In vivo cross-linking combined with Affinity-Purification Mass Spectrometry and label-free quantification to study human proteasome complexes: Subcellular distribution, composition, and 20S proteasome assembly

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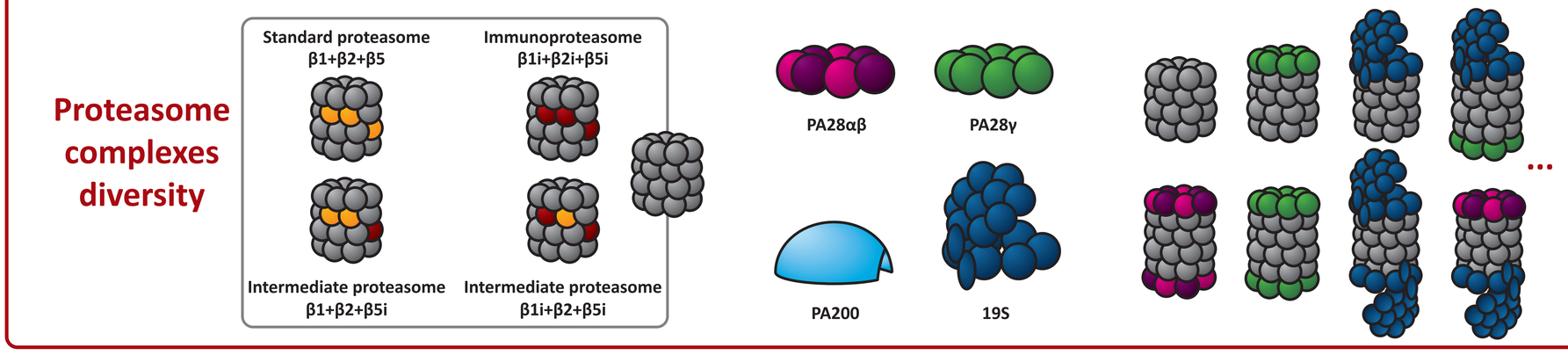
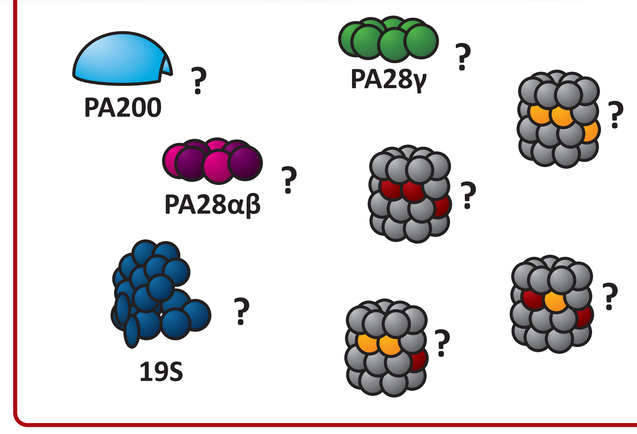
Introduction and objectives

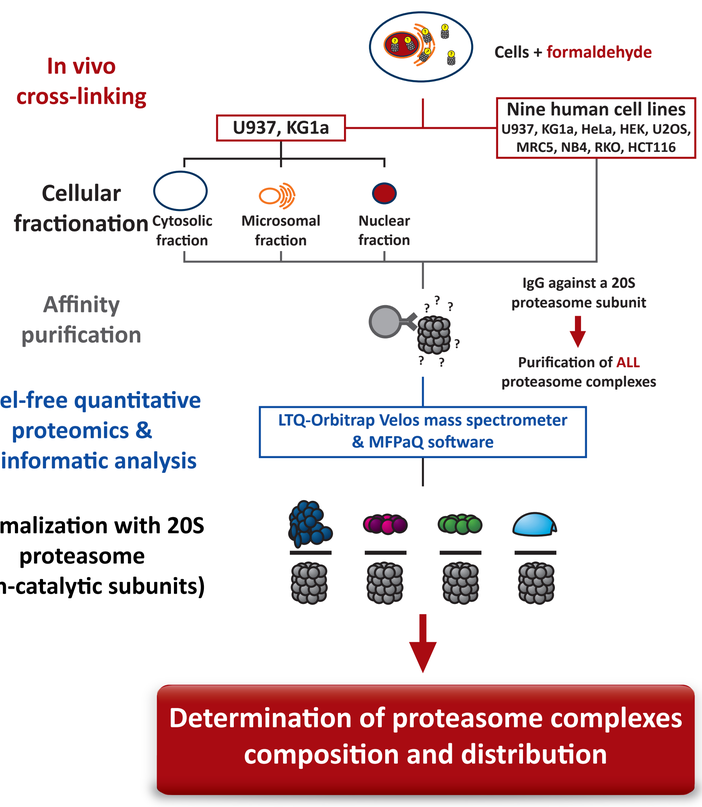
The proteasome is a large protein complex involved in the degradation of intracellular proteins. It thus plays a crucial role in the regulation of many cellular processes and in antigenic peptides presentation. The proteasome displays a high heterogeneity in protein subunit composition as it results from the dynamic association of several subcomplexes. A 20S core particle is composed of 14 different subunits organized to form a barrel-like structure of four stacked rings (α7β7β7α7) that contains the catalytic activity. It can be associated to one or two regulatory particles (RPs) of identical or different protein composition. In higher eukaryotes four subtypes of 20S complexes containing either standard catalytic subunits (β1, β2, and β5), immunosubunits (β1i, β2i, and β5i), or a mix of both subunit types, have been described, and four RPs are known (19S, PA28αβ, PA28γ, and PA200). Other proteins called proteasome interacting proteins (PIPs) can also regulate proteasome activity. Although the structure of proteasome complexes has been well characterized, the distribution of 20S proteasome subtypes, of RPs associated to the 20S proteasome core, and of PIPs remains to be further studied.

