International Journal of Pharmaceutical Development & Technology

www.ijpdt.com

e ISSN - 2248 - 910X

Print ISSN - 2248 - 9096

A REVIEW ON MOLECULAR CHAPERONES AND CHAPERONE OVERLOAD

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ABSTRACT

The molecular chaperones are the molecules that help the cell in effective refolding of misfolded proteins and also removal of proteins that cannot be refolded properly. These chaperones are produced in the cell only under some specific signals like the stress conditions and other unfavourable conditions faced by the cell. However, there must be a balance between the misfolded proteins and the chaperones present in the cell. Sometimes unknowingly, the chaperones can also cause a great damage to the cell. As we know that mutations occur naturally in a cell's DNA, some of them may be deleterious to the cell. If the mutation is deleterious, the cell undergoes apoptosis and if the mutation is resistible, the cell survives. In such resistible mutations, the chaperones silence the effect of mutation and allow the cell to survive. When the organism grows old, the chaperones become inefficient and the silenced mutations get exposed all at once leading to a large number of complications.

INTRODUCTION

Protein folding is a process where a protein gets a specific structure. Native state of a protein corresponds to the structure that is thermodynamically most stable under physiological conditions. The wide variety of highly specific structures that result from protein folding and that bring key functional groups into close proximity has enabled living systems to develop astonishing diversity and selectivity in their underlying chemical processes. In addition to generating biological activity, however, we now know that folding is coupled to many other biological processes, including the trafficking of molecules to specific cellular locations and the regulation of cellular growth and differentiation. In addition, only correctly folded proteins have long-term stability in crowded biological environments and are able to interact selectively with their natural partners.

Native states of proteins almost always correspond to the structures that are most thermo- dynamically stable under physiological conditions. The folding process does not involve a series of mandatory steps between specific partly folded states, but rather a stochastic search of the many conformations accessible to a polypeptide chain.

On average, native-like interactions between residues are more stable than nonnative ones, they are more persistent and the polypeptide chain is able to find its lowest-energy structure by a process of trial and error. Moreover, if the energy surface or 'landscape' has the right shape only a small number of all possible conformations needs to be sampled by any given protein molecule during its transition from a random coil to a native structure.

Monitoring the effects of specific mutations on the kinetics of folding and unfolding has proved to be a seminal technique, because of its ability to probe the role of individual residues in the folding process. Particular insight has come from the use of this approach to analyze the transition states for folding, namely the critical regions of energy surfaces through which all molecules must pass to reach the native fold. The results of many studies of these species suggest that the fundamental mechanism of protein folding involves the interaction of a relatively small number of residues to form a folding nucleus, about which the remainder of the structure rapidly condenses.

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Despite a high degree of disorder, these structures have the same overall topology as the native fold. In essence, interactions involving the key residues force the chain to adopt a rudimentary native-like architecture [1].

The essential elements of the fold are likely to be determined primarily by the pattern of hydrophobic and polar residues that favours preferential interactions of specific residues as the structure becomes increasingly compact. Once the correct topology has been achieved, the native structure will then almost invariably be generated during the final stages of folding. This mechanism therefore acts also as a 'quality control' process by which misfolding can generally be avoided [2].

Secondary structure, the helices and sheets that are found in nearly every native protein structure, is stabilized primarily by hydrogen bonding between the amide and carbonyl groups of the main chain. The formation of such structure is an important element in the overall folding process, although it might not have as fundamental a role as the establishment of the overall chain topology. Contact order is the average separation in the sequence between residues that are in contact with each other in the native structure. There are relatively simple underlying principles by which the sequence of a protein encodes its structure. The structural properties of intermediates provide important evidence about the folding of these larger proteins. In particular, they suggest that these proteins generally fold in modules, in other words, folding can take place largely independently in different segments or domains of the protein. In such cases, interactions involving key residues are likely to establish the native-like fold within local regions or domains and also to ensure that the latter then interact appropriately to form the correct overall structure. The fully native structure is only acquired when all the native-like interactions have been formed both within and between the domains; this happens in a final cooperative folding step when all the side chains become locked in their unique close-packed arrangement and water is excluded from the protein core. This modular mechanism is appealing because it suggests that highly complex structures might be assembled in manageable pieces [3].

