CHAPTER 1

The Lysosomal System: Physiology and Pathology

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Introduction

The lysosome and its constituent parts – what has been referred to as the greater lysosomal system [1] – constitute a major metabolic regulatory network in eukaryotic cells. This system includes secretory streams transporting newly synthesized enzymes and other proteins to lysosomes, endosomal and retromembral streams contributing to signal transduction and related processing, autophagic streams delivering intracellular material for lysosomal degradation, and salvage streams facilitating egress of lysosomal degradation products to other sites in the cell for reutilization. Operating in close parallel are additional proteolytic mechanisms such as the ubiquitin–proteasome system (UPS), which assists in efficient protein turnover. The central coordinator of this remarkable intracellular network is ultimately the lysosome itself, an acidic membrane-bound organelle that functions to degrade and reprocess a vast array of cellular material. Hydrolytic enzymes localized to the lysosomal lumen are optimally active at an acidic pH and have the capacity to degrade most macromolecules including proteins, carbohydrates, lipids, RNA and DNA. Following the breakdown of this material, the resultant amino acids, sugars, simple glycolipids, cholesterol and nucleotides are salvaged by transport through the lysosomal membrane with the aid of specific transporter proteins for delivery to other cell organelles and membranes for subsequent use in biosynthetic processes. Although traditionally depicted as a terminal compartment, this role in recycling molecular precursors brings the importance of the lysosome full circle. Taken as a whole, the greater lysosomal system therefore functions at the very hub of cellular metabolic homeostasis. With the recent discovery of an overarching gene regulatory network referred to as CLEAR (Coordinated Lysosomal Expression and Regulation) and its master gene transcription factor EB (TFEB), many components of the greater lysosomal system have been shown to be linked at the transcriptional level [2]. Indeed, these studies further establish the lysosomal system as a highly efficient and coordinated network. As such, proper lysosomal function is essential since failure of this system leads, inexorably, to catastrophic consequences for cells, organs, and individuals, with nearly 60 different types of lysosomal diseases documented to date (see Classification in Chapter 5).

The greater lysosomal system

Our understanding of the lysosome and its role in cells has evolved significantly since its discovery by Christian de Duve more than 50 years ago. This organelle and the constituent streams or pathways to which it is linked comprise a processing and recycling centre essential to all cells. While each component is typically defined separately, it is important to conceptualize the various parts functioning as a highly orchestrated cellular mechanism (Figure 1.1).

Endocytosis

The endolysosomal pathway consists of the major delivery streams and molecular machinery necessary for the internalization of cell surface and extracellular material linking
cells with their external environment. The full scope of the complexity of the endocytic system continues to evolve with the characterization of diverse forms of endocytosis and the elucidation of key molecular components associated with these pathways. Endocytic processes are often grouped by the morphological characteristics of the invagination of the membrane. Clathrin-mediated endocytosis (CME) is defined by clathrin-coated pits localized to the plasmalemma that internalize receptor/cargo complexes into vesicles that are sorted and targeted to various intracellular locations. In the CNS, neurons rely on CME for the cycling of neurotransmitter receptors regulating signaling and activity-dependent neuroplasticity. Clathrin-independent endocytosis has also been described and most often occurs at flask-like invaginations along the plasmalemma called caveolae. Caveolae are long-lived plasma membrane microdomains composed of caveolins, cholesterol, sphingolipids including glycosphingolipids (GSLs) and sphingomyelin, GPI-anchored proteins and various receptor proteins. Such specialized membrane structures which are ultimately processed by the endolysosomal system are known to play a critical role as platforms for cell signalling and as regulators of lipid components within the plasmalemma.

The canonical endocytic pathway progresses along an increasing lumen-acidic gradient from early endosomes retrogradely trafficked from the plasma membrane, to multivesicular bodies or late endosomes, and finally to perinuclear-localized lysosomes. Deviating from this pathway, early endosomes can be recruited back to the plasmalemma or to other organelles as sorting/recycling endosomes. These divergent streams allow for the recycling and reinsertion of cell surface receptors, delivery of signaling ligands throughout the cell, and internalization...
of membrane components to be reorganized. Such carefully orchestrated processing and its related signal transduction events may be interrupted in diseases of the lysosomal system in which endocytosed components including cholesterol and GSLs accumulate. While this accumulation is typically associated with late endosomes and lysosomes, recent evidence has emerged suggesting additional involvement of early endosomal compartments [3], raising the likelihood that the consequences of lysosomal disease extend well beyond the lysosome itself.

