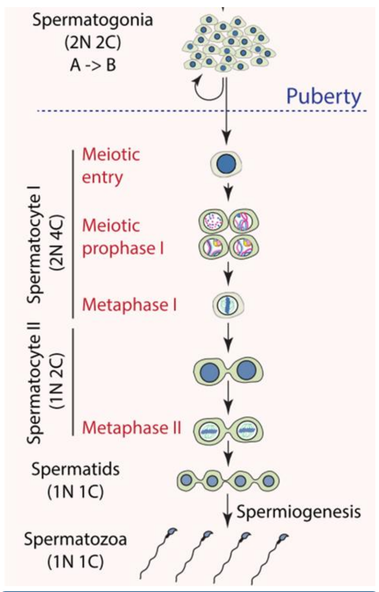
Proteasome complexes experience profound structural and functional rearrangements throughout mammalian spermatogenesis

Dušan Živković [1], Angelique Sanchez Dafun [1], Thomas Menneteau [1], Adrien Schahl [1], Sandrine Lise [1], Christine Kervarrec [2], Ana Toste-Rêgo [3], Paula da Fonseca [3], Matthieu Chavent [1], Charles Pineau [2], Burlet-Schiltz Odile[1] Marcoux Julien [1], Bousquet Marie-Pierre [1]

[1] Institute of Pharmacology and Structural Biology, CNRS UMR 5089, [2] Univ. Rennes, Inserm UMR_S1085, [3] MRC Lab. Of Molecular Biology Cambridge UK

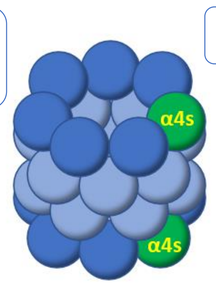
the article link

Spermatogenesis: a male gamete differentiation



- Very difficult system to study: not possible in vitro
- KOs infertile

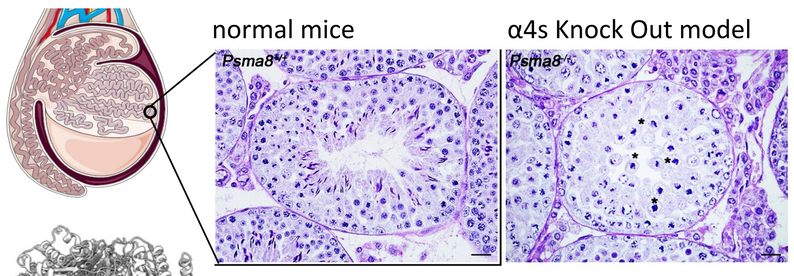
The spermatoproteasome

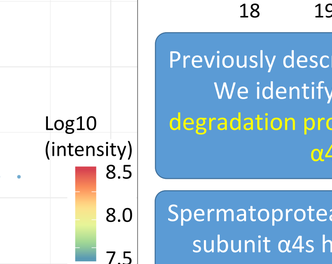


Exists in male and female gonads. Observed in germ cells entering meiosis. **α4s replaces α4 → spermatoproteasome** [1]

Crucial for spermatogenesis:

The KO germ cells stop differentiating at the metaphase I of the spermatocyte stage → **infertility** [2]:

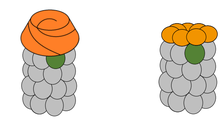




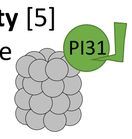
Differences between α4 and α4s (model in yellow) are focused in two C-terminal regions that face outwards (in red). To «grab» interactors??

