



## **The misfolding diseases unfold**

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### ***Introduction***

It has become increasingly apparent that there are a number of diseases which, although they have very different symptoms and aetiologies, may have in common a perturbation of protein folding. Here we briefly examine the 'protein misfolding diseases' and speculate on the therapeutic implications of the concept of misfolding as a causative factor in disease.

### ***Protein folding***

Protein folding is the process by which a string of amino acids (the chemical building blocks of protein) interacts with itself to form a stable three-dimensional structure during production of the protein within the cell. The process is roughly analogous to the ways in which a length of wire may be twisted onto or against itself to form various functional entities, for example a spring, a paperclip or a coathanger. Folding occurs very rapidly, probably within milliseconds of production of the string of amino acids, and results in 3-D conformations which usually are quite stable, with specific biological functions.

The folding of proteins thus facilitates the production of discrete functional entities, including enzymes and structural proteins, which allow the various processes associated with life to occur. Importantly, folding not only allows the production of structures which can perform particular functions in the cellular milieu, but also it prevents inappropriate interactions between proteins, in that folding hides elements of the amino acid sequence which if exposed would react non-specifically with other proteins. Restriction of interactions to those which are necessary and desirable for life is crucial in the intracellular environment where many thousands of proteins are present and required to perform precisely specified functions. Evolutionary pressure thus has favoured those proteins which fold in such a way that appropriate reactive elements are exposed and unwanted reactivities are hidden.

### ***Protein misfolding***

On one (reductive) level, life may be thought of as the co-ordinated activity of proteins, and disease as an imbalance of proteins that adversely affects the quality of life – either through too little of a particular protein being present, or too much of a protein, or a protein being produced or rendered dysfunctional, or produced at the wrong place or the wrong time. Inappropriate folding is one way in which a protein imbalance may arise – the misfolded protein may be non-functional or suboptimally functional, or it may be degraded by cellular machinery, or the misfolding may expose epitopes which lead to dysfunctional interactions with other proteins. There are a number of serious diseases which have a common aspect in that they all appear to involve inappropriate folding of a particular protein. These diseases are sometimes lumped together under the heading of the protein misfolding diseases.

## ***Protein misfolding diseases***

In many cases, misfolded proteins are recognised to be undesirable by a group of proteins called heat shock proteins, and consequently directed to protein degradation machinery in the cell. This involves conjugation to the protein ubiquitin, which acts as a tag that directs the proteins to proteasomes, where they are degraded into their constituent amino acids. Hence many protein misfolding diseases are characterised by absence of a key protein, as it has been recognised as dysfunctional and eliminated by the cell's own machinery. Diseases caused by lack of a particular functioning protein, due to its degradation as a consequence of misfolding, include cystic fibrosis (misfolded CFTR protein), Marfan syndrome (misfolded fibrillin), Fabry disease (misfolded alpha galactosidase), Gaucher's disease (misfolded beta glucocerebrosidase) and retinitis pigmentosa 3 (misfolded rhodopsin). In addition, some cancers may be associated with misfolding, and hence ineffective functioning, of tumour suppressor proteins such as von Hippel Lindau protein or p53.

Many protein misfolding diseases are characterised not by disappearance of a protein but by its deposition in insoluble aggregates within the cell. Diseases caused by protein aggregation include Alzheimer's disease (deposits of amyloid beta and tau), Type II diabetes (deposits of amylin), Parkinson's disease (deposits of alpha synuclein), and the spongiform encephalopathies such as Creutzfeldt-Jakob disease (deposits of prion protein). Hereditary transthyretin amyloidosis is caused by the deposition of transthyretin in various tissues, eg the heart (leading to congestive heart failure), or the nerves (leading to peripheral neuropathy).

The diagnostic feature common to the protein aggregation diseases is the deposition of insoluble protein aggregates called amyloid fibrils, hence the generic term amyloidosis. The fibrils may themselves be assembled into discrete bodies, termed plaques. In each disease the misfolded protein is unique (ie a different protein is associated with each disease), and a unique set of tissues is affected, but the deposited amyloid fibrils produced by aggregation of the misfolded protein appear structurally very similar. These deposits are found in various subsets of cells, frequently neurons, and may cause their dysfunction and eventual death, with all the typical symptoms of the disease in question. However it should be noted that it is not completely clear whether amyloid fibril deposition is always the cause of disease, or merely a symptom of it; and if it is a cause, it is not always clear whether consequent pathology is due to non-functioning of the parent protein or to direct toxicity of the aggregate.