**Autophagy**

In addition to endocytic pathways, autophagic streams feed into the lysosome and are involved in targeting intracellular material including effete organelles, long-lived proteins and pathogens for degradation [4]. Autophagy, which is often activated following starvation stress, is divided into three distinct subtypes – microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy – defined by the delivery method of substrate to lysosomes. Microautophagy involves the direct engulfment of cytosolic material by the lysosome either by direct invagination of the lysosomal membrane, or projected arm-like extensions that sequester material into intralysosomal vesicles. CMA is selective for soluble monomeric proteins containing the peptide signalling motif Lys-Phe-Glu-Arg-Gln (KFERQ). This motif allows for binding by the heat shock cognate 70 protein (HSC70). HSC70 and co-chaperones then promote protein unfolding and translocation across the lysosomal membrane via the lysosome-associated membrane protein type 2A (LAMP2A). Macroautophagy has been traditionally characterized as a vesicle-mediated bulk degradation mechanism activated in response to nutrient deprivation. Recently, however, it has been shown that macroautophagy is also constitutively active and selective. When macroautophagy is stimulated, double membrane vesicles known as autophagosomes form to engulf cytosolic material. This material, including dysfunctional organelles and oligomerized proteins, is selectively recognized by chaperone complexes and adapter molecules including heat-shock proteins (HSPs), ubiquitin, and p62/SQSTM1 (p62) which allow for the specific uptake of these substrates within autophagosomes. To date several lysosomal diseases have been found to exhibit alterations in autophagy, however the downstream consequences for disease progression remain unclear. Of particular interest is evaluating how impaired autophagy contributes to neurodegenerative processes.

**Salvage**

Degradation products resulting from lysosomal processing must be efficiently removed from the organelle for utilization elsewhere in the cell. This salvage process involves numerous lysosomal membrane proteins that act as transporters, including cystinosin, sialin, cobalamin transporter and NPC1 protein (see Chapter 17). For example, a defect in the salvage of cobalamin (also known as vitamin B12) leads to cobalamin F-type disease, a disorder characterized in children by megaloblastic anaemia, failure to thrive and neurological deficits [5]. The inability of cobalamin to leave the lysosome and enter the cytosol means that it is unavailable for conversion into enzyme cofactors methylcobalamin and adenosylcobalamin, which are critical for multiple metabolic processes. Another example of salvage compromise involves the reutilization of lysosomal degradation products directly in synthetic pathways in the Golgi and elsewhere in the cell. In Niemann–Pick type C disease (NPC), lack of the NPC1 protein and the resulting compromise in egress of unesterified cholesterol to other sites in the cell is known to cause compensatory increases in cholesterol synthesis [6] and a similar phenomenon involving glycosphingolipids (GSLs) may occur following sequestration of GM2 and GM3 gangliosides [3]. Following synthesis in the Golgi and transport to the plasmalemma, GM1 and other complex gangliosides are eventually endocytosed and degraded in lysosomes. Salvage of simple gangliosides (e.g., GM2 and GM3) from lysosomes and their transport back to the Golgi allows for efficient production of complex gangliosides without the need for complete de novo synthesis from ceramide. Even in other lysosomal disease caused by defects in degradation rather than egress – such as Sandhoff disease in which GM2 accumulates following absence of β-hexosaminidase – neurons may similarly be deprived of this precursor for complex ganglioside synthesis. Reutilization of simple molecular components derived from lysosomal degradation would be energetically favourable over full de novo synthesis. What storage may lead to in terms of cell energy consumption and altered regulatory processing is only now beginning to be explored in depth. Thus, while lysosomal diseases have been historically viewed as states of overabundance of non-degraded material, it is only logical to assume that they are also likely deficiency diseases in which critical components for multiple metabolic pathways are in reduced supply.