Folding in vivo is in some cases co-translational; that is, it is initiated before the completion of protein synthesis, whereas the nascent chain is still attached to the ribosome. Others fold in specific compartments, such as mitochondria or the endoplasmic reticulum (ER), after trafficking and translocation through membranes. Many details of the folding process depend on the particular environment in which folding takes place. But because incompletely folded proteins must inevitably expose to the solvent, at least some regions of structure that are buried in the native state, they are prone to inappropriate interaction with other molecules within the crowded environment of a cell.

Some chaperones interact with nascent chains as they emerge from the ribosome, whereas others are involved in guiding later stages of the folding process. Molecular chaperones often work in tandem to ensure that the various stages in the folding of such systems are all completed efficiently. The best characterized of the chaperones studied in this manner is the bacterial complex involving GroEL, a member of the family of 'chaperonins', and its 'co-chaperone' GroES.

Molecular chaperones do not themselves increase the rate of individual steps in protein folding; rather, they increase the efficiency of the overall process by reducing the probability of competing reactions, particularly aggregation. However, there are several classes of folding catalyst that accelerate potentially slow steps in the folding process. The most important are peptidyl prolyl isomerases, which increase the rate of cis–trans isomerization of peptide bonds involving proline residues, and protein disulphide isomerases, which enhance the rate of formation and reorganization of disulphide bonds [4].

The concentrations of many of these species are substantially increased during cellular stress; indeed, the designation of many as heat shock proteins (HSPs) reflects this fact. It is also clear that some molecular chaperones are able not only to protect proteins as they fold but also to rescue misfolded and even aggregated proteins and enable them to have a second chance to fold correctly. Active intervention in the folding process requires energy, and ATP is required for most of the molecular chaperones to function with full efficiency.

The ER contains a wide range of molecular chaperones and folding catalysts, and in addition the proteins that fold here must satisfy a 'quality-control' check before being exported. This quality-control mechanism involves a remarkable series of glycosylation and deglycosylation reactions that enables correctly folded proteins to be distinguished from misfolded ones.

A large fraction of all polypeptide chains synthesized in a cell fail to pass this test and are targeted for degradation. Like the 'heat shock response' in the cytoplasm, the 'unfolded protein response' in the ER is also stimulated (upregulated) during stress and, as we shall see below, is strongly linked to the avoidance of misfolding diseases [5].

Folding and unfolding are the ultimate ways of generating and abolishing specific types of cellular activity. In addition, processes as apparently diverse as translocation across membranes, trafficking, secretion, the immune response and regulation of the cell cycle are directly dependent on folding and unfolding events. Failure to fold correctly, or to remain correctly folded, will therefore give rise to the malfunctioning of living systems and hence to disease.

Amyloid fibrils are just one of the types of aggregate that can be formed by proteins, although a significant feature of this particular species is that its highly organized hydrogen-bonded structure is likely to give it unique kinetic stability. Thus, once formed, such aggregates can persist for long periods, allowing a progressive build-up of deposits in tissue, and indeed enabling seeding of the subsequent conversion of additional quantities of the same protein into amyloid fibrils. Evolutionary selection has tended to avoid amino-acid sequences, such as alternating polar and hydrophobic residues that favour a β-sheet structure of the type seen in amyloid fibrils [6].

Aggregation process that results in amyloid fibrils is nucleated in a similar manner to that of folding, but that the residues involved might well be located in different regions of the sequence from those that nucleate folding. Such 'kinetic partitioning' means that mutations that occur during evolution could be selected for their ability to enhance folding at the expense of aggregation.

However, it is apparent that biological systems have become robust not just by careful manipulation of the sequences of proteins but also by controlling, by means of molecular chaperones and degradation mechanisms, the particular state adopted by a given polypeptide chain at a given time and under given conditions. The aberrant behavior of the chaperone and other machinery regulating polypeptide conformations can contribute to misfolding and aggregation diseases.