Protein misfolding appears at least in some cases to be due to mutations (missing or incorrect amino acids) in the protein which destabilise it such that it is more likely to fold incorrectly. It may be that the more destabilising the mutation, the earlier the onset of disease in the patient, as illustrated by the correlation between onset and the various mutations of the protein transthyretin in familial amyloidotic polyneuropathy. Such destabilising mutations do not always appear inherently to alter the structure or function of the protein, but may simply make it more likely to adopt an inappropriate conformation at some point, and hence to provide the opportunity for inappropriate aggregation. In other cases mutations in the amino acid sequence may directly promote amyloid formation, in that they may increase the likelihood of aggregation of a misfolded protein, rather than increasing the likelihood of it misfolding.

However, in many cases amyloid diseases are not clearly associated with genetic mutations; eg Alzheimer's is quite common in the aged but cases of Alzheimer's with a clear genetic causation are relatively rare. This may simply be because

there are a very wide range of genetic mutations which can give rise to the same disease, of which very few have been identified. Alternatively, the misfolding could occur due to progressively lower levels of chaperone proteins in ageing neurons. Chaperone proteins, such as the heat shock proteins, protect other proteins against misfolding by stabilising them, and usually remove them when misfolded. Lower levels of chaperone proteins, or less effective chaperoning, could be one element of a progressively less well-controlled cellular environment that may be a feature of ageing. It may also be that mutations or other changes in the chaperone proteins themselves cause them to actually promote misfolding, rather than guard against it.

### ***Implications for therapy – “rechaperoning”***

If the protein misfolding diseases share a common aetiology in that proteins are misfolded then there may be common features to successful therapies for these diseases. Thus entities which can stabilise the protein in its appropriate conformation may prevent or delay the onset of fibril deposition. For example, familial amyloid polyneuropathy I may be amenable to approaches using analogues of the thyroid hormone which bind to and stabilise misfoldable transthyretin, thus maintaining the protein in its desired conformation. In effect, therefore, the misfolding diseases may be treatable by augmenting or replacing the natural chaperoning functions which may be ineffective for one reason or another.

As many of the misfolding diseases are manifest in very large numbers of cells, and tend not to be anatomically localised, any such ‘rechaperoning’ therapies would need to diffuse throughout the affected tissues into most cellular compartments, implying a small molecule approach. In addition, as the rechaperones would at best prevent or delay progression of disease, rather than curing the cause of the disease, patients would have to use the therapy for the rest of their lives. If effective, the therapy might also be used prophylactically in symptomless patients to prevent onset of disease. The above would suggest that we have a potential market profile that is ideal for most big pharmaceuticals – a small molecule therapy taken by a very significant proportion of the population over long periods of time. We might therefore expect to see some significant pharma activity in this field.

Of course, even if the concept of rechaperoning is accurate in theory, creating a therapy will not be straightforward. Firstly, there is the small matter of identifying non-toxic small molecules which can stabilise an appropriate protein *in vivo* without side-effects, and which can be manufactured in appropriate formulations. Then it must be considered that some of the misfolding disorders may be the result of large numbers of different mutations (for example, over 90 mutations are associated with FAP). If each mutation has a subtly different effect on the protein such that effective stabilisation requires a different rechaperoning molecule in each case, then the patient population will in effect be stratified into smaller subpopulations that may make the economics of new drug development unattractive. Finally, it should be said that under normal circumstances the chaperone molecules are proteins, and it is not clear that the function of a protein in stabilising another protein can always be substituted for by a small molecule.

So, in summary, although the concept of protein misfolding as a factor in the causation of many diseases may one day lead to a new class of therapeutics, the rechaperones, nevertheless the search for cures for highly complex diseases such as Alzheimer’s will not be easy, and we can look forward to a no holds barred struggle between medical research and misfolded proteins.

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