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Proteome folding and aggregation

Michele Vendruscolo

The description of protein folding at the proteome level requires further principles in addition to those that govern this phenomenon for individual molecules. An important aspect of the increased complexity of the folding process in the cellular environment is that proteins tend to be metastable against aggregation, as they are often expressed at levels at which they are poorly soluble. The maintenance of the solubility of the proteome requires the coordinated intervention of a range of quality control mechanisms, which include molecular chaperones, trafficking and degradation pathways, post-translational modifications and transcriptional and translational control. As these regulatory mechanisms should always be active to keep proteins in their soluble state, their impairment upon ageing or environmental stress can lead to the disruption of protein homeostasis resulting in uncontrolled widespread aggregation and disease.

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Introduction

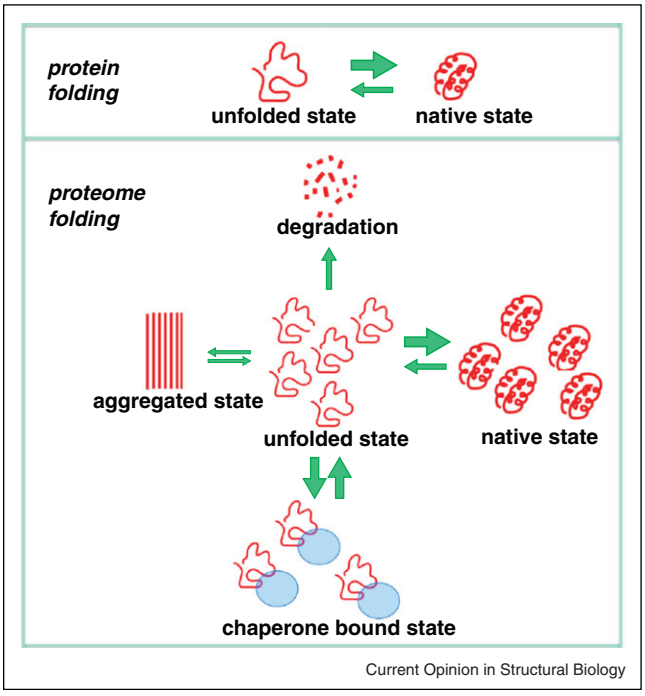
Proteins are involved in nearly all the biochemical reactions that take place in living organisms. In order to carry out their functions the majority of them fold into well-defined three-dimensional native structures by a spontaneous process that is relatively robust against perturbations [1,2]. The task of reaching and maintaining such native states amongst the vast multitude of possible alternative conformations that proteins can adopt is facilitated by the presence of an energetic bias towards the native states themselves [3,4]. Most of this knowledge, however, comes from *in vitro* studies, which concern diluted buffer solutions, and thus provide a picture that tends to apply to individual molecules in isolation. With the advent of quantitative methods to monitor the behaviour of proteins in living systems, it is becoming increasingly clear that these molecules *in vivo* do not follow just the relatively small set of rules defined from experiments carried out in the test tube, but a considerably more complex code [5–15]. In the cell, proteins are expressed at very high concentrations and they interact with a heterogeneous environment. Although the fundamental nature of the process by which individual protein molecules fold is similar *in vitro* and *in vivo*, the description of the manner in which folding is achieved at the proteome-wide level requires further concepts to be established. As put by Phil Anderson, 'more is different' [16], so that complex systems are not only regulated by just the fundamental laws that govern their component parts, but also by additional principles that describe their collective behaviour. In this sense, protein folding and proteome folding are different phenomena, as the former refers to a molecular-level process, whilst the latter to a cellular-level one.

Proteome folding

Proteins fold *in vivo* in a complex environment in the presence of a multitude of other macromolecules and metabolites [5–15]. As they emerge from the ribosome as nascent polypeptide chains, before reaching their native states, proteins are exposed to a series of potentially dangerous interactions. Despite the fact that folding is a spontaneous and robust process, in order to facilitate and protect it, a battery of molecular chaperones, disulfide and peptidyl-prolyl isomerases, post-translational modification enzymes and other ancillary factors are present [5–12]. Thus, although proteins experience a variety of challenges whilst they fold, they can still reach safely their native states provided that appropriate control mechanisms are in place. Overall, protein synthesis, folding and degradation are balanced carefully in order to guarantee that the vast network of biochemical reactions sustaining living organisms can operate in the correct manner [6,17–21]. It is also particularly important to avoid dysfunctional interactions leading to misfolding and aggregation [20,22,23], which are processes often associated with disease [2,24].

