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Chaperone overload is a possible contributor to 'civilization diseases'

Peter Csermely

Molecular chaperones dampen the effect of damaging mutations that would otherwise be removed from the population by natural selection. Here, I propose that 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.

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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. Another recent development showed that defective protein folding is the source of numerous monogenic diseases², emphasizing the importance of

the correct balance between molecular chaperones (the proteins that help to refold damaged proteins^{3–5}) and their targets. The investigations of Rutherford and Lindquist⁶ connected chaperone-overload with evolution, identifying one of the most abundant cytoplasmic chaperones, the 90-kDa heat shock protein (Hsp90) as a capacitor of morphological evolution (Box 1)⁶. Recently, the *Drosophila* chaperone Hsp70 (Ref. 7) and the *Saccharomyces cerevisiae* prion⁸ [PSI⁺] were also shown to have roles in the stress-related exposure of pre-existing genetic variations. Furthermore, chaperones can have more-direct effects on DNA recombination and repair: integrating several mechanisms, Hsp70 has been recently proposed as a facilitator of evolution⁹.

'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 (Box 1)⁶. 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

Box 1. The abundant chaperone, Hsp90 is a capacitor of morphological evolution of fruit flies

In fruit flies that have not been stressed, the 90-kDa heat shock protein (Hsp90) acts as post-translational 'silencer' of several genetic changes by assisting in the efficient repair of folding defects. However, severe stress leads to the accumulation of damaged, misfolded proteins. New stress-induced chaperone targets compete with existing malformed proteins, which leads to a decline in chaperone-mediated repair of conformational defects. Similar effects are also achieved by introducing a defective Hsp90 or by Hsp90 inhibition^a. As a consequence, a fraction of these polygenetic changes escape chaperone repair, and are manifested phenotypically during stress. Manifestation of the functional defects leads to a burst of heritable phenotypic changes and enhances the adaptation potential of the affected population^a.

Reference

- ^a Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336–342

be efficiently cleansed from the genome of the whole population.

The 'chaperone-overload' model

Molecular chaperones comprise one of the most-conserved protein families, in terms of both their structure and their function^{3,4}. This makes it probable that the chaperone-mediated silencing of certain mutations described in *Drosophila*^{6,7} and yeast⁸ also occurs in humans. I propose that 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 mortality¹⁰. 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 impaired¹¹. 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. 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). 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^{12,13}. The proposed chaperone-overload model also gives a novel explanation for the environmental determination and variability of disease aetiology.

Possible disproof of the model

Protein damage leads to an increased expression of chaperones². An obvious response to the increased background of misfolded proteins, according to the model, would be continuously elevated amounts of chaperones. Thus, over the past two centuries, increases in everyday stress from environmental poisoning, medicinal side-effects, high calorie intake, overcrowding, multiple infections enhanced by globalization, and so on, could have caused a rise in chaperone expression. This would produce an efficient buffer for the 'silencing' of gradually accumulating conformational defects and attenuating the effects of chaperone overload. However, in reality, long-term stress leads to a decline in chaperone synthesis^{14,15}, most probably because of the toxic effects of permanently high chaperone amounts¹⁶. There are a few examples where the induction of certain chaperones can serve as an *in vivo* adaptation for a continuously high presence of unfolded proteins^{17–19}, the usual cellular response for a massive and occasional protein damage. However, an increase in chaperone capacity is not generally used to protect against a permanent, but low, increased background of misfolded proteins. Moreover, the inadequate chaperone response against the continuously present misfolded proteins becomes critical during the ageing process, where chaperone capacity and induction declines.

Limitations of the model

There are several limitations that affect the present model:

- (1) The role of defective protein folding in several monogenic diseases² is well-established. However, there are only sporadic examples where the involvement of conformational instability in the aetiology of multigene diseases has been demonstrated directly; for example, the 'carcinogenic' destabilizing mutations of the p53 protein²⁰, and a point mutation of the human nucleoside diphosphate kinase A, which is associated with cancer progression, does not affect in enzyme activity but does cause a partial unfolding of the molecule²¹. Therefore, we cannot estimate how many of the known disease-linked mutations cause a folding problem and make the host protein a potential target of molecular chaperones.
- (2) Several pathways have a 'robust' behaviour^{22,23} where their complexity buffers mutations even when they are exposed phenotypically. This behaviour reduces the manifestation of exposed silent mutations even in aged subjects and also reduces the probability of genome cleansing.
- (3) Stressful conditions provoke an increase in genetic instability by several mechanisms, such as DNA uptake, hypomethylation, decrease in proofreading, transposon activation, coding sequence fusion, recombination errors²⁴. Thus,