Many of the mutations associated with the familial forms of deposition diseases increase the population of partially unfolded states, and hence the propensity to aggregate, by decreasing the stability or co- operativity of the native state Other familial diseases are associated with the accumulation of amyloid deposits whose primary components are fragments of native proteins; such fragments can be produced by aberrant processing or incomplete proteolysis, and are unable to fold into aggregation-resistant states [7].

In the prion disorders such as Kuru or Creutzfeldt–Jakob disease, it seems that ingestion of pre-aggregated states of an identical protein, for example by voluntary or involuntary cannibalism or through the use of contaminated pharmaceuticals or surgical instruments, can markedly increase the inherent rate of aggregation through seeding and hence can generate a mechanism for transmission.

Molecular chaperones are also able to alter the partitioning between harmful and harmless forms of aggregates. If the efficiency of these protective mechanisms is impaired, however, the probability of pathogenic behavior increases. Such a process would explain why most of the amyloid diseases are associated with old age.

It seems that such partly folded forms, which have exposed hydrophobic regions and are therefore prone to self-aggregation, can exist in equilibrium with the native protein. In the case of the mutant lysozymes, the molten-globule-like intermediates have persistent structure in the a-domain but lack stable, native-type structure in the β-domain. It has therefore been proposed that the transient, partly folded forms of the mutant lysozymes associate through their unstable β-domains.

The emergence of stable structure through such intermolecular self-association might provide a template (seed) for the recruitment of additional peptide chains that ultimately form the hydrogen-bonded, mainly cross β sheet core structure of the insoluble amyloid fibril. In agreement with this model, the NMR structure of a domain of the prion protein (PrP) indicates that amino acids that are mutated in inherited prion diseases are involved in the maintenance of the hydrophobic core [8].

Chaperones

The folding of most newly synthesized proteins in the cell requires the interaction of a variety of protein cofactors known as molecular chaperones. These molecules recognize and bind to nascent polypeptide chains and partially folded intermediates of proteins, preventing their aggregation and misfolding. There are several families of chaperones; those most involved in protein folding are the 40-kDa heat shock protein (HSP40; DnaJ), 60-kDa heat shock protein (HSP60; GroEL), and 70-kDa heat shock protein (HSP70; DnaK) families. The availability of high-resolution structures has facilitated a more detailed understanding of the complex chaperone machinery and mechanisms, including the ATP-dependent reaction cycles of the GroEL and HSP70 chaperones. For both of these chaperones, the binding of ATP triggers a critical conformational change leading to release of the bound substrate protein. Whereas the main role of the HSP70/HSP40 chaperone system is to minimize aggregation of newly synthesized proteins, the HSP60 chaperones also facilitate the actual folding process by providing a secluded environment for individual folding molecules and may also promote the unfolding and refolding of misfolded intermediates [9].

The basic paradigm of molecular chaperones is that they recognize and selectively bind nonnative, but not native, proteins to form relatively stable complexes. In most cases, the complexes are dissociated by the binding and hydrolysis of ATP. In addition, there are "specific" molecular chaperones that typically are involved in the assembly of particular multiprotein complexes.

Chaperones prevent irreversible aggregation of nonnative conformations and keep proteins on the productive folding pathway. In addition, they may maintain newly synthesized proteins in an unfolded conformation suitable for trans-location across membranes and bind to nonnative proteins during cellular stress, among other functions.

The need for chaperones 1) to prevent aggregation and misfolding during the folding of newly synthesized chains, 2) to prevent nonproductive interactions with other cell components, 3) to direct the assembly of larger proteins and multiprotein complexes, and 4) during exposure to stresses that cause previously folded proteins to unfold, becomes evident.

The molecular chaperones involved in the folding of newly synthesized proteins recognize nonnative substrate proteins predominantly via their exposed hydrophobic residues. A number of other proteins involved in the folding of many newly synthesized proteins are often considered to be molecular chaperones; these include protein disulfide isomerase and peptidyl prolyl isomerase, which catalyze the rearrangement of disulfide bonds and isomerization of peptide bonds around Pro residues, respectively, and are perhaps better considered to be folding catalysts rather than chaperones [10].

