Ubiquitin, lysosomes and neurodegenerative diseases

R. John Mayer, Lajos Laszlo, Aliya Middleton, Michael Landon, James Hope* and James Lowe†

Departments of Biochemistry and Pathology; †University of Nottingham Medical School, Queen’s Medical Centre, Nottingham NG7 2UH, U.K. and AFRC/MRC Neuropathogenesis Unit,* Ogston Building, King’s Building Site, West Mains Road, Edinburgh EH9 3JF, Scotland, U.K.

Ubiquitin−filament disorders

Ubiquitin−protein deposits were first demonstrated immunohistochemically in association with neurofibrillar tangles in Alzheimer’s disease [1−3]. Neurofibrillary tangles are based on paired helical filaments that are now known to contain abnormally phosphorylated forms of the neuronal microtubule-associated protein, tau [4]. The reason for the coexistence of abnormal forms of the microtubule-associated protein, tau, together with ubiquitin−protein conjugate(s) in neurofibrillary tangles, is unknown. Ubiquitin or ubiquitin−protein conjugates have been reported to be normal components of assembled microtubules [5] and may correspond to a novel ubiquitinated 30 kDa microtubule−associated protein [6]. Recent biochemical and immunofluorescence studies with polyclonal antiantiodyptic antibodies raised to anti-ubiquitin−protein conjugates have demonstrated binding sites for ubiquitin or ubiquitin−protein conjugates on both α- and β-tubulins and on intact microtubules (R. J. Mayer, unpublished work). The binding sites may be for the 30 kDa microtubule-associated protein, free ubiquitin or other ubiquitinated proteins. Neurofibrillary tangles could represent relics in diseased neurones of a family of microtubule-associated proteins, some of which are ubiquitinated.

The reason for the accumulation of the relics will only be understood when the roles of ubiquitin in microtubule function are fully characterized. There are two obvious hypotheses. Ubiquitinated proteins or ubiquitin may play a role in microtubule assembly or disassembly. Alternatively, binding sites on microtubules may serve as ‘collection points’ for ubiquitinated proteins before degradation. Ubiquitinated proteins may be degraded either within lysosomes, which are known to move along microtubules in a saltatory manner, or by the ATP− and ubiquitin−dependent non-lysosomal degradation system.

Other components of neurofibrillary tangles are less well defined; for example, it is still contentious if tangles contain any neurofilaments. Neurofilaments are one of the family of intermediate filaments, which are the element of the cytoskeleton most sensitive to a variety of stresses, including heat-shock, poisons and radiation [7]. Intermediate filaments reversibly collapse around the cell nucleus in response to such deleterious stimuli. The same response occurs during the internalization of viral membrane proteins from the plasma membrane for eventual lysosomal degradation or ejection from the cell [7]. In cells producing large quantities of a single viral membrane protein, irreversible collapse of intermediate filaments and inclusion formation can occur [8].

In those chronic degenerative disease inclusions to which intermediate filaments definitively contribute, ubiquitin−protein conjugates are also found. Such inclusions include the neurofilament-containing Lewy bodies in Lewy body dementia [9], the glial filament-containing Rosenthal fibres in cerebellar astrocytomas [10], the cytokeratin-containing Mallory bodies in alcoholic liver disease and the desmin-containing cytoplasmic inclusions in a cytoplasmic body myopathy [9]. Ubiquitin−protein deposits are also a feature of filamentous inclusions of unknown origin, first seen by ubiquitin immunocytochemistry in amyotrophic lateral sclerosis (motor neurone disease [10, 11]).

Ubiquitin immunocytochemistry has been important not only in unravelling commonalities in ostensibly unrelated diseases but also in diagnosis.
The true incidence of cortical Lewy bodies was only appreciated through staining for ubiquitin–protein conjugates [12–14] where the technique has revealed that 25–33% of all the cases of dementia coming to brain autopsy possess large numbers of these structures. Lewy body dementia, with cortical Lewy bodies as well as extracellular amyloid plaques, is therefore the second most common cause of dementia after Alzheimer’s disease (which accounts for approximately 50% of cases).

