# Ubiquitin and Neurodegenerative Diseases

***UBIQUITI'N VE NORODEJENERATIF HASTALIKLAR***

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## UBIQUITIN

In 1975, during the characterisation of calf thymus peptide hormones, Gideon Goldstein et al. Identified a protein which appeared to induce B-lympocyte differentiation (1). Interestingly, antibodies raised to this protein cross reacted with proteins of other organisms such as yeast and plants. Hence, the protein was termed ubiquitous immunopoietic peptide, later to be renamed ubiquitin (Ub). Ubiquitin was first sequenced by Schlesinger et al (2). The sequence is comprised of 76 amino acids, secondary structure includes a single alfa-helix, with four strands of B-sheet. The primary structure is highly conserved in nature and differing by only three amino acids from animal to yeast. This degree of conservation emphasises the fundemental role played by Ub in cells. Ub is extremely stable to increased temperature, changes in pH and proteolysis (3) .

Cellular Ub is found in both free and conjugated forms, greater than 50% of the Ub in a particular cell may be of the free form.

Ub can from both stable and unstable conjugates with a variety of different proteins in-vivo. The stable Ub-protein conjugates include:

i. Histones; H2A and H2B, approximately 11 % of H2A and 1.5% of H2B was found to be ubiquitinated (4) .

ii. Cell surface receptors; the lymphocyte homing receptor is ubiquitinated on its external domain. It is possible that the Ub may modulate the affinity of the receptor, or may be involved in signal transduction (7.8).

Unstable Ub-protein conjugates are conjugates which are normally soon degraded after formation. In this case, the ubiquitination is targeting proteins for degradation, the Ub is acting as a cofactor of selective protein catabolism. It has been suggested that nearly 90% of the proteolysis of short lived proteins in mouse mammalian carcinoma cells may proceed through the Ub catalyzed pathway (9-11).

The enzymes involved in conjugation of Ub to a target protein are imaginatively termed E1, E2 and E3. E1 is Ub activating enzyme, E2s are Ub carrier proteins and E3s are proteins thought to recognize the target protein substrate (12-18). Conjugated Ub to abnormal proteins is then removed by enzymes known as Ub-C-terminal hydrolyses. Ub-dependent protein degradation system with their enzymes is summarized in Fig. 1. Using DEAE column chromatography and rapid HPLC assay, three peaks of activity of Ub specific esterases were identified and separated from calf thymus (19). It has also been shown that the abundant neuron specific protein PGP 9.5 is in fact a Ub-C-terminal hydrolyze (20-22).

Ub was implicated in ATP dependent proteolysis. It was proposed that the attachment of Ub can signal degradation of the target protein. It has recently been suggested that Ub protein conjugates may be directed towards the lysosome for degradation, hence Ub may be involved in two major catabolic system. Doherty and others showed that when fibroblasts were incubated with the thiol protease inhibitor E64, which is known to inhibit lysosome cysteine catephsins, Ub-protein conjugates accumulate in multivesicular bodies related to lysosomes (23-25). There have also been recent reports of the localization of free Ub in lysosomes of hepatoma cells (26). It now appears that Ub-protein conjugates are also enriched in lysosomes of normal cells with enrichments of up to 12 fold of the concentrations seen in all other cytoplasmic organelles. This shows that the lysosomal accumulation of Ub-protein conjugates is likely to be a normal cellular event. Ub gene organization shows some interesting features. In yeast, four different genes which code for Ub are known, termed Ubi1, Ubi2, Ubi3 and Ubi 4 (27,28). In humans, three different genes code for the Ub sequence. The genes are termed UbiA, UbiB and UbiC

