014, 134:3027.
(3) **Fabre *et al.*** Mol Syst Biol. 2015, 11:771.
(4) **Chen *et al.*** Mol Cell. 2007, 26:843-52.