**Ubiquitin–Proteasome System (UPS)**

In addition to pathways directly feeding into and out of the lysosome, the lysosomal system includes mechanisms functionally allied in maintaining proteolytic quality
control, namely the UPS. As the primary degradative pathway for most soluble short-lived proteins, the UPS plays a pivotal role in cellular regulatory processes, including the endoplasmic reticulum associated degradation system (ERAD). The ERAD system is responsible for turning over aberrantly misfolded proteins immediately following their translation thereby serving as a quality control check-point. Additionally, the UPS has been found to coordinate proteolysis with the autophagic/lysosomal system in certain instances. For example, UPS inhibition promotes the upregulation of macroautophagy in an apparent effort to redirect and sustain efficient protein degradation through the lysosome. Furthermore, the UPS and autophagic/lysosomal systems rely on several of the same key molecules – most notably ubiquitin and p62 – selectively targeting substrates for degradation. This complementation is limited, however, as the UPS only has the ability to degrade monomeric proteins that have been properly unfolded and fed into the proteasome catalytic core, while macroautophagy is capable of bulk degradation of organelles and oligomerized proteins. In lysosomal disease states in which the degradative capacity of the lysosome is compromised it is conceivable that the UPS may be employed to compensate for some of the proteolytic load, although the molecular machinery and sequence of events involved in this cross-talk is largely unknown. Finally, it is unclear whether there is a threshold at which the UPS may too become overwhelmed, perhaps contributing to ER stress and thereby causing complete proteolytic failure in lysosomal disease states.

**Lysosomal diseases**

After more than a half century of clinical recognition and classification, lysosomal storage diseases were conceptualized as disorders resulting from deficiencies in single lysosomal hydrolases followed by the subsequent accumulation of a specific substrate for that enzyme [7]. Today, lysosomal diseases are known to include nearly 60 monogenic disorders with a combined frequency of approximately 1: 7000 live births. They are known to be caused by deficiencies not only in lysosomal hydrolases, but also in other lysosomal and non-lysosomal proteins including enzymes, soluble non-enzymatic proteins, and membrane-associated proteins critical for proper function of the greater lysosomal system [8]. Categorizing lysosomal disorders is not completely straightforward as several diseases exhibit significant overlap of pathological features, storage material, and so forth. However, grouping lysosomal diseases based on the traditional biochemical nature of the primary storage material is often preferred; these include the lipidoses, mucopolysaccharidoses (MPSs), glycogenoses, neuronal ceroid lipofuscinoses (NCLs), mucolipidoses (MLs), glycoproteinoses and others (see Classification in Chapter 5).

In general, most lysosomal diseases afflict children. Age of onset and clinical course can vary significantly, but nearly all lysosomal diseases have a delayed non-congenital onset, and progressive course ultimately leading to premature death. Even as our grasp of the genetic, molecular and biochemical bases of these disorders has advanced in recent years there are still many gaps in our understanding as to pathogenic mechanisms and the reversibility of disease-induced cell damage. Some of the most important questions in this regard involve the brain.

**CNS Involvement** (See also Chapter 21) Adding to lysosomal disease complexity is the broad systemic involvement of multiple tissues and organs. Clinical presentation often involves bone, muscle, liver, kidney and spleen, while nearly two-thirds of these disorders also exhibit extensive neurological impairment (see Chapter 2). Intellectual disability, dementia, seizures, motor system deficits, visual impairment and hearing loss are common manifestations associated with several lysosomal diseases. The progressive clinical and pathological course presented in these disorders highlights the indispensible role of the lysosome, especially in post-mitotic neurons which are predominately affected. Interestingly, this vulnerability becomes manifest in a highly specified manner with different neuronal subtypes and brain regions exhibiting distinct pathophysiological changes.

One of the more perplexing phenomena associated with lysosomal disease is neurodegeneration. While many of these disorders exhibit some degree of patterned neuronal loss, the question remains why some neurons appear more vulnerable to this fate than others and what effect neurodegeneration has on disease course. A common theme implicated in motor system impairment is Purkinje cell (PC) death. PC death in mice with NPC begins early and progresses in an anterior to posterior lobe pattern within the cerebellum. This cell loss is severe and nearly total, with the conspicuous exception of lobule X (flocculonodular lobe in humans) where PCs are well preserved. Remarkably, almost identical patterns of PC loss occur across a wide spectrum of lysosomal diseases, including mucolipidosis type IV and the gangliosidoses. It should also be noted that in the case of NPC disease that both PCs and cerebrocortical neurons exhibit extensive pathological
storage of cholesterol and ganglioside, however unlike PC degeneration, cortical neuron death is often not as conspicuous in the early stages of the disease.