The earliest stages of folding involve hydrophobic collapse to a relatively compact state and formation of metastable secondary structure. It is most likely that both proceed concurrently. The intermediates are more prone to aggregate than the unfolded state because in the latter the hydrophobic side chains are scattered relatively randomly in many small hydrophobic regions, whereas in the partially folded intermediates, there will be large patches of contiguous surface hydrophobicity that will have a much stronger propensity for aggregation. The tendency of partially folded intermediates to associate or aggregate is exacerbated as the protein concentration increases.

Chaperone overload

Molecular chaperones dampen the effect of damaging mutations that would otherwise be removed from the population by natural selection. The development of modern medical practice depressed this process, leading to a rise of phenotypically silent mutations in the genome. The background of misfolded proteins increases during ageing and, by competition, prevents the chaperone-mediated buffering of silent mutations.

Phenotypically exposed mutations contribute to a more-abundant manifestation of multigene diseases. This 'chaperone overload' hypothesis emphasizes the need for efficient ways to enhance chaperone capacity in ageing subjects, and will hopefully lead to the identification and 'repair' of silent mutations.

Recently, a surprising ~60 000 single nucleotide polymorphisms (SNPs) were found in exons of the human genome1 (i.e. an average of two per gene). Some of these variations, together with other mutations, could cause the affected proteins to fold incorrectly [11].

Another recent development showed that defective protein folding is the source of numerous monogenic diseases, emphasizing the importance of the correct balance between molecular chaperones (the proteins that help to refold damaged proteins) and their targets.

'Genome cleansing'

Under normal conditions, chaperones repair the conformational defects of some mutated proteins, thus reducing their phenotypic effects and dampening genome cleansing – the elimination of damaged genes from the gene pool of a population, which would normally occur through natural selection. After severe stress, however, chaperones become occupied by stress-damaged proteins and several mutations could begin to affect phenotypes [12].

If the functional consequence of the stress-exposed mutation(s) is life threatening, the organism could die as a result of the combined challenge (stress and mutation). These lethal competitions between genetically encoded folding defects and stress-induced chaperone occupancy usually occur before the organism reaches reproductive age, therefore the mutation is not inherited in later generations. Thus, stress exposes potentially dangerous mutations, allowing them to be efficiently cleansed from the genome of the whole population [13].

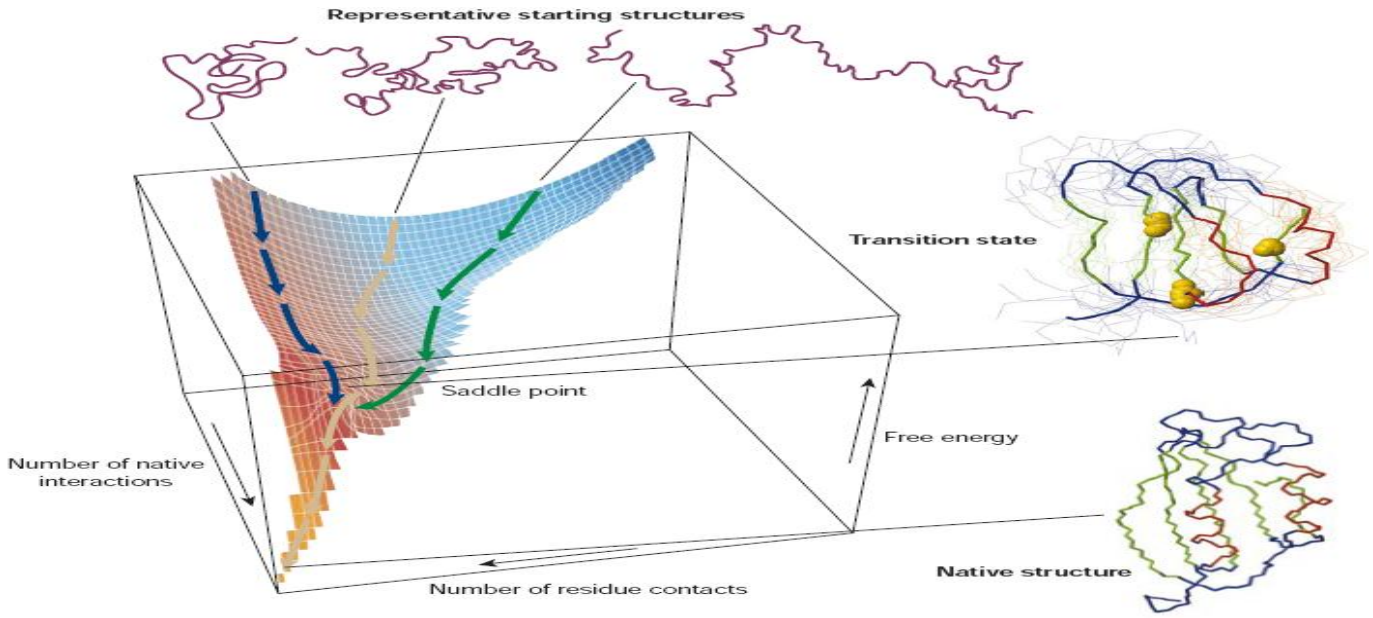
Molecular chaperones comprise one of the most conserved protein families, in terms of both their structure and their function. This makes it probable that the chaperone-mediated silencing of certain mutations described in Drosophila and yeast also occurs in humans. The development of modern medical practice has inhibited stress-induced genome cleansing of the human population by its groundbreaking achievements in the reduction of infant and prenatal mortality [14].

