Clearance of intracellular aggregation-prone protein fragments

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CHAPTER 1

Introduction and outline

INTRODUCTION

Proteins are involved in the regulation of many cellular processes. Therefore, cells need to regulate their protein levels carefully for cellular homeostasis. Protein levels are determined by the balance between protein synthesis and degradation. For correct function of newly-synthesized proteins, they have to be correctly folded, modified and assembled. These processes are assisted by molecular chaperones that stabilize non-native conformations, thereby preventing aggregation of non-native proteins. However, a considerable amount of newly-synthesized proteins is defective and therefore quickly degraded. Two pathways have evolved that can degrade intracellular proteins, the ubiquitin-proteasome system (UPS) and autophagy. The majority of intracellular proteins are degraded via the UPS, where selective poly-ubiquitination serves as a signal for proteasomal degradation. Proteasomes cleave proteins into peptides, which are recycled into amino acids by aminopeptidases acting downstream of the proteasome. Autophagy is the lysosome-dependent degradation of mainly long-lived cytoplasmic proteins, protein complexes and cell organelles. Despite the involvement of these pathways in protein quality control, various neurodegenerative diseases are hallmarked by the accumulation and aggregation of proteins leading to neuronal loss. These neurodegenerative diseases include Alzheimer's disease, Parkinson's disease and various polyglutamine (PolyQ) disorders such as Huntington's disease (Goldberg, 2003; Li et al., 2008; Rubinsztein, 2006).

In the present thesis, we focus on Alzheimer's disease and polyQ disorders. These disorders are initiated by the accumulation of protein fragments instead of full-length proteins. Alzheimer's disease is the most common form of dementia and is hallmarked by extracellular senile plaques and intracellular neurofibrillary tangles in post-mortem brains of Alzheimer's disease patients. Alzheimer's disease is initiated by the aggregation-prone amyloid-β (Aβ) peptide, which is generated by sequential cleavage of the amyloid precursor protein. Extracellular plaques composed of Aβ peptides were considered to initiate toxicity, but evidence is accumulating that oligomeric intermediates of intracellular Aβ are associated with the early stages in the pathogenesis of Alzheimer's disease (LaFerla et al., 2007; Selkoe, 2004).

PolyQ disorders are a group of nine dominantly inherited, slowly progressive neurodegenerative disorders. These disorders are caused by an expansion of the polyQ tract over 40 glutamines within the disease-related protein. The length of the polyQ tract is inversely related with the onset of disease, which becomes manifest around midlife (Orr and Zoghbi, 2007). Aggregates in brains of patients and mice suffering from various polyQ disorders mainly contain proteolytic fragments of the polyQ-expanded protein that includes the polyQ tract (Butler et al., 1998; DiFiglia et al., 1997; Schilling et al., 1999). These fragments containing the expanded polyQ tract are more aggregation-prone and more toxic than their full-length analogues (Cooper et al., 1998; Ellerby et al., 1999; Gafni et al., 2004; Haacke et al., 2006; Mangiarini et al., 1996; Schilling et al., 1999; Young et al., 2007). These data resulted in the toxic fragment hypothesis which states that proteolytic fragments initiate aggregation and cause toxicity. This is similar to Alzheimer's disease where fragments initiate aggregation and toxicity.

The protein quality control system declines with age, even in the absence of disease (Morimoto and Cuervo, 2009). Therefore, aggregation-prone proteins, such as Aβ peptides and expanded polyQ protein fragments may become less efficiently cleared with increasing age

leading to their accumulation and subsequent aggregation. This may also explain why neurodegenerative disorders are late-onset disorders. So far, the majority of studies have been focused on the prevention of the generation of these toxic fragments (Gafni et al., 2004; Selkoe and Wolfe, 2007; Wellington et al., 2000). However, we aim to study the behavior of aggregation-prone protein fragments in living cells and to stimulate their clearance via the UPS, peptidases and autophagy, as well as the assistance by chaperones.

OUTLINE

The UPS can degrade most proteins efficiently into peptides, but little is known about the way cells deal with peptides that show resistance to degradation by downstream aminopeptidases. In **chapter 2**, we designed a fluorescent peptidase-resistant peptide. We introduced this peptide into living cells via micro-injection and analyzed whether cells have alternative mechanisms to cope with these peptides.

Proteasomes are able to degrade polyQ-containing proteins, but fail to cleave within expanded polyQ tracts. As a result, polyQ peptides are released into the cytoplasm. In **chapter 3**, we mimic this proteasomal release of polyQ peptides in living cells and examine whether these polyQ peptides are resistant to degradation by cytoplasmic proteases. We show here that expanded polyQ peptides accumulate and are sufficient to induce aggregation and toxicity. Therefore, we focus in **chapter 4** on prevention of aggregation and enhancing clearance of these expanded polyQ peptides by overexpression of various chaperones. In **chapter 5**, we study the dynamics of polyQ peptides in more detail and compare polyQ peptides with larger polyQ-expanded huntingtin fragments by studying their interactions with various components that are sequestered in aggregates. In **chapter 6**, we focus on the aggregation-prone Aβ peptide causing Alzheimer's disease and study whether cytoplasmic Aβ peptides are peptidase-resistant or become efficiently degraded.

Finally, we review and discuss in **chapter 7** how aggregation and cytotoxicity of intracellular toxic fragments can be prevented, focusing on the UPS and autophagy as degradation machineries and on assisting chaperones.

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