Cystinosis: from gene to disease

Vasiliki Kalatzis¹ and Corinne Antignac¹,²

¹Inserm U423 and ²Département de Génétique, Université René Descartes, Hôpital Necker-Enfants Malades, Paris, France

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Introduction

Lysosomes are intracellular sacs of enzymes that are responsible for the digestion of macromolecules. The products of the hydrolytic digestion process then leave the lysosome via specific transporters in its membrane, to be either reused by the cell or excreted outwards. The general consensus about cystinosis has been that it is an inherited multi-systemic disease resulting from failure of lysosomal cystine transport. However, it has only been since the cloning of the causative gene, CTNS (short for cystinosis), and the study of the encoded protein, cystinosin, that the molecular basis of this disorder has been understood.

Clinical description

Cystinosis (MIM 219800) is the most common inherited cause of the renal Fanconi syndrome. The most severe form, infantile cystinosis, manifests generally between 6 and 12 months of age by fluid and electrolyte loss, aminoaciduria, glycosuria, phosphaturia, renal tubular acidosis, rickets and growth retardation [1]. Glomerular impairment leads to end-stage renal failure by 10 years of age. Cystinosis was first thought to be a purely renal disease; however, with the advent of renal replacement therapy it was found to lead to widespread organ damage. This includes ocular, endocrinological, hepatic, muscular and central nervous system complications. Two less severe and less frequent forms have also been described, juvenile cystinosis (MIM 219900) and ocular cystinosis (MIM 219750). Individuals with juvenile cystinosis present with a glomerular impairment between 12 and 15 years of age but do not suffer from severe tubulopathy or growth retardation. The progression to end-stage renal failure is slow and it is reached at variable ages. Individuals with ocular cystinosis do not present any renal anomalies. The current treatment for cystinosis is by the drug cysteamine, which cleaves cystine molecules, thereby allowing their clearance from the lysosome.

Underlying metabolic defect

Cystine, the disulphide of the amino acid cysteine, accumulates in the organs of affected children. The site of cystine storage was determined to be the lysosome in the 1960s, by differential centrifugation studies of cystinotic leukocytes [2] and fibroblasts [3], which showed that cystine co-sedimented with lysosomal enzymes. As no lysosomal enzyme systems metabolizing cystine exists, it was suggested that lysosomal cystine transport was the probable metabolic defect of this disorder. Support for this hypothesis came in the 1980s from studies performed on whole lysosomes artificially loaded with high levels of cystine. This work showed that cystine is rapidly lost from artificially loaded normal lysosomes, whereas cystine efflux from cystinotic lysosomes is almost non-existent [4,5]. Furthermore, these studies showed that cystine transport must be carrier-mediated, because of the demonstration that cystine efflux is saturable [5] and that cystine counter-transport exists across the lysosomal membrane [6]—two hallmarks of carrier-mediated transport. These important studies set the stage for deciphering the molecular basis of cystinosis.

CTNS and causative mutations

Our understanding of cystinosis did not progress significantly, however, until 1998, with the positional cloning of the causative gene, CTNS [7]. CTNS is localized to 17p13 and is composed of 12 exons, the first two of which are non-coding. The remaining 10 exons encode a 367-amino-acid protein, cystinosin, which is predicted to contain seven transmembrane domains (TM), a 128-amino-acid N-terminal region bearing seven N-glycosylation sites, and a 10-amino-acid
cystinosin was determined by fusing a green fluorescent protein (GFP) tag to the C-terminal end of the protein and following the fluorescence of the cystinosin–GFP fusion protein in transfected cells [18]. The fluorescence was localized to intracellular vesicles, which were identified as lysosomes by co-localization studies with an antibody directed to a known lysosomal membrane protein, LAMP-2. The next step was to determine the signals responsible for the targeting of cystinosin to this organelle. In the C-terminal tail of cystinosin resides a GYDQL motif that resembles the lysosomal targeting motif found in the tail of other lysosomal membrane proteins [19]. Mutation or deletion of this motif, in particular of the Y and L residues, resulted in partial redirection to the plasma membrane. A signal remained in the lysosomes suggestive of a second lysosomal targeting motif, which was determined to lie in the fifth inter-TM loop [18]. Deletion of the first half of this loop, in particular a YFPQA pentapeptide, coupled to the deletion of the GYDQL motif, resulted in a complete relocalization of cystinosin to the plasma membrane. In contrast, point mutations of the YFPQA motif had a less dramatic effect on the relocalization of cystinosin, suggesting that the conformation of this motif is more important than its specific sequence. This suggests that it is probably recognized as part of a secondary structure formed by the 5th inter-TM loop. What is particularly interesting about this second motif is that it is one of the first examples of a lysosomal sorting signal situated elsewhere than in the cytoplasmic tail. It is tempting to speculate that the YFPQA motif represents a rescue pathway by which cystinosin can be sorted to lysosomes.