CNS inflammation is an additional feature of lysosomal diseases that in many cases has been shown to be spatially and temporally correlated with neuronal dysfunction and neurodegeneration. Of particular interest is how this initial protective response to disease may become deleterious with chronic induction, ultimately leading to secondary damage and exacerbation of pathogenic processes. In the normal brain, microglia play an essential house-keeping role intimately coupled to neuronal function. As the resident macrophage of the CNS, microglia are also critical for synapse maintenance, axon and spine pruning, clearing extracellular debris and apoptotic cells, glutamate and trophic factor regulation, and probing their surroundings for homeostatic deviations in the extracellular environment. Conversely, during disease/injury activated microglia proliferate, and, along with infiltrating macrophages, can generate cytotoxic components including reactive oxygen species, nitric oxide and pro-inflammatory cytokines. Microglia and macrophages exhibit altered morphological states in many lysosomal diseases, while activated astrocytes are also a prominent feature contributing to the pathological landscape in the CNS. Some NCLs, gangliosidoses, MPSs, MLs and neuronopathic Gaucher disease exhibit neuroinflammatory features. A study in Sandhoff disease mice showed that microglial activation precedes neuronal cell death, and that bone marrow transplantation ameliorates the expansion of microglia and neurodegeneration [9]. Interestingly, however, this improvement did not coincide with any significant decrease in GM2 ganglioside storage, suggesting that microglial activation is a significant component of neuronal death independent of storage. As such, the use of anti-inflammatory drugs to treat lysosomal diseases may be a relevant therapeutic strategy for providing benefit in certain instances. Future efforts to decipher the protective roles of microglial activation and inflammation from pathogenic stimulating events, and to determine the temporal window for using anti-inflammatory drugs in the treatment of lysosomal disease states, will be critical.

**Intracellular storage**

Ever since the concept of lysosomal disease was developed by H.G. Hers, intracellular storage material has been a defining characteristic of these disorders. As the complexity of this pathological feature has evolved well beyond the original single enzyme/single substrate theory, research has focused on understanding how storage contributes to pathogenic cascades. The relationship between primary and secondary storage warrants clarification. Primary storage may be defined as the lysosomal buildup of biochemical components that accumulate as a direct result of a failure of degradation within, or egress of, degradation products from the lysosomal system. Secondary storage is material accumulating from subsequent downstream compromise in lysosomal function. Given a number of lysosomal diseases caused by proteins of unknown or poorly understood function, however, the classification of specific storage material as primary or secondary continues to evolve. Neuronal storage is often found in perinuclear regions of the perikarya, although it may extend into dendrites and the axon hillock. Large swellings in the latter are known as meganeurites (described below).

At its simplest, the buildup of primary storage material probably further compromises the degradative capacity of lysosomes and thereby exacerbates the accumulation of secondary storage components in a deleterious cycle. More complex is the question of how storage may affect the lysosomal system as a whole through such expanding downstream events. These include the regulation of appropriate lysosomal pH, proper coordination of fusion events between autophagosomes, endosomes and lysosomes, unabated signal transduction along the endocytic pathway, and efficient and appropriate lysosomal salvage for regulating biosynthetic processes. Like selective neurodegeneration, differences in storage can also occur in distinct neuronal populations with regional specificity. For example, in mucolipidosis (ML) IV mice, different gangliosides accumulate throughout the CNS; in the hippocampus, however, gangliosides appear to be confined to specific regions – storage of GM3 occurs in the CA3 region, while GD3 is found in the CA1–CA2 regions only [10]. What this region-dependent storage represents remains unknown.

