

Role of cystinosin in vesicular trafficking and membrane fusion

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Research project conducted at Inserm U983 (Necker Hospital, Paris)

Principal investigator : Corinne Antignac

Persons working on the project :

Zuzanna Andrzejewska (PhD student, funded by the Cystinosis Research Foundation)

Dr Véronique Chauvet (funded by the EU grant Eunefron)

Nathalie Nevo (technician, Inserm funded)

Background and objectives

The global aim of the research project is to characterize intracellular trafficking of cystinosin and to identify possible novel functions of cystinosin, especially in membrane fusion. The specific aims of the projects are:

- 1. To characterize how cystinosin is sorted to the lysosome**
- 2. To identify the possible cystinosin partners involved in vesicle fusion**

Update on the progress of research plan :

Previous research demonstrated that cystinosin, the lysosomal cystin transporter, is targeted to the late endosomes and lysosomes by two sorting signals, the classical tyrosine-based GYDQL lysosomal sorting motif in its C-terminal tail, and a novel conformational, localized to the 5th inter-TM loop, both of which are oriented toward the cytoplasm (Cherqui et al, 2001). We also showed that cells transiently or stably overexpressing a cystinosin-GFP fusion protein display striking aggregation of lysosomes into a few large juxtannuclear structures and a diminution of the usual pattern of small discrete intracytoplasmic vesicles characteristic of lysosomes. The number of these structures was drastically decreased when cystinosin C-terminal tail, its 5th inter-TM loop, or both motifs were altered. The enlarged lysosomes are reminiscent of what is observed in cells overexpressing hVam6p, a protein of the Vamp (Vesicle associated membrane protein) family, which has been identified as a mammalian tethering/docking factor with an intrinsic ability to promote lysosome fusion *in vivo*.

Altogether, this led to the hypothesis that cystinosin has important roles apart from cystine efflux, in particular that it may be involved in intracellular vesicular trafficking and lysosomal fusion, and that these effects might be mediated by its 5th inter-TM loop or the C-terminal tail.

Moreover, little is known about the way the multispinning transmembrane proteins, like cystinosin, are targeted to lysosomes. Four heterotetrameric adaptor protein complexes (AP-1 to AP-4) are involved in selection of cargo molecules in mammalian cells by their ability to recognize the sorting motifs (Robinson and Bonifacino, 2001). The tyrosin-based motif located at the C-terminal tail of cystinosin presents similarities to those contained by proteins interacting with various AP complex sub-units. The studies on lysosomal targeting of family of lysosome-associated membrane proteins (LAMPs) and CLN3 indicate the existence of different possible pathways by which proteins can be sorted to these organelles mediated by distinct AP complexes. The second part of the project will focus on the cystinosin trafficking to the lysosomes and the role of AP complexes in this process.

Characterization of cystinosin intracellular sorting :

1. In order to identify the AP complexes interacting with cystinosin, a direct two hybrid screen is being performed. The C-terminal cystinosin sequence (RKRPGYDQLN) was cloned into LexA plasmid to allow the expression of cystinosin tyrosine based motif fused to DNA binding domain of GAL4. The subunits of AP complexes: μ 1A (AP1), μ 2 (AP2), μ 3 (AP3), μ 4 (AP4), α (AP2) fused to activating domain of GAL4 transcription factor were kindly provided by J Bonifacino. The cystinosin C terminus containing construct or empty LexA pasmid was cotransformed into L40 yeast strain with constructs expressing differen APs subunits. Our preliminary data indicate the interaction between cystinosin and AP3 complex, as the blue coloration in β -galactosidase assay and the growth on limiting histidine deprived medium was observed only for colonies containing the cystinosin tyrosine based motif and AP3 μ subunit. The identified interaction will be further confirmed by immunoprecipitation performed on lysates of HeLa cells transfected with cystinosin GFP expressing construct.
2. To analyze the role of AP complexes on cystinosin trafficking in cellular model, the possible cystinosin mislocalization will be studied by cell surface biotinylation and immunofluorescence in cell lines depleted in subunits of AP complexes. For this purpose, we generated stable mocha ($\Delta\delta$ AP3) and 3T3 cell lines expressing cystinosin GFP fusion protein. We are now performing the colocalization studies of cystinosin GFP protein with markers of different cellular compartments in these cell lines. The HeLa cells deficient in AP1 or AP2 complex are in preparation.

Identification of cystinosin interaction partners

In order to get insight into the possible implication of the 5th inter-TM loop of cystinosin this domain was used for a large screen against a mouse kidney cDNA library (collaboration with Hybrigenics). We identified different putative partners and will focus on two of them: Vps39 and Snf8 (the murine homolog of Vps22) both being implicated in membrane fusion and trafficking respectively(Caplan et al, 2001; Progida et al, 2006). We are now trying to confirm these interactions by immunoprecipitation and colocalization with cystinosin in HeLa cell line. As we found that transient cotransfections were not efficient for these studies, we generated stable cell lines expressing VPS39 or SNF8 GFP fusion proteins that will be transfected with cystinosin HA construct. Both immunoprecipitations using anti-GFP and anti-HA antibodies will be performed.

References:

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