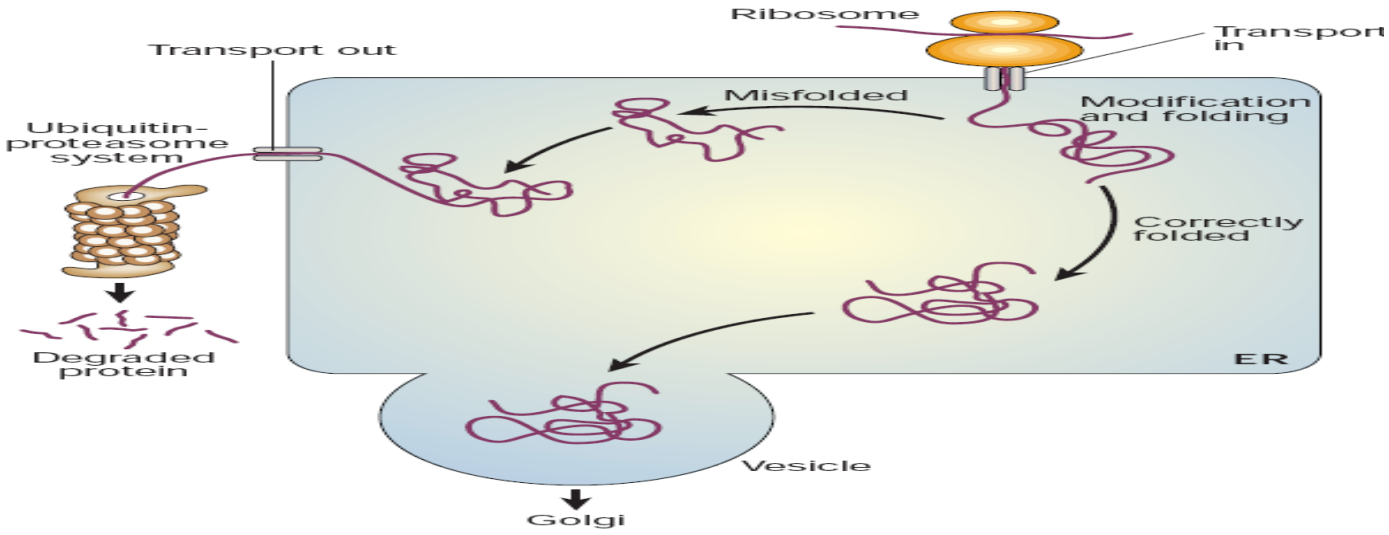
As a consequence, we probably carry increasing numbers of chaperone buffered, silent mutations from generation to generation. The chance of manifesting these mutations phenotypically increases in aged individuals, where protein damage is abundant and chaperone induction is impaired. Furthermore, medical practice has significantly increased our chance of surviving to the age when increasing protein damage would expose any previously silent mutations carried from former generations [15].