Determining whether lysosomal storage *per se* is a fundamental cause of neuronal dysfunction remains an important question. Studies in NCL disease have shown no direct correlation between the accumulation of saposins A and D and subunit c of mitochondrial ATP synthase (SCMAS) and neuron loss (Figure 1.2). In fact, storage pathology in NCL and other lysosomal diseases is typically prevalent long before the onset of any behavioural phenotype in animal models [8]. Similarly, neurons in many lysosomal diseases present significant storage pathology early, and yet survive for extended periods of time (decades) suggesting that accumulation is
not immediately cytotoxic. Yet this does not mean that neurons remain functionally normal. Indeed, as described below, given the presence of metabolic compromise and of axonal, dendritic and synaptic abnormalities, it is highly likely that affected neurons are not optimally functioning even from early time points in the storage process. Eventually the presence of such variously malfunctioning neurons in given neural networks would be anticipated to reach a tipping-point at a systems level, with clinical disease emanating as a result, even in the absence of frank neurodegeneration. Such clinical deficits would be solidified, and possibly worsened, with the eventual death of neurons that participate in these neural circuits. It is essential to understand these issues in the face of emerging therapies that may rescue the storage phenotype long after it has been established, but before neuron death. Interestingly, substrate reduction therapy either aimed at inhibiting the biosynthesis of GSLs in gangliosidoses [11], or at enhancing the egress of cholesterol and GSLs in NPC disease, has proven effective in delaying clinical onset and increasing life-span [12]. These studies clearly indicate that reducing storage is beneficial in lysosomal disorders but reveal little about the precise link between storage and brain dysfunction.

In contrast to a primary role in disease pathogenesis, storage may have a more indirect and broader influence on hindering metabolic homeostasis in cells. Impairment in lysosomal salvage probably represents a progressive and deleterious metabolic burden forcing cells to expend energy synthesizing simple molecules to replace those trapped within residual lysosomes. Owing to its tight regulation of peripheral energy stores via the hypothalamus, as well as to additional local support from astrocytes, the brain in general, and neurons in particular, are considered resistant to starvation events. Lysosomal diseases, however, may represent a unique pathophysiological state within the CNS characterized by chronic energy depletion. This could perhaps explain why neurons which need to sustain high levels of activity are particularly susceptible in these disorders. It is also interesting to consider how this scenario might be further compounded in lysosomal disorders that have been speculated to involve suboptimal mitochondrial function (e.g. NCL disorders and MLIV), and whether this could be a cause, or a result, of chronic energy strain. Once again, clues as to how storage correlates with CNS dysfunction and why specific neuronal subtypes are more susceptible to pathological changes in lysosomal diseases may lie in the different metabolic requirements of individual neuronal populations. These so-called metabolic signatures may determine a cell’s vulnerability to downstream deleterious events like starvation stress, oxidative stress, ER stress and even the capacity to tolerate protein aggregates and storage accumulation.

In addition to interfering with metabolic homeostasis, lysosomal storage may inhibit the destined function of the stored material within the cell. For instance, glycosaminoglycans (GAGs) are the predominant storage material in MPS diseases. Normally, mature proteoglycans are decorated with GAG side chains and localized to the outer leaflet of the plasma membrane to function in intercellular signalling cascades. Specifically, proteoglycans play a significant role in CNS development during axon guidance...
and synapse formation through their interaction with growth factors and other extracellular components. Although MPS disorders, like other lysosomal diseases, are not believed to be associated with abnormal brain development per se, it is conceivable that the buildup of GAGs in MPS disease may over time hinder appropriate signalling events – eventually reaching a tipping-point for neuron and neural circuit dysfunction as described above.

**Axonal spheroids**

Neuroaxonal dystrophy, also known as axonal spheroid formation, occurs in a wide spectrum of lysosomal diseases, from the gangliosidoses to the glycoproteinosis [8]. Notably, this feature appears to occur predominantly in γ-aminobutyric acid (GABA)ergic neurons, particularly PCs in the cerebellum (Figure 1.3). Morphologically, spheroids are characterized as focal swellings along axons where mitochondria, multivesicular and dense bodies, tubulovesicular profiles and possibly autophagic vacuoles accumulate. Interestingly, this material is ultrastructurally similar across different lysosomal diseases, yet distinct from the storage found in neuronal cell bodies. Given the large surface area of neurons and their highly polarized nature, functional anterograde and retrograde trafficking is essential. Spheroids appear to reflect a compromise in such transport and may as a result hinder critical growth factor support for the development, maintenance, and survival of target neurons. They are also large enough to impact normal action potential propagation and thereby contribute to neuronal dysfunction. Indeed, the incidence of spheroids appears to correlate with the onset and progression of CNS impairment in animal models, suggesting a similar, significant role in human clinical neurological disease [8].