**Ubiquitin and the lysosome system**

Protein ubiquitination has been studied extensively in ATP-dependent non-lysosomal proteolysis [15], but the idea that ubiquitin might have any role in lysosome function was not considered previously. One of the first indications that ubiquitin could be involved in lysosome function came from the observation that ubiquitin–protein conjugates were enriched in areas of granulovacular degeneration in hippocampal neurones in Alzheimer’s disease [9]. As the name suggests conventional histochimistry shows that such neurones contain granular material in some form of vacuole. These structures may correspond to secretory granules or lysosome-related autophagic vacuoles. There is no evidence for the former notion, but it has been reported recently that the granulovacuoles are indeed autophagic vacuoles [16]. There have been several experimental findings which indicate that ubiquitin–protein conjugates are enriched in the lysosomes of different cell types, including neurones [17–21]. Quantitative electron microscopy shows that ubiquitin–protein conjugates may be enriched some 10–12-fold in the lysosomes of normal fibroblasts [18]. As well as ubiquitin–protein conjugates, some free ubiquitin has been detected in lysosomes by immunogold microscopy [22]. This procedure has also been used to detect ubiquitin–protein deposits in functionally compromised lysosomes [17] as well as in normal lysosomes (e.g. in fibroblasts and polymorphonuclear neutrophils [18, 19]) and in lysosomes of cells transformed by Epstein–Barr virus [20]. Cells with a temperature-sensitive mutation in the enzyme responsible for the activation of ubiquitin for protein-conjugation fail to load ubiquitin–protein conjugates into lysosomes at the non-permissive temperature [23]. Protein ubiquitination therefore appears to have a dual role in intracellular protein degradation: the well known role in ATP-dependent, non-lysosomal protein catabolism and a much less well characterized function in lysosomal protein degradation [24].

These experimental findings underpin the notion that ubiquitin–protein conjugates in hippocampal neurones in Alzheimer’s disease are probably in some form of lysosome-related autophagic vacuole.

The ubiquitin–protein deposits that have been described as ‘dot-like immunoreactivity’ in the neuropil, and which increase in density both in normal ageing [25, 26] and in neurodegenerative diseases [27, 28], are also lysosome-related bodies. The coarse ubiquitin–protein deposits observed immunohistochemically adjacent to amyloid plaques in Alzheimer’s disease [9] and in scrapie-infected mouse brain [29] probably represent pleomorphic lysosomes in dystrophic neurites surrounding the amyloid plaques [27]. This notion is reinforced by immunohistochemical and histochemical observations that coarse deposits (similar to those observed by ubiquitin immunocytochemistry) corresponding to several lysosomal cathepsins can be detected in the periphery of amyloid plaques in Alzheimer’s disease. The enzymes appear to spill out from the lysosome-related structures into the plaque periphery [30].

In summary, light microscopy with sensitive immunohistochemical techniques has indicated that there are at least three forms of ubiquitin–protein deposit in the human idiopathic and human and animal transmissible neurodegenerative diseases. The initial observations concentrated on the association of ubiquitin–protein deposits with filamentous inclusions; the molecular nature of these deposits is currently unknown. Ubiquitin immunohistochemistry has also revealed dot-like structures, within and adjacent to neuronal cell bodies and in the neuropil, which correspond to some form of pleomorphic lysosome-related organelles. Finally, the finding of both ubiquitin–protein deposits and lysosomal cathepsins in coarse distorted structures adjacent to amyloid plaques suggests that pleomorphic lysosomes are in dystrophic neurites in the plaque periphery. Lysosomes are therefore likely to have an important role in both idiopathic and transmissible neurodegenerative diseases. Recently, as noted below, a central role for lysosomes has been put forward in the generation of spongiform change in a prion encephalopathy, scrapie-infected mouse brain, based on immunogold electron microscope studies [21].

**Lysosomes and prion encephalopathies**

Ubiquitin immunohistochemistry predictively shows ubiquitin–protein conjugates in coarse...
deposits surrounding prion or protease-resistant protein (PrP) plaques in mouse brain infected with the ME7 strain of scrapie. There are also coarse dot-like structures adjacent to neuronal perikarya (which may be large intraneuronal autophagic vacuoles or lysosome-related organelles in axosomal processes abutting onto neuronal perikarya) and dot-like structures in the neuropil [29]. Again, as in the idiopathic neurodegenerative diseases, there are at least three types of ubiquitin–protein deposit in diseased brain.

