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Review

Longitudinal measures of proteostasis in live neurons: Features that determine fate in models of neurodegenerative disease

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ABSTRACT

Protein misfolding and proteostasis decline is a common feature of many neurodegenerative diseases. However, modeling the complexity of proteostasis and the global cellular consequences of its disruption is a challenge, particularly in live neurons. Although conventional approaches, based on population measures and single "snapshots", can identify cellular changes during neurodegeneration, they fail to determine if these cellular events drive cell death or act as adaptive responses. Alternatively, a "systems" cell biology approach known as longitudinal survival analysis enables single neurons to be followed over the course of neurodegeneration. By capturing the dynamics of misfolded proteins and the multiple cellular events that occur along the way, the relationship of these events to each other and their importance and role during cell death can be determined. Quantitative models of proteostasis dysfunction may yield unique insight and novel therapeutic strategies for neurodegenerative disease.

1. Introduction

Proteostasis involves a dynamic and highly integrated network of millions of proteins. Multiple cellular processes, intricately integrated, ensure homeostasis [1]. Breakdown of the network leads to cellular dysfunction and cell death [2]. Much effort has focused on determining if disruption of proteostasis is causally linked to neurodegenerative diseases, including Parkinson's disease (PD), Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD) [3]. Neurodegenerative diseases normally present late in life with different symptoms, but they all involve deposits of insoluble protein in the brain [4]. At a molecular level, these diseases, also termed proteinopathies, are caused by distinct proteins, but they all undergo protein misfolding, show similarities in the multiple cellular pathways that are disrupted, and eventually lead to neuronal death [5]. Despite this convergence in cellular consequences, strategies to enhance proteostasis have not been translated into therapies.

Recently, considerable interest has been directed towards modeling disease to capture early changes and the temporal and spatial progression of dysfunction and adaptive responses, and ultimately, to relate these events to cell death [6,7]. Models that more faithfully recapitulate the complexity of the disease may improve the success rate of biomedical drugs [8]. Here, we will discuss the properties of proteostasis and neurodegeneration that make them difficult to model and describe a "systems" biology approach to model their complexity.

1.1. Modeling the complexity of proteostasis in single cells

Given the complexity of proteostasis, determining how proteins misfold and why cells fail to handle them is a challenge. The presence of misfolded proteins is probably a consequence of opposing pressures on structural stability and functional flexibility [9]. As the abundance of a protein imposes a stronger evolutionary pressure on its coding sequence than its actual function [10], the cost of protein misfolding to the fate of the cell may be high. In a cell, the accumulation of misfolded protein reflects a decline in the cell's ability to maintain proteome stability. In some models, only a 4 °C increase is enough to destabilize at least 16% of the proteome [11], suggesting that even small perturbations can greatly affect the proteome. Common causes of neurodegenerative disease favor protein misfolding, including mutations in disease-associated proteins and exposure to environmental stimuli, such as oxidative stress [12,13].

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Most of our knowledge about the folding and unfolding dynamics of proteins is based on in vitro biochemical approaches and in silico studies [14,15]. These methods have provided a wealth of knowledge and shaped a number of concepts surrounding protein folding, including the role for intermediate species [16] and folding energy landscapes [17,18]. These studies often focus on proteins that fold rapidly and rarely misfold and aggregate [16]. However, in neurodegenerative diseases, conformational instability and aggregation prevail [19] and the folding landscapes and intermediate species of disease-related misfolded proteins may require a different understanding. A remarkable number of disease-associated proteins are intrinsically unstructured and exhibit conformational promiscuity. Although this allows for multitasking, mutations that disrupt function may lead to collapse of a network of cellular processes [20].

In vitro biochemical and in silico approaches also lack the cellular milieu that is essential for protein dynamics. Post-translational modifications and intracellular crowding of macromolecules, including chaperones, affect protein interactions [21] that, in turn, influence the folding rates [22], stability, and function of proteins [23–25]. Ideally, physiologically relevant measurements of proteostasis should be carried out in live cells. There is a growing interest in modeling biological systems through a "middle-out" approach, in which the cell is the basic unit of the system and contains spatiotemporal information at multiple levels [26]. Information quantified at each level of the cell can be used to build predictive models that measure the effects of misfolded proteins on the cell. At a genetic level, modifiers can influence the cell's capacity to cope with misfolded proteins. For example, in an ALS model, temperature-sensitive mutations in various unrelated genes enhance misfolding of superoxide dismutase 1 (SOD1) [12]. At a molecular level, misfolded proteins can be measured to determine how processes, such as transcription, translation, folding, trafficking, and degradation affect their dynamics [1]. Conformational sensors can be used to measure rates of misfolding [27] or the effects of protein misfolding on proteome stability [28]. Reporters can also provide readouts for the activation of adaptive strategies, including the heat shock and unfolded protein responses [29], or mechanisms that target misfolded proteins for degradation [30]. These pathways are critical for modulating protein misfolding and toxicity in multiple models of neurodegeneration, such as ALS, PD, and HD [31–34]. Molecular complexes and organelle dynamics can also be measured in a cell, providing insight into the multiple cell processes that coincide with the build-up of misfolded proteins, including mitochondrial dysfunction, aberrant trafficking, synapse dysfunction and altered signaling [35].