**Calcium signalling**

Calcium plays a critical role as a second messenger involved in a wide range of cellular functions, and altered...
calcium homeostasis and signalling have provided clues to lysosomal disease mechanisms. Indeed, calcium is critical for intraorganellar fusion events along the endocytic and autophagic pathways. Not surprisingly, several lysosomal diseases show defects in endosome/autophagosome/lysosome fusion that may be attributed in part to abnormal calcium homeostasis. A number of diseases have been characterized by elevated neuronal intracellular calcium levels as a result of aberrant ER calcium channel function affected by storage accumulation. In NPC1, sphingosine storage has been reported to lead to reduction in lysosomal calcium stores, altered pH, trafficking defects and the storage of cholesterol, sphingomyelin and GSLs [13]. Altered calcium homeostasis may also be linked to the excitotoxic stress seen in NCL disease, with cortical excitatory neurons unable to recover from depolarization due to high intracellular levels of calcium.

**Meganeurites and ectopic dendritogenesis**

The buildup of storage within the basilar region of neurons can extend into the axon hillock resulting in the aberrant formation of a meganeurite. This striking feature can be visualized by silver or Golgi stains, for example, in cerebral cortex in ganglioside storage diseases. A clear functional consequence of meganeurites is the distal displacement of the axonal initial segment rich in sodium channels (the action potential trigger zone) away from the cell body. This displacement may result in inappropriate action potential initiation, with serious repercussions for neuronal function. Notably, meganeurites are morphologically distinct from axonal spheroids. Whereas meganeurites occur at the axon hillock proximal to the initial segment and contain storage bodies characteristic of a particular lysosomal disease, spheroids occur distal to the initial segment in the axon proper and contain a generic accumulation of organelles and other structures as described earlier [8].

Ectopic dendritogenesis, which often is seen in conjunction with meganeurite formation, is a phenomenon in which a subset of excitatory cortical pyramidal neurons exhibit new dendrite and synapse formation [8]. This unprecedented event is both mislocalized to the axon hillock and occurs distinctively after the normal temporal window of dendritogenesis during early development. Ectopic dendritogenesis has been documented in several lysosomal diseases, all of which are characterized by primary or secondary storage of gangliosides. The precise relationship, however, between ectopic dendritogenesis, endosomal/lysosomal dysfunction and GSL metabolism remains to be determined.

**Autophagy dysfunction**

Autophagy dysfunction marked by a buildup of autophagosomes in brain tissue has been closely linked to neurodegeneration, and not surprisingly several lysosomal diseases have been found to exhibit alterations in this critical homeostatic mechanism [14]. This buildup can represent a pathogenic failure in autophagosome degradation, a physiological induction in macroautophagy flux, or, in some cases, both a blockage and induction. Given the primary disease defect and general compromise of the lysosome, impaired autophagosome maturation is a predicted downstream consequence of lysosomal storage accumulation. Trafficking and fusion deficits between autophagosomes and lysosomes, as well as failures in degrading autophagic material, have been attributed to the observed increases in autophagosomes in specific disorders. The presence of autophagosomes implies that the molecular machinery for macroautophagy initiation is intact and functional, while later steps in the pathway are compromised. Danon disease, which is caused by a deficiency in LAMP2, represents a classic example of failure in autophagosome maturation [14]. Studies in mice have found LAMP2 to be critical for the fusion of autophagosomes and lysosomes, resulting in significant accumulation of autophagic vacuoles in disease tissue. Also worth noting is the importance of the LAMP2A isoform in mediating CMA, although potential dysfunction along this pathway in Danon disease has not been thoroughly explored.