**Function of cystinosin**

The next problem that needed to be addressed was what role does cystinosin play in the lysosome. Although it has been accepted that the underlying metabolic defect of cystinosis is cystine transport, it was unknown whether this protein was directly or indirectly responsible. It was possible that cystinosin represented the lysosomal cystine transporter itself; however, neither its sequence nor its predicted topology was similar to known transporters. Due to its similarity with G-protein-coupled receptors, which also have seven TM, it was suggested that it could be part of a complex that binds, rather than transports, cystine [12,20]. Thus its function needed to be determined at the molecular level. As the lysosomal lumen is not easily accessible for transport experiments, cystinosin was redirected to the plasma membrane by deletion of the C-terminal GYDQL motif (cystinosin–ΔGYDQL), thereby creating a cellular model in which the ability of cystinosin to transport cystine could be examined using whole cells (Figure 1). Cells expressing cystinosin–ΔGYDQL were equivalent to giant ‘inside-out’ lysosomes and cystinosin activity could be assayed by its ability to take up 35S-labelled cystine from...
the extracellular medium [21]. This work showed that when incubated in a neutral extracellular medium, cystinosin–ΔGYDQL-expressing cells did not significantly accumulate 35S-cystine. In contrast, when these cells were placed into an acid extracellular medium, there was a dramatic increase in cystine uptake. This observation provided the first direct proof that cystinosin is the lysosomal cystine transporter. Interestingly, when the transmembrane pH gradient existing between the acid extracellular medium and the neutral cytosol is disrupted, cystine transport is abolished, demonstrating that cystinosin co-transports cystine and protons (cystine\textsuperscript{H}\textsuperscript{q}\textsuperscript{symporter}). Thus the H\textsuperscript{+}-translocating ATPase that acidifies the lysosomal lumen actively drives the cystinosin-mediated transport in the efflux direction (Figure 1). This explained early observations that cystine efflux from whole lysosomes was stimulated by Mg-ATP [22]. In addition, cystinosin-mediated cystine transport is saturable and follows Michaelis–Menten kinetics with a mean KM of 278 ± 49 μM [21], which is consistent with studies performed on the native cystine transporter [6], demonstrating the reliability of this in vitro model. Finally, cystinosin is highly specific for l-cystine [21]. It does not transport other amino acids, including the monosulphide cysteine, which distinguishes it from the plasma membrane cystine transporters 4F2hc/\textsuperscript{XCT} and rBAT/b\textsuperscript{0\textsubscript{AT}}, and its activity is not stimulated by other ions. In conclusion, cystinosin defines a novel family of transporters characterized by a seven-TM topology.

**Perspectives**

Although the function of cystinosin has been determined, it is still not known how cystine accumulation leads to the clinical signs. The associated tubulopathy, which appears prior to massive cystine accumulation and which is not significantly improved by cysteamine therapy, is particularly intriguing and may represent a secondary metabolic consequence of cystine storage rather than a direct effect. It has been suggested that cystine accumulation in proximal tubular cells leads to a decrease in ATP levels; this in turn would inhibit NaK-ATPase activity and reduce sodium-coupled transport of other solutes into these cells [23].
Determining the molecular basis of the phenotypic differences underlying the cystinosis forms may help address this question. The in vitro cystinosin model was used to test the effect of a point mutation, G308R, associated with infantile cystinosis in several families on cystine transport [21]. G308R, situated in the sixth TM, abolished cystine transport without affecting the subcellular localization, accounting for the severe phenotype and providing the first insight into a region of cystinosin critical for cystine transport. Similarly, this model could now be used to test the effect on cystine transport of all the CTNS mutations associated with the infantile, juvenile and ocular forms. This will allow a better understanding of the critical regions of cystinosin and perhaps provide an answer for the variations observed with regards to kidney disease.

References