Objectives:
- **Determination of the composition of proteasome complexes at the subcellular level and in various human cell lines**
- **Determination of the fraction of 20S proteasome that are engaged in the assembly process**



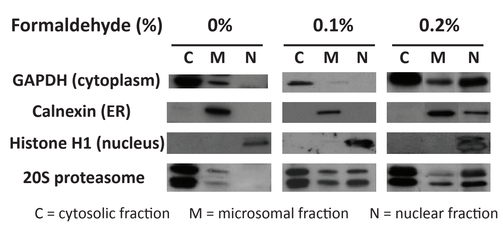


Strategy



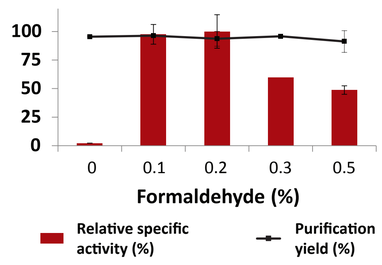
Subcellular distribution

Fractionation efficiency (Western blot)

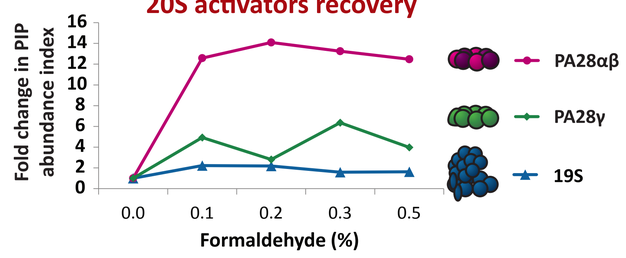


Efficient fractionation of cells cross-linked with 0.1% formaldehyde. Cross-linking avoids 20S proteasome leakage from the nucleus.

Proteasome specific activity and purification yield

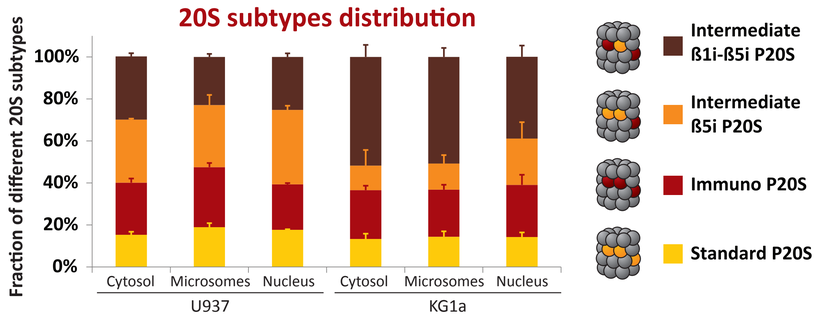


20S activators recovery

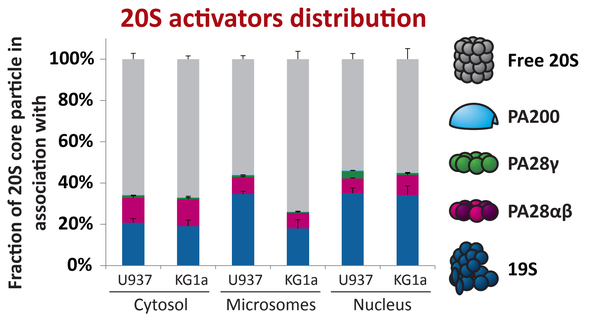


Cross-linking leads to different stabilization levels of 20S activators associated to the 20S complex, PA28αβ being the most affected.