By combining the conceptual advances made through *in vitro* and *in vivo* studies, it is becoming clear that protein folding in the cell should be considered as a collective phenomenon, which involves the proteome as a whole. From the point of view of a cell, folding, rather than a process concerning individual molecules, is a phenomenon that requires the participation of a variety of different molecules, which by working in close collaboration ensure that the whole system functions properly (Figure 1). The maintenance of a functional proteome requires hundreds of genes, if not more [6], which regulate the processes of synthesis, folding, trafficking

Figure 1



More is different. The laws that regulate the folding process of individual proteins in the test tube are not sufficient to describe the collective behaviour of these molecules in living systems. In the cellular environment, additional principles are required to specify how protein homeostasis is maintained through a balanced interplay between expression, folding, aggregation, regulation and degradation processes.

and degradation mechanisms, thus establishing protein homeostasis [8,9,25].

Proteome aggregation

The aggregation of proteins is progressively being recognized as one of the most fundamental processes involving these molecules [2,26], and consequently as a widespread phenomenon in the cellular environment [9,27••,28,29•,30]. It has been observed that hundreds of proteins in *Escherichia coli* are at high risk of aggregation, and that a small but significant fraction (perhaps about 1–3%) of these proteins form, at least transiently, aggregates under mildly destabilizing conditions [31,32,33•]. Proteome-level aggregation has also been observed in yeast in the stationary phase. Under these conditions, nearly 200 proteins were found to self-associate into deposits, which in some cases were observed to revert to a soluble form when normal conditions were restored [29•]. These observations open the intriguing possibility that the direction of misfolded proteins into large and perhaps relatively inert aggregates could represent a last-line of defense to cope with stress conditions [23,29•,34].

The existence of a metastable sub-proteome has been further illustrated by a recent study in which a wide number of human proteins have been shown to be at risk of co-aggregating with amyloid-forming polypeptide chains [35••]. These problematic proteins were found to be relatively large in size and only weakly hydrophobic, as well as to exhibit elevated structural flexibility and enrichment in disordered regions. All these features render such proteins particularly prone to experiencing aberrant interactions, as they are unable to protect effectively their aggregation-prone regions, either as a consequence of their large sizes, which make them fold slowly after biosynthesis, or because of the presence of extended disordered regions even after reaching the native state [35••]. In a related study, a pool of metastable proteins with destabilizing temperature-sensitive mutations was observed in *Caenorhabditis elegans* to form deposits upon aggregation of polyglutamine expansions [28]. In a vicious circle, such proteins, which normally exhibit an apparently normal behaviour, under stress conditions were found to further enhance the aggregation of the polyglutamine proteins [28]. These results indicate that when a particular protein undergoes misfolding and aggregation, other proteins that share the same regulatory pathways become at risk themselves [11,25,28].

These observations indicate that proteomes are intrinsically metastable and that under stress their predisposition to aggregate can be increased. It has been suggested that at the concentrations at which they are expressed in the cell, proteins are only marginally soluble [30]. This observation is particularly important since the maintenance of proteins in a soluble state represents one of the most fundamental principles that regulate the behaviour of proteins in living organisms (Table 1). The very high concentration at which proteins are expressed in relation to their intrinsic tendency to aggregate, creates a challenging situation

Table 1

Anfinsen's thermodynamic hypothesis [70], which states that the native structures of proteins are encoded in their amino acid sequences, is well established for dilute solutions [1–5]. At the concentrations typically found in living organisms, however, the folding process becomes prone to errors, often resulting in misfolding and aggregation [2,5,26]. Indeed, according to the 'life on the edge hypothesis' proteins are only just soluble at the levels at which they are expressed in the cell [30]. Since under these circumstances aggregation is widespread, quality control mechanisms, including molecular chaperones, degradation pathways and post-translational modifications, are crucial to maintain proteins in a soluble state. These quality control mechanisms are required to be constantly active, giving rise to a Red Queen effect.