The proteasome activators in spermatogenesis

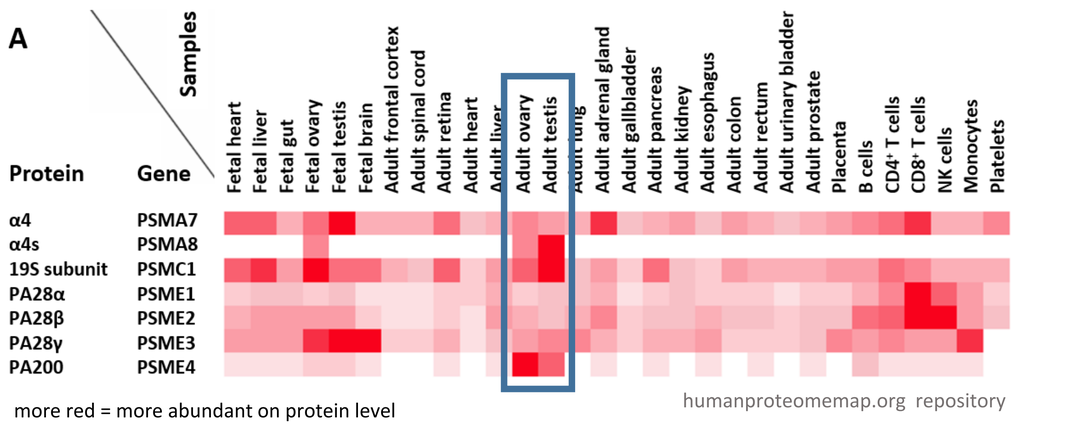
- PA200/PA28γ dKO models are infertile [3]
- **PA200 targets the acetylated histones** through bromodomain-like regions.
- PA200 helps in histone removal (in spermatids)! [4]



PI31 is an important proteasome partner, essencial for fertility [5]



- an adaptor for proteasome transport [6]
- activator, inhibitor
- Very context-dependant [7]

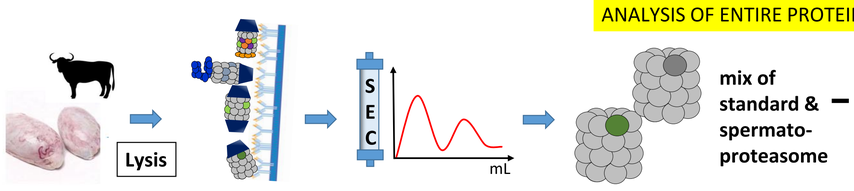


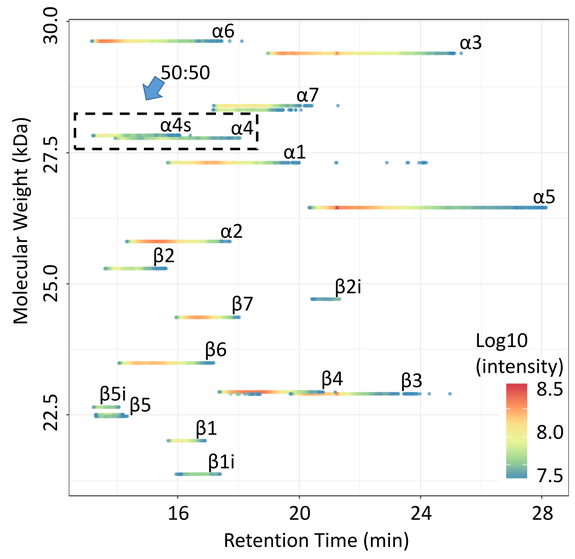
more red = more abundant on protein level

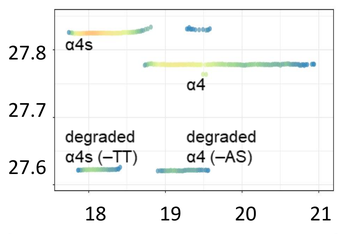
humanproteomemap.org repository

Proteoform study using Top-Down Proteomics (TDP)