20S subtypes distribution



20S activators distribution

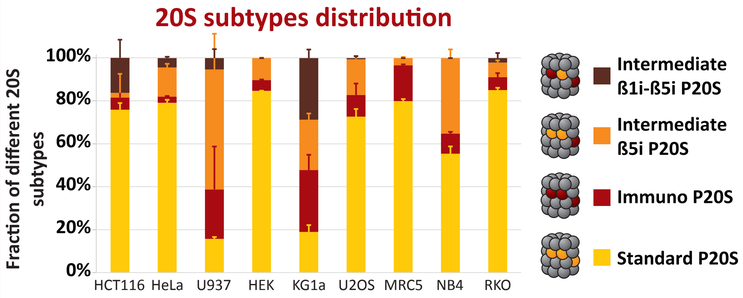


The cytosolic and microsomal fractions of U937 and KG1a cells have different proteasomal compositions. 20S Proteasome subtypes have a similar subcellular distribution in the two cell lines.

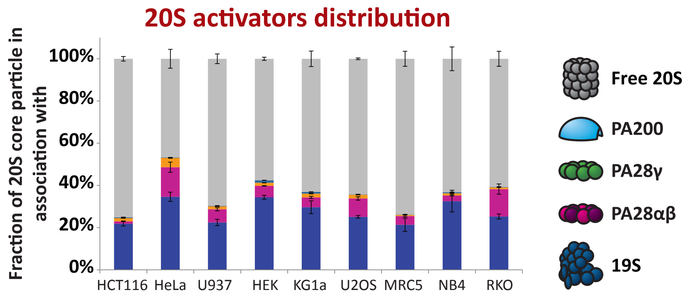
More than 50% of proteasome complexes correspond to free forms of 20S proteasome. The 19S regulatory particle is the most abundant activator bound to the 20S.

Proteasome composition and 20S stoichiometry in 9 human cell lines

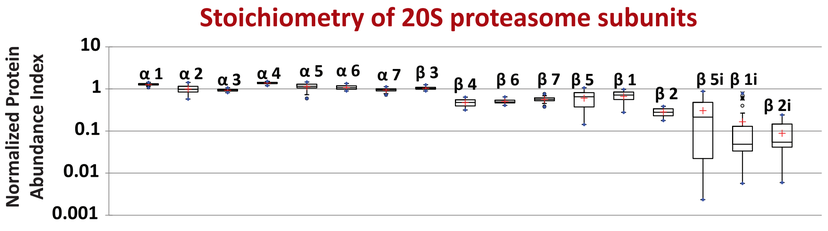
20S subtypes distribution



20S activators distribution

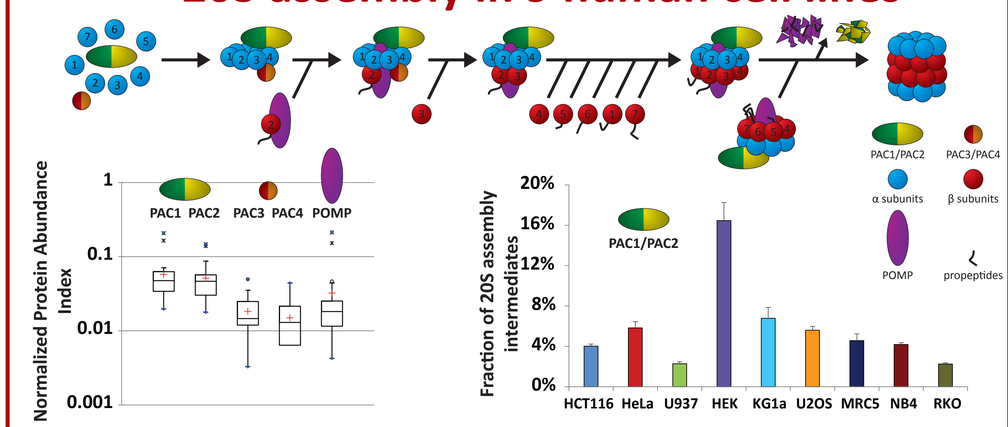


Stoichiometry of 20S proteasome subunits



Standard proteasome is the most abundant 20S subtype in most human cell lines studied and there is a high variability in the composition of the 20S catalytic subunits.
20S proteasome is mainly present as a free particle (≈64%). 19S is the major regulator associated with the 20S core particle.

20S assembly in 9 human cell lines



PAC1 and PAC2 as well as PAC3 and PAC4 show the same stoichiometry, as expected for dimers. PAC1/PAC2 proportion may be a good approximation of the fraction of 20S proteasome in the course of assembly because they are associated to all assembly intermediates. Significant variations in the proportions of PAC1/PAC2 are observed in the different cell lines suggesting variations in proteasome assembly intermediates across cell lines.

Conclusions

- **Subcellular distribution and composition of proteasome complexes determined using an integrated strategy including *in vivo* cross-linking, cell fractionation, proteasome immuno-purification, and robust label-free quantitative proteomics.**
- **First extensive study of proteasome complexes composition in nine different human cell lines.**
- **The 19S complex is the most abundant regulator bound to the catalytic 20S core particle.**
- **An important proportion (~65%) of 20S proteasome is found as a free particle in human cell lines.**
- **Different fractions of 20S proteasome in the course of assembly across nine human cell line.**

References

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