Principles of proteome folding	
Protein folding	Thermodynamic hypothesis
Protein solubility	Life on the edge hypothesis
Protein quality control	Red Queen hypothesis

not only because of crowding (i.e. excluded-volume) effects [36–39] and hydrodynamic (i.e. solvent-mediated) interactions [40,41], but also because it is only through the balance between functional and dysfunctional interactions that native states can emerge from the competition between folding and aggregation [42,43]. As common disturbances such as mistranslations, mutations, overexpression and dysregulated post-translational modifications can precipitate proteins into insoluble deposits, in particular in proximity of mitochondria, where the production of reactive oxygen species can often cause molecular damage [44,45], a variety of housekeeping mechanisms, such as the heat shock response in the cytoplasm and the unfolded protein response in the endoplasmic reticulum, are required to enable proteins to remain soluble [6,10,17,19,20,34,46]. Indeed, since misfolding imposes a burden on the cell, highly expressed proteins tend to be more soluble than others [30,47–51], and avoidance of aggregation is a major force that shapes protein evolution [47,48,52–55].

Proteome aggregation and disease

The view that the healthy state of a proteome is only metastable and that aggregation is a pervasive phenomenon in the cell suggests that misfolding diseases represent essentially unavoidable conditions associated with ageing and stress. With the impairment of the housekeeping mechanisms and the accumulation of defects such as those caused by reactive oxygen species, metal ions or mutations, aggregation becomes even more widespread and difficult to control. A recent study in *C. elegans* has shown that the age-related decline of protective mechanisms against aggregation results in the formation of aberrant assemblies by several hundred proteins [27••]. Inhibition of the insulin/insulin-like growth factor (IGF-1) pathways, which regulate the action of a variety of quality control mechanisms, in addition to slowing down ageing, was found to decrease the extent of aggregation, thus suggesting that down-regulation of the more aggregation-prone proteins could be associated with prolonged lifespans [27••]. In another study, thermally destabilized mutants promoted the collapse of proteins homeostasis in *C. elegans* by putting the chaperone system under stress, thus causing premature ageing [56••]. On a more abstract level, an intriguing theoretical analysis has also suggested that the average propensity of aggregation of a proteome correlates with the lifespan of the corresponding organism [57].

The importance of maintaining cellular regulatory systems in a fully functional state has prompted the quest for strategies involving small molecules capable of acting as 'chemical chaperones' that can support the action of the natural defenses against misfolding and aggregation [8,9,58•]. Such therapeutic interventions may be most effective if they are targeted at the prevention of disease, rather than at its direct treatment, as once started, the collapse of protein homeostasis can lead to a cascade of harmful events that is very difficult to control [24,59]. Thus, it has been demonstrated that activation of stress–response anti-aggregation pathways increases the lifespans of *C. elegans* and mice [11,56••,60], and protects from the cytotoxicity associated with the presence of Aβ deposits [61]. Drug-induced activation of proteasome-associated pathways was also found to favour the degradation of misfolded proteins, in particular those that exhibit oxidative damage, thus reducing the level of toxic aggregates [62]. In a recent study, a screen of about 900,000 small molecules was carried out to identify new types of small molecules that can restore protein homeostasis by imitating the molecular signals that enhance the activity of heat shock transcription factor-1 (HSF-1) and can thus induce the expression of the molecular chaperones that are regulated by it [58•].