ANALYSIS OF ENTIRE PROTEINS



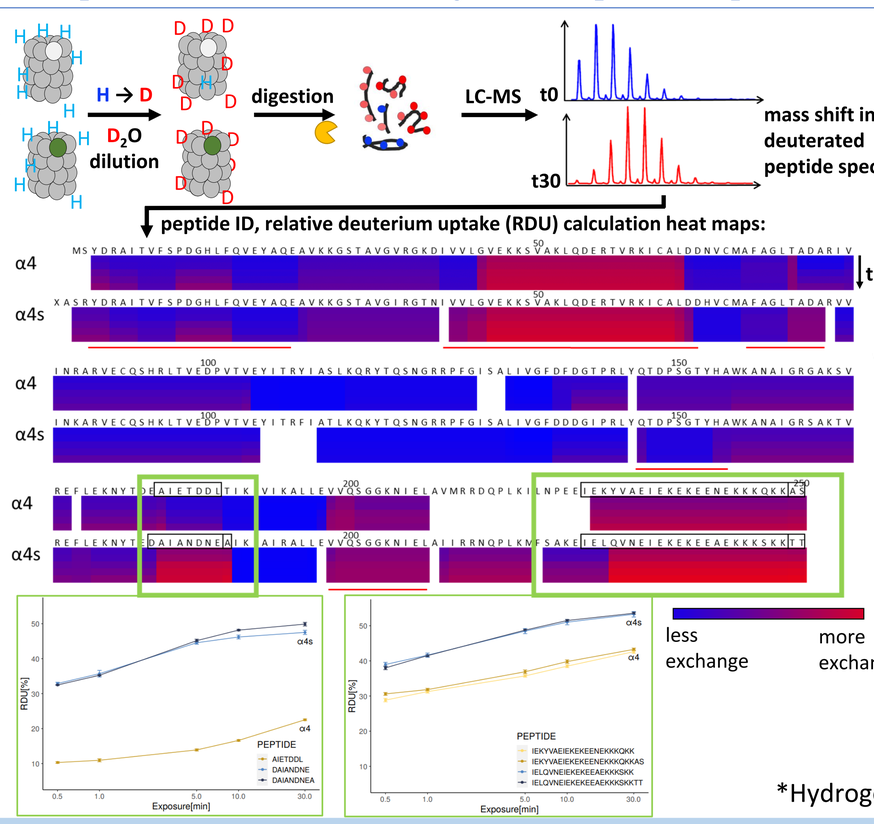




Previously described isoform - We identify as a C-ter degradation product of α4s or α4.

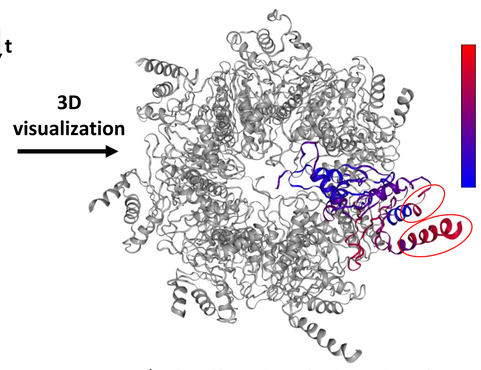
Spermatoproteasome-specific subunit α4s has the same PTMs as the α4: loss of Met & N-Acetyl

Comparative structural study of the spermatoproteasome using HDX-MS*



relative deuterium uptake is observed multiple timepoints are collected

HDX gives us information on:
- interaction surface
- domain flexibility
- conformational info

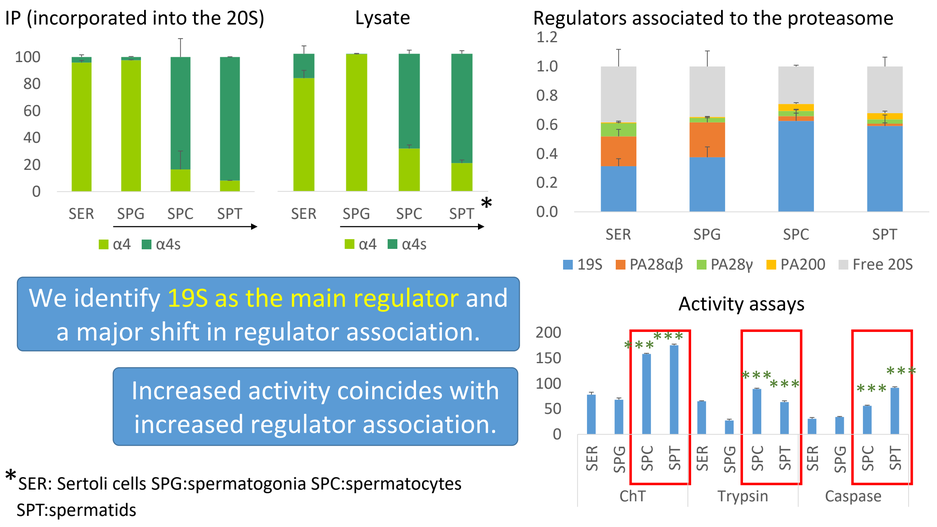


α4s deuteration at 5 minutes – top view

Much higher flexibility of two above-mentioned regions (helices) in α4s compared to α4!