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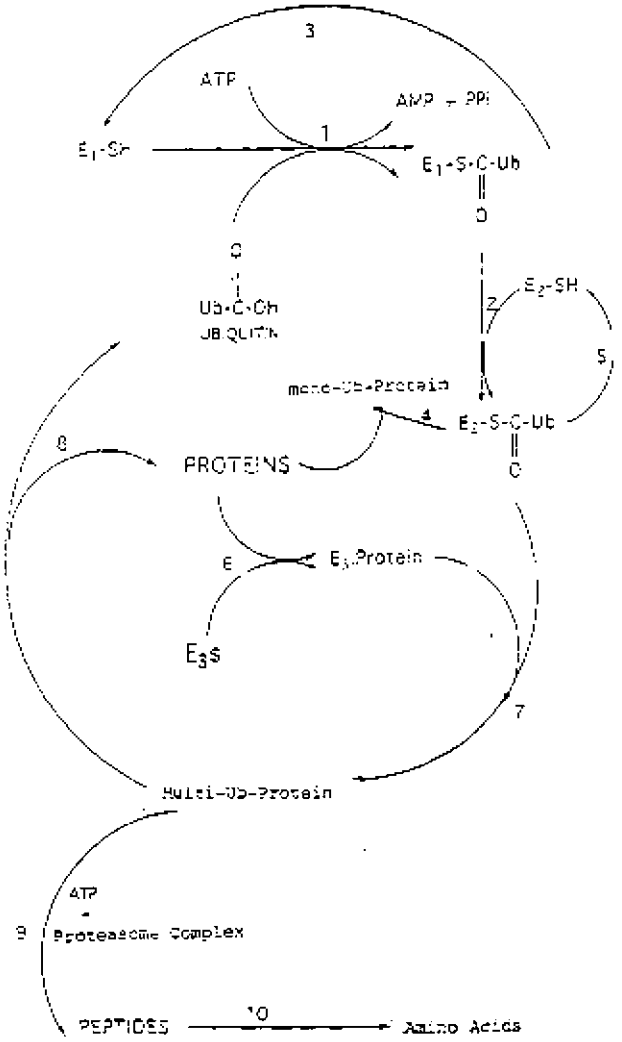


**Figure 1.** Proposed sequence of events in the conjugation and degradation of proteins via the ubiquitin system

1. Activation of ubiquitin by E1.
2. Transfer of the high-energy ubiquitin intermediate to E2.
3. Recycling of E1.
4. Conjugation of ubiquitin directly to the protein from E2.
5. Recycling of E2.
6. In the case of particular proteins, involvement of E3.
7. Conjugation of further molecules of ubiquitin to the protein substrate.
8. Recycling of an intact substrate and ubiquitin.
9. ATP-dependent degradation of conjugates into peptides by the 26S proteasome complex.
10. Release of amino acids from peptides.

(29,30). UbiB and UbiC genes code for polyUb se¬quences of three and nine copies of Ub respectively.

## WHAT IS THE RELATION BETWEEN UBIQUITIN AND NEURODEGENERATIVE DISEASES?

Much interest in the etiology and pathogenesis of Alzheimer's disease (AD) has arisen since the realization that AO is responsible for approximately half of the cases of dementia seen. In 1987, Ub was found to be a component of the paired helical filaments (PHP') which are intermediate sized filaments (IF) found in the brains of patients with AD (31-34). PHF are the prencibal constituent of neurofibrillary tangles (NFT) (a type of inclusion body) of AD and also occur as bun¬dles in dystrophic neurites which form plaques in the brains of AD patients. Immunochemical studies have identified the microtubule associated phosphoproteins tau as a major component of PHF. Ub is thought to be conjugated to this tau protein. Ub has also been detected in granulovascular bodies in hippocampi pyra¬midal neurons in AD (32-34). When other neurodege-nerative diseases were studied, using polyclonal anti¬bodies with a high affinity to conjugated Ub as a tool to detect Ub-protein conjugates, other cellular inclu¬sions bodies of man were found to be immunoreactive (35,36). These Ub reactive inclusions are shown in Table 1.

Example, Lewy bodies of Parkinson's disease; brainstem Lewy bodies stained intensely for both Ub and phosphorylated neurofilament proteins at the peri¬phery. Cortical Lewy bodies stain more homogeneous¬ly for Ub. Immunohistochemistry has been used to de¬monstrate that diffuse Lewy body disease is more common than previously thought, accounting for ap¬proximately a third of dementia cases (37).