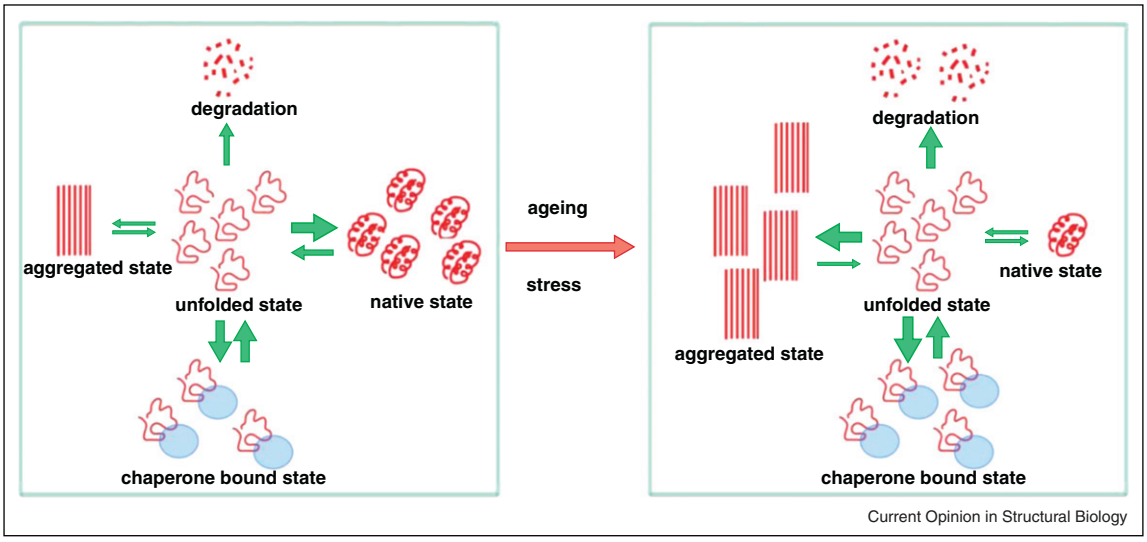
A 'Red Queen effect' in protein homeostasis

If the view that native states are metastable against aggregation [30,63••,64] will receive further support, conceptual frameworks to describe protein homeostasis will have to include the notion that quality control pathways need to be switched on at all times to avoid aggregation (Figure 2). As revealed by the Red Queen in Lewis Carroll's 'Through the Looking-Glass', 'it takes all the running you can do, to keep in the same place'. In the cell, since aggregation is widespread, this phenomenon appears to be at play, since housekeeping mechanisms have to be always active, rather than being just called in to respond to stress or other insults. Thus, proteome folding and aggregation are regulated by a series of principles more articulated than those at play in the test tube under diluted conditions (Table 1).

The constant activity of quality control mechanisms is essential to maintain the balance of molecular processes that results in protein homeostasis. The normal state of a cell is characterized by a steady-state condition characterised by nearly constant populations of the different states in which proteins can be found [65–68]. This situation is achieved through the presence of a variety of interconnected processes that are responsible for the synthesis, degradation, folding, aggregation, trafficking and regulation or proteins (Figure 2). Aggregation events may therefore not represent by themselves a critical challenge to cellular health, as long as the fluxes of proteins in and out of aggregated states is balanced, which normally requires the levels of protein deposits to remain low. Although the maintenance of such steady-state conditions is quite robust with respect to perturbations, acute or persistent offences, such as environmental or genetic factors, or ageing, can eventually disrupt the balance and lead to disease.

When looked at from an evolutionary perspective, this type of steady-state conditions is the result of

Figure 2



A Red Queen effect. Since proteins in the cell are close to their solubility limits, aggregation is a common event. Quality control mechanisms that prevent aggregation and dissolve existing aggregates should therefore be active always, and not just under stress conditions. Age-related impairment of such mechanisms or environmental factors can lead to a disruption of protein homeostasis resulting in widespread aggregation and disease.

evolutionary processes in which proteins have co-evolved with their cellular environment to acquire properties, such as solubility and affinity for chaperones, that make them highly adapted to carry out their biochemical tasks in living organisms. In this sense, the Red Queen effect in molecular biology is analogous to the corresponding one that has been proposed to describe the constant evolutionary arms race between competing species in ecosystems [69].

Conclusions

Recent studies on the phenomena of protein folding and aggregation in the cell have indicated that the proteome is metastable against aggregation. The presence of a variety of housekeeping mechanisms is thus required to maintain proteins in their soluble and functional states. Eventually, however, because of acute or chronic stress, or just as a consequence of normal ageing, protein homeostasis will inevitably become compromised, resulting in disease. Our hope is that this fate can be at least retarded, if not reversed, thus prolonging the duration of healthy lives, by pharmacological interventions developed to support the activities of cellular quality control systems.

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