*Hydrogen- Deuterium eXchange coupled to Mass Spectrometry

Dynamics of the complexes throughout spermatogenesis



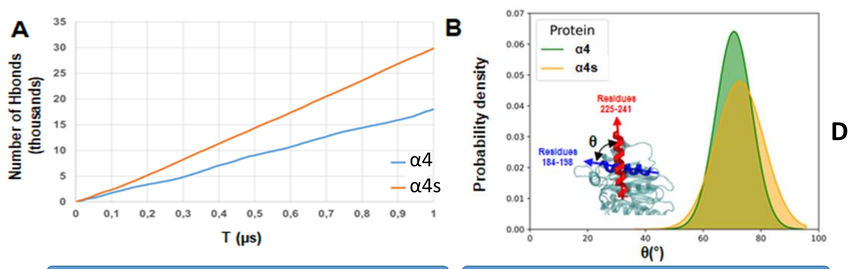
We identify 19S as the main regulator and a major shift in regulator association.

Increased activity coincides with increased regulator association.

*SER: Sertoli cells SPG:spermatogonia SPC:spermatocytes SPT:spermatids

Molecular dynamics to complement the HDX-MS

by Matthieu Chavent & Adrien Schahl



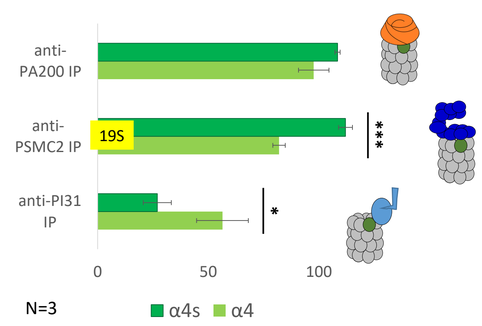
More H-bonds with water = more accessible structure of α4s.

Broader θ distribution = more dynamic structure of α4s.

Six AA residues participate in the stabilization of the two C-ter helices.

More H-bonds between sidechains = more stable structure of α4.

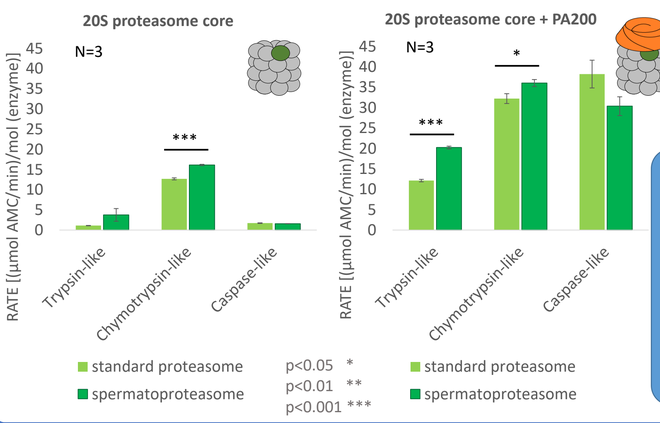
Pull Down Assay to measure relative affinity of activators



19S has higher affinity for spermatoproteasome = stronger activation?

PI31 has higher affinity for standard proteasome.