Example, Pick's bodies of Pick's disease; these rounded inclusion bodies stain for Ub in a punctuate and weak staining pattern. Tau has also been detec¬ted in Picks's bodies. Pick's bodies are thought to be a mixture of microtubules and neurofilaments which possibly sets them aside from the family of pure IF in¬clusion bodies.

Example, Rosenthal fibres within astrocytic tu¬mors; these hyaline inclusion bodies have granular ir¬regular cores surrounded by bundles of IF. The peri¬phery of the bodies stains strongly for Ub, the core has been found to contain the HSP B-crystallin. Glial fibrillary acidic protein (GFAP) has also been detected in the Rosenthal fibre. It is thought the fibre may be the degenerated center of a ubiquitinated GFAP IF in¬clusion body.

Example, Cytoplasmic bodies within muscle; these fibrillary inclusions are found in diseased states such as rare congenital myopathy. They stain strongly for Ub and are also immunoreactive for desmin.

Example, Mallory bodies of alcholic liver disease; several structural types of these bodies are known, they contain cytokeratin with other non cytokeratin pro¬teins. Type III Mallory bodies have a central amor¬phous core which does not stain for Ub, and a strong Ub staining periphery. B-crystalline has also been de¬tected in Mallory bodies. Thus, Ub is common to many IF inclusions characteristic of major diseases, and is li¬kely to have a fundamental but as yet unexplained

**Table 1. Disease with Ubiquitin immunoreactive intracellular inclusions**

| Disease | Type of cell | Inclusion body | intermediate filament type |
|---|---|---|---|
| Alzheimer's disease | Neuron | Neurofibrillary tangle | (Paired helical filaments) |
| Parkinson's disease | Brainstem neuron | Brainstem lewy body | Neurofilament |
| Lewy body dementia | Cortical neuron | Cortical lewy body | Neurofilament |
| Pick's disease | Cortical neuron | Pick body | Neurofilament |
| Motor neurone disease | Spinal anterior horn neuron | Motor neurone disease inclusion | Not known |
| Cerebellar astrocytoma | Astrocyte | Rosenthal fibre | Glial fibrillary acidic protein |
| Alcholtc liver disease | Hepatocyte | Mallory body | Cytokeratin |
| Cytoplasmic body myopathy | Muscle | Cytoplasmic body | Desmin |

role in the metabolism of the IF inclusions. It is apparent that different types of IF inclusions exit which stain differently with the anti-Ub antibody. The rarer granular cytoplasmic staining of Ub in some cells suggests that protein ubiquitination may actually occur before the inclusion body formation. The mechanism of Ub IF inclusion body formation is however unknown, but relevant information might be gained from studying metabolism of IF in virally infected cells (35-38).

Anti-Ub immunohistochemistry has been extended to study other disease states. In motor neuron disease a novel inclusion body has been identified in spinal anterior horn neurons (39). The inclusion body exists as;

i. loose tangles or skeins of Ub positive material in the cytoplasm

ii. tight balls of Ub positive filaments which stain strongly for Ub

iii. multiple small rounded bodies, 2-7 um in diameter.

The inclusion bodies appear closely associated with classical Bunina bodies of motor neuron disease e.g. Ub positive material sometimes surrounds the Bunina body area. The Bunina bodies may be equivalent to the amorphous core often found in Ub filamentous inclusions (35). In motor neuron disease patients there has been shown to be a two fold increase in the expression of the poly UbiC gene in the motor cortex and spinal cord (40). This suggests the cells response is activated in this neurodegenerative disease, which may lead to the formation of the Ub filamentous inclusion.

## CONCLUSION

It has been shown that the formation of filamentous inclusion bodies is a general cellular response in many chronic neurodegenerative diseases. Ub seems to be a common factor in these inclusion bodies. There are likely to be different mechanisms for inclusion body formation, but the occurence of Ub may be the key which leads to the elucidation of a common pathway in inclusion body formation and an explanation of the role of IF in neurodegenerative diseases. A through study of the human enzymes involved in Ub metabolism e.g. E1, E2 is a logical starting point for further investigations into the mechanism of Ub IF inclusion body formation. A thorough understanding of this mechanism could give insights into the initial stimuli for inclusion body formation, thus directing further research into therapeutic strategies to combat the diseases.

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