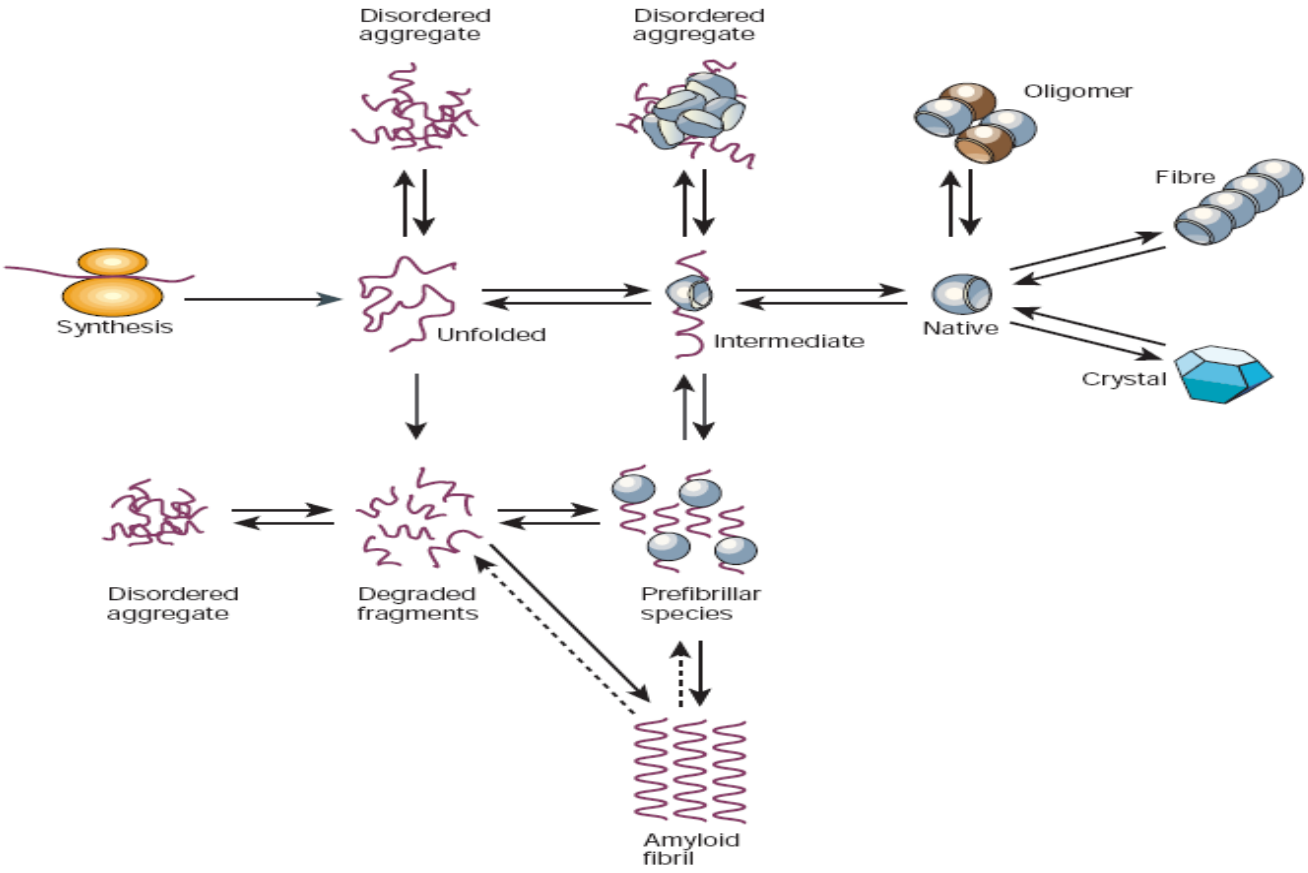
Exposed mutations could contribute to an increase in multigene diseases, such as atherosclerosis, autoimmune-type diseases (e.g. asthma, lupus, psoriasis and arthritis), cancer, diabetes, hypertensive cardiovascular disease and several psychiatric illnesses (e.g. Alzheimer's disease and schizophrenia) [16,17].