Evidence of an induction or overactive macroautophagy is also commonly seen in many lysosomal diseases, although the reason for this response is not entirely clear. Perhaps most relevant to consider is an upregulation of macroautophagy in an effort to counter energy deprivation stress incurred within cells due to salvage failure. In Pompe disease, a deficiency of α-glucosidase and the consequent failure to breakdown glycogen in lysosomes stimulates macroautophagy, while the buildup of storage concomitantly impedes autophagosome maturation. This resulting accumulation of autophagosomes in Pompe disease severely hinders muscle contraction and contributes to muscle deterioration [14]. Interestingly, overactive autophagy in type-II muscle fibres is believed to interfere with enzyme replacement therapy in Pompe, where recombinant enzyme is taken up in accumulating autophagosomes, preventing efficient trafficking to the lysosome. Significantly, studies have shown that suppression of macroautophagy in skeletal muscle corrects enzyme delivery deficits and attenuates glycogen storage in mouse models of Pompe disease. Several other lysosomal diseases have shown evidence of upregulated
macroautophagy, but whether this initial response is detrimental or protective remains unknown.

An additional scenario to consider in regard to autophagy impairment is a failure to initiate the macroautophagy pathway or target appropriate cargo to autophagosomes. As opposed to inefficiencies late in autophagosome maturation and flux, the accumulation of oligomerized proteins and effete organelles is suggestive of general proteolytic compromise and failures in constitutive macroautophagy initiation. Several lysosomal diseases (MPS IIIA, MSD, NPC, MLIV, several NCLs and several gangliosidoses) have been found to exhibit increased levels of proteins preferentially degraded by macroautophagy, most notably the autophagosome adaptor protein p62 (Figure 1.4). Often interpreted simply as an indirect marker of autophagy function, the accumulation of cytosolic p62-positive aggregates may more appropriately signify a deficiency in autophagosome formation and/or engulfment of material by autophagosomes. These p62 aggregates are widely distributed throughout the CNS in both neuronal and glial cell populations depending on the disease. It remains unclear however, what role aggregates play and how they contribute to the pathological milieu of lysosomal disorders. In addition to aggregate formation, macroautophagy initiation impairment and cargo recognition failure directly result in the inefficient turnover of damaged mitochondria, also known as mitophagy. Accumulation of dysfunctional mitochondria can have a significant deleterious effect on cells including altered calcium homeostasis and the generation of reactive oxygen species (ROS). Most importantly, dysfunctional mitochondria can stimulate the release of cytochrome c initiating intrinsic apoptotic cell death pathways. Several lysosomal diseases are associated with mitochondrial abnormalities, although how mitophagy deficits contribute to this phenotype remains unclear. Future studies will need to focus on whether alterations in autophagy are consistent with an induction or blockage in a given disease state, what the repercussions are for protein aggregate accumulation, and importantly how compromise of mitophagy may contribute to neurodegeneration. In addition to evaluating how lysosomal compromise affects pathways that feed into the lysosome, it will also be prudent to consider how systems allied in maintaining proteolytic quality control, like the UPS, may be affected downstream in lysosomal disease pathogenesis. To date, little is known about this relationship.

Concluding remarks

Delineation of the pathogenic cascades underlying lysosomal diseases continues to reveal the intricate nature of the greater lysosomal system and its role in cell

Figure 1.4 p62 accumulation in end-stage cerebellum of the Npc1<sup>−/−</sup> mouse model. (a) Confocal immunofluorescence image of lobule X Purkinje cell from Npc1<sup>−/−</sup> cerebellum showing p62 aggregates. Scale bar represents 10 μm. (b) Western blot probed for p62 in soluble and SDS-insoluble fractions from cerebellar homogenate of wildtype and Npc1<sup>−/−</sup> showing increases in both protein fractions. β-actin was used as a loading control.
homeostasis. Novel genes and proteins contributing to lysosome function continue to be identified and the list of lysosomal and lysosomal-associated diseases continues to grow. Studies of the molecular and cellular pathogenesis of lysosomal diseases have also never been more compelling, and are progressively opening up new avenues for therapy. Furthermore, parallels across groups of traditionally unrelated neurological disorders have emerged, providing insight into CNS dysfunction and reshaping the way we think about the role of the lysosomal system in neuron function. Indeed, in recent years pathophysiological boundaries have blurred between lysosomal diseases and other neurological disorders including both early-onset neurogenetic diseases like Angelman syndrome [15], to late-onset neurodegenerative diseases like Alzheimer’s [16] and Parkinson’s [17]. Emerging commonalities in lysosomal dysfunction and their links across this spectrum of conditions may indeed provide valuable insights toward the goal of developing therapeutic strategies for a host of presently untreatable neurological conditions.

References