Immunogold electron microscopy reveals a central role for lysosome-related organelles in scrapie-infected mouse brain, where gold particles corresponding to ubiquitin–protein conjugates can be seen to decorate dense pleomorphic multivesicular bodies and tubulovesicular bodies in the neuropil [21]. These types of organelles probably represent different stages in the condensation of endosome-like structures into denser lysosome-related bodies. Large numbers of gold particles can also be seen to be associated with remnants of dense lysosome-related organelles in areas of rarefaction. These regions appear to be the beginnings of areas of spongiform change. The disruption of lysosome-related organelles and release of lysosomal enzymes thus seem to be a cause of the tissue lesions in prion encephalopathies [21].

Ubiquitin–protein conjugates are found not only in vesicular bodies in neuritic processes in the neuropil (corresponding to the dot-like structures revealed at light microscope level) but also in pleomorphic multivesicular bodies adjacent to neuronal perikarya, as well as in microtubule-bearing axons near to nerve cell bodies [21]. The pleomorphic multivesicular bodies adjacent to neurones correspond to the coarse deposits of ubiquitin–protein conjugates visualized by ubiquitin immunocytochemistry [29] and represent lysosome-related bodies in axosomal processes.

Gold particles corresponding to a lysosomal marker enzyme β-glucuronidase are seen in lysosome-related dense bodies adjacent to large spongiform lesions as well as with debris in areas of spongiosis [21]. Heat-shock 70 cognate proteins have been shown to be involved in the uptake of proteins into lysosomes in stressed cells [31]. Gold particles specific for a heat-shock protein 70 (hsp70) can be seen spilling from dense bodies beside small areas of rarefaction and also associated with debris in large spongiform areas [21]. Presumably, distended pleomorphic lysosome-related vesicular bodies rupture to release the hsp70. These observations on the distribution of β-glucuronidase and hsp70 [21] support those on ubiquitin–protein conjugates; release of proteins from lysosome-related dense vesicular bodies leads to the generation of the earliest type of spongiform lesions in the prion encephalopathies.

The reason for the disruption of lysosomal organelles in scrapie-infected brain may be that the abnormal isoform of the prion protein (PrPSc) accumulates in neuronal lysosome-related dense bodies. The PrPSc can be specifically detected by immunogold electron microscopy with special fixation and processing procedures employing guanidinium thiocyanate [32]. The PrPSc can be seen in dense bodies in the neuropil and in dense bodies in axons [21] as well as in secondary lysosome remnants in areas of rarefaction. The PrPSc is present, therefore, inside lysosome-related organelles: progressive accumulation of PrPSc in these structures may lead to the disruptive events indicated by the distribution of immunospecific gold particles corresponding to ubiquitin–protein conjugates, β-glucuronidase and hsp70 [21]. None of the lysosome-related organelles seen in scrapie-infected mouse brain are visible in non-infected controls.

The immunogold microscopical studies on scrapie-infected mouse brain provide the in vivo corroborations of in vitro studies on scrapie-infected neuroblastoma cells, where PrPSc is seen to accumulate in lysosome-related organelles [32, 33].

The transmissible encephalopathies pose a fascinating set of problems for modern molecular biology in that an enormous amount of effort has failed to find nucleic acid associated with the infectious agent. We are therefore faced with a transmissible genetic disease where the mode of replication of the infective agent is unknown [34].

The agent is likely to be a novel type of infectious particle based on the PrPSc isoform of the normal cell protein PrP. The basic questions to address, therefore, are where can infectious particles containing PrPSc form and how can the PrPSc be produced in the infected cells?

The demonstration that PrPSc is found in lysosome-related organelles both in infected cells [32, 33] and in brain tissue [21] may provide the answers to these questions. The lysosome-endosome system is a site for the generation of the PrPSc from PrP. The interior milieu of lysosome-related organelles provides the ideal environment for adventitious protein–protein interactions which will result in the ‘hijacking’ of PrP by secondary structural interactions with PrPSc leading to the biogenesis of more PrPSc: in this way the lysosome can serve as a ‘bioreactor’ for PrPSc formation [21].
We would like to thank the Agricultural and Food Research Council, the Motor Neurone Disease Association, the Wellcome Trust and the Parkinson’s Disease Society of Great Britain for support of this work.


Received 16 April 1992