Proteasome Activity Assay



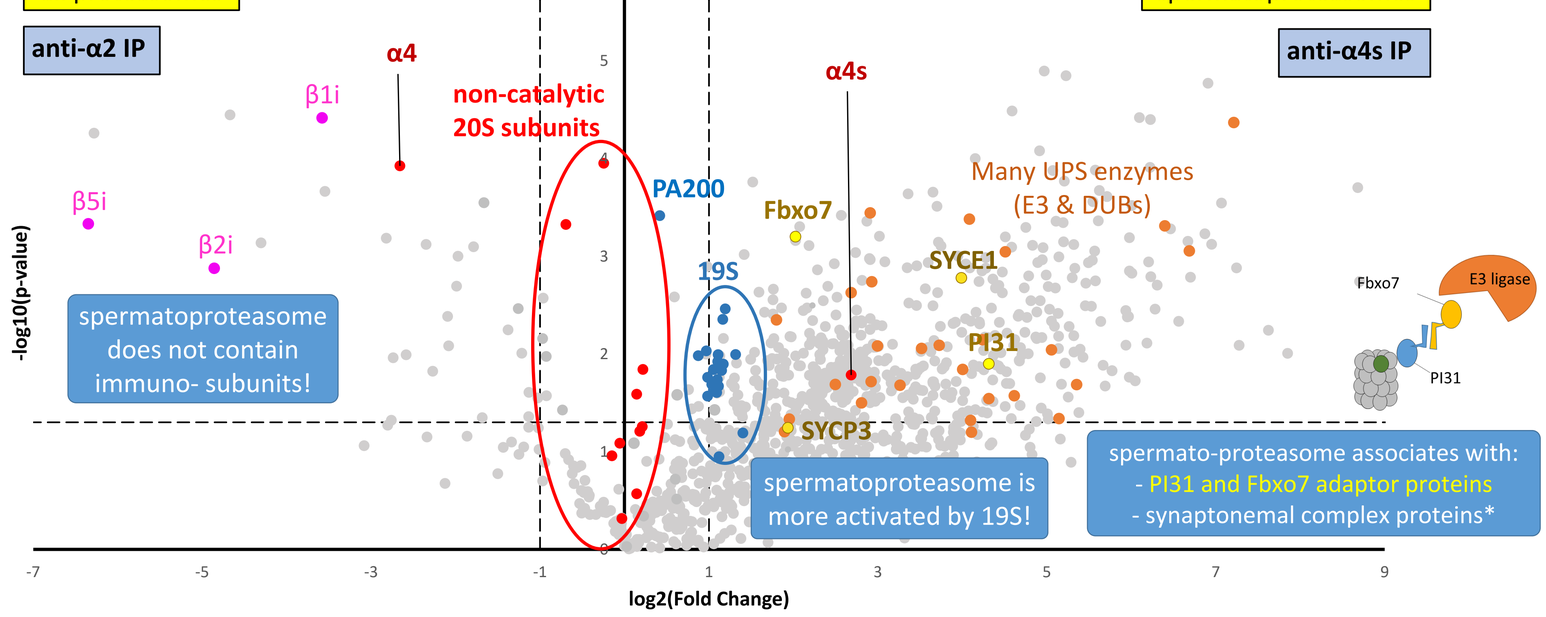
19S & PI31 activity assays are on their way..

Activated spermatoproteasome has much higher trypsin-like activity.

In order to better degrade histones?

Interactome study using Bottom-Up Proteomics

testis proteasome CoIP with **anti-α2 antibody (total proteasome) or anti-α4s antibody (spermatoproteasome only)** followed by bottom-up proteomics identification to obtain complementary datasets



spermatoproteasome does not contain immuno- subunits!

spermatoproteasome is more activated by 19S!

spermato-proteasome associates with:
- PI31 and Fbxo7 adaptor proteins
- synaptonemal complex proteins*

REFERENCES:
[1] [Uechi, H. *et al.*, 2014., *J. Biol. Chem.*;
[2] Gómez-H, L. *et al.*, 2019. PLOS Genetics;
[3] [Huang L. *et al.*, 2016., *Sci. Rep.*];
[4] Quian, L. *et al.*, 2019. *PLOS Genetics*;
[5] Rathje, C. C. *et al.*, 2019, *Frontiers in Physiology*;
[6] Liu, K. *et al.*, 2019, *Developmental Cell*;
[7] Nelson, D. E., *et al.*, 2013. *Open Biology.*

You need bioinformatic tools for your BU, TD proteomics or structural MS?
Visit us at: https://masstools.ipbs.fr/