Diseases due to Chaperone overload

Exposed mutations could contribute to an increase in multigene diseases such as Atherosclerosis, Autoimmune-type diseases (e.g. asthma, lupus, psoriasis and arthritis), Cancer, Diabetes, Hypertensive cardiovascular disease and Several psychiatric illnesses (e.g. Alzheimer's disease and schizophrenia) [18].



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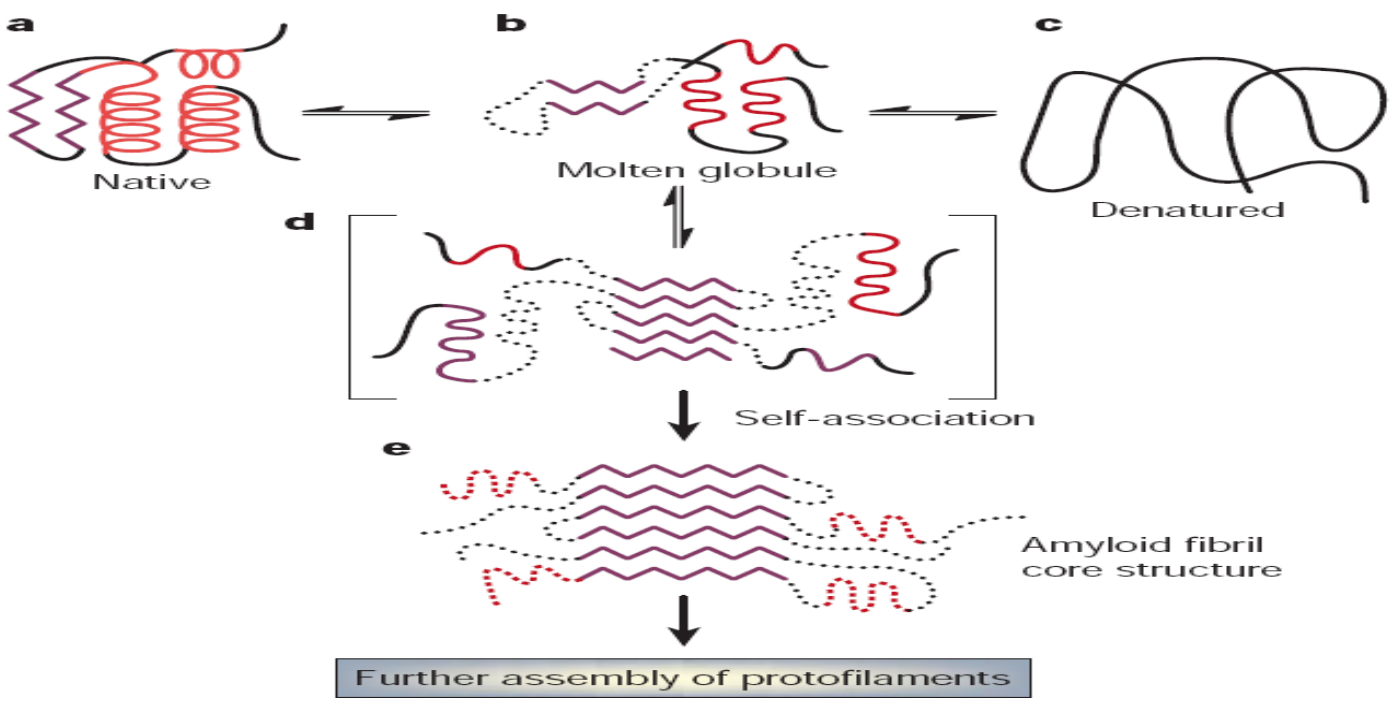
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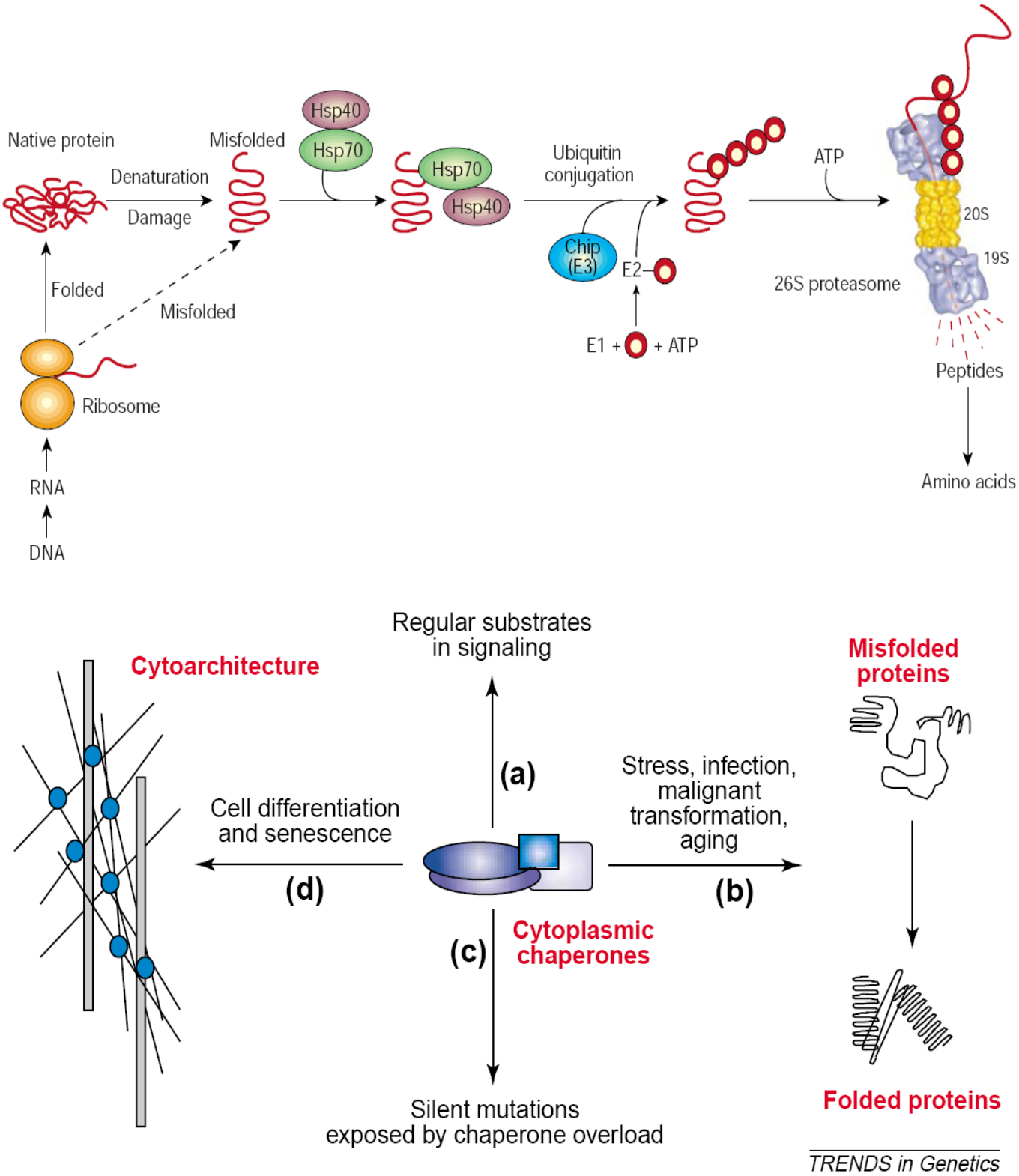

Fig 1. **Diseases due to Chaperone overload**

CONCLUSION

The possible involvement of chaperones in the development of these diseases gives another common element to their aetiology – in addition to being polygenic, having several stages of progression, and having a mix of genetic, nutritional, psycho-social, environmental and viral factors that contribute to their pathology. The proposed chaperone-overload model also gives a novel explanation for the environmental determination and variability of disease aetiology.

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