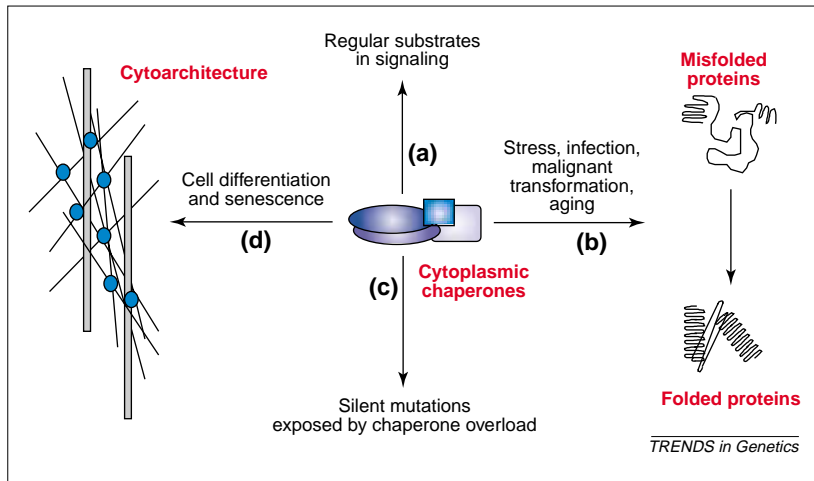


Fig. 1. Hypothetical mechanisms competing for chaperone occupancy. (a) Cytoplasmic chaperones of eukaryotic cells participate in the maintenance of the conformation of some selected protein substrates. Most of these unstable proteins are parts of various signalling cascades^{3,4}. (b) In case of environmental stress, the amount of damaged, misfolded proteins is increased, which requires the participation of chaperones in the sequestration and refolding of these damaged proteins^{3,4}. (c) Phenotypically buffered, silent mutations also require the assistance of chaperones to rescue them from folding traps⁶. (d) Chaperones might also form low affinity and highly dynamic extensions of the cytoskeleton participating in cellular traffic and in the organization of the cytoarchitecture^{37,38}. Following severe environmental stress, chaperones are occupied by damaged proteins (b) and silent mutations (c) become exposed⁶. Exposure of silent mutations probably occurs if damaged proteins become gradually more abundant; for example, during the ageing process¹¹. The delicate balance between various tasks of chaperones might also expose hidden mutations after viral infections, in malignant transformation, extensive signalling, cell differentiation or during cellular senescence (figure adapted from Ref. 38).

besides increased genetic buffering, medically minimized stress reduces the amount of genetic change.

- (4) Over the past 200 years, when medicine has gradually developed to such a level that it can significantly dampen stress-induced genome cleansing, there have been only 6–10 human generations, which has allowed only a modest accumulation of silent mutations.
- (5) A large part of the recent enormous reduction in infant mortality is achieved by conquering various postnatal infections and does not necessarily 'rescue' silent mutations.
- (6) Silent mutations are usually located in heterozygous loci and masked by wild-type alleles. However, stressful conditions are known to lead to higher genetic variation²⁵.
- (7) Obviously, numerous other factors influencing the conformational homeostasis of proteins, and the development of multigenic diseases, such as environmental poisoning, infections, eating habits, physical fitness, mental stress, and so on, changed dramatically in the past 200 years, which make the population analysis of the chaperone-overload model difficult. Moreover, changes in diagnostic methods and treatment protocols further aggravate the analysis of previous mortality records.

The above examples show that the contribution of chaperone-overload to the aetiology of polygenic diseases could be almost negligible at the moment, if compared with environmental factors, but it is

steadily rising with new generations and with the advances of medical science.

Observations supporting the model

There are several findings and observations that indirectly support the chaperone overload model:

- (1) Chaperone overload leads to a significant disturbance in protein folding homeostasis²⁶, this situation might be present in transgenic mice expressing the hepatitis B virus X protein, which are more sensitive to hepatocarcinogens without showing an increase in mutation rate²⁷.
- (2) There are numerous – mostly monogenic – diseases, where point mutations impair protein folding. Chaperones often bind to the mutant protein redirecting it from its final destination to be rapidly degraded^{3,28}. In these illnesses, an expansion of chaperone capacity, for example by the administration of chemical chaperones (small molecules that stabilize the native conformation of mutant proteins²⁹) significantly improves the conditions of affected individuals.
- (3) Chaperones bind to several mutant forms of 'inherited cancer genes', such as the p53 protein, or retinoblastoma proteins; the unpredictable frequency of retinal tumour development in low-penetrant, heat-sensitive retinoblastoma mutants³⁰ could reflect changes in chaperone–mutant protein association during heat stress.
- (4) A number of identified human disease genes encode large proteins where mutations have a greater probability of inducing folding defects^{31,32}.
- (5) There are many examples of genes that increase health risks, but that are generally maintained as selectively neutral variants³³.

Experiments to test the validity of the model

Several approaches are possible to test the validity of the current hypothesis.

Change in the level of chaperone overload

Organ-specific expression of misfolded proteins in conditionally transgenic animals should enhance the spontaneous or environmentally induced occurrence of cancer and other diseases with a multigenetic background. Conversely, a decrease in the overall amount of misfolded proteins by chemical chaperones^{28,34} should attenuate the development of polygenic diseases. Also, a general, but not complete decrease in chaperone function (e.g. by partially debilitating point mutations) should enhance the occurrence of polygenic diseases. Conversely, a sustained and non-toxic increase of chaperone capacity^{35,36} should attenuate the development of multigenic diseases. Finally, inhibition of Hsp90 chaperone function by specific inhibitors, such as geldanamycin⁶ should cause an accelerated development of polygenic diseases in animal models.

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