Using the cell as the basic unit of the system to measure the impact of protein misfolding requires the simultaneous capture of both the dynamics of the misfolded protein and the stochastic cellular changes that result. In addition to identifying how misfolded proteins cause cell dysfunction, it is also critical to determine which cellular events drive neurodegeneration. Although some cellular events may be harmful, some changes may be incidental or even adaptive responses to more subtle maladaptive changes elsewhere in the cell.

1.2. Separating pathogenic events from adaptive strategies

Neurodegeneration is progressive and may occur along a single pathway or multiple distinct cellular pathways that arise from the same initial insult [36]. In addition, the roles of the cellular events during neurodegeneration may differ. Some may reflect true pathogenic insults, whereas others may be beneficial, adaptive strategies that are up-regulated to cope with the build-up of misfolded proteins [29] (Fig. 1). The extent to which a true coping response is activated is dependent on the pathogenic event that incites it.

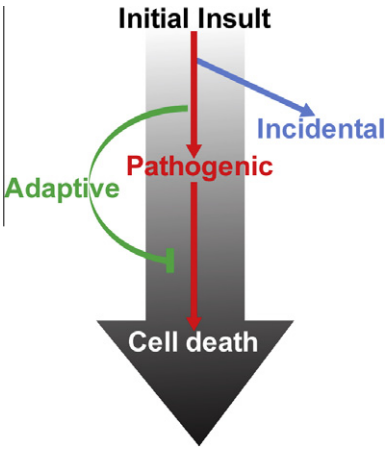

Fig. 1. The different roles of cellular events during neurodegeneration. Schematic shows how, during the course of neurodegeneration, cellular changes may be pathogenic. Alternatively, some changes may be incidental events, while others may be adaptive responses that occur to cope with the pathological events within the cell. As these events may occur in parallel and correlate with cell death, distinguishing their roles can be difficult.

Therefore, pathogenic events and coping responses will occur in parallel, and both will correlate with cell death, making separating their roles during the disease process very difficult.

For example, amyloid-like structures form as a common feature of many neurodegenerative diseases [3]. Early reports implicated amyloid deposits as the toxic species because they were consistently found in the brains of deceased patients. At the time, this seemed to be a reasonable conclusion. The distribution of various pathologies provides temporal resolution of the activities of disease-related proteins within those pathological changes. However, any given sample represents only a single "snapshot" in the life of the protein. In addition, conclusions based on pathological events from postmortem tissue might represent a bias of ascertainment: the tissue comes from patients who have already lost many of the specific neurons affected by the disease. Furthermore, although amyloid deposits were found in many deceased patients, many non-toxic proteins also form amyloid structures [37]. Mounting evidence suggests that amyloid structures sequester toxic misfolded conformers and principally serve as a coping response by the cell [38–40]. This adaptive response is becoming an increasingly common theme in the study of the major neurodegenerative disorders [41].

The unfolded protein response might also be activated in response to rising levels of misfolded protein in the cell [42]. It is mediated, in part, by phosphorylation of the α-subunit of eukaryotic translation initiation factor (elf2α-P), which is found in greater amounts in AD and PD patients than in non-patients and causes the transient shutdown of protein translation, including that of the misfolded protein [43,44]. Protein translation requires a great deal of energy [45], and repressing translation allows reallocation of molecular chaperones to detect and respond to protein misfolding elsewhere in the cell [46] and to promote the selective translation of stress-response genes [47]. However, repressing translation for too long can be detrimental to the cell. Accumulation of the misfolded prion protein is associated with synaptic dysfunction, neurodegeneration and persistent translational repression of global protein synthesis by elf2α-P. Interestingly, irrespective of the presence of the misfolded prion protein, stimulating protein translation preserved synapses and rescued neurodegeneration [48]. It remains unknown if restoring translation prevents neurodegeneration in the long term, even if the initial insult, misfolded prion protein accumulation, is not directly addressed.