9 The First International Workshop on Comparative Genome Organization (1996) Comparative genome organization of vertebrates. *Mamm. Genome* 7, 717–734
10 Lahn, B.T. and Page, D.C. (1999) Four evolutionary strata on the human X chromosome. *Science* 286, 964–967
11 Ohno, S. (1967) *Sex Chromosomes and Sex Limited Genes*, Springer-Verlag
12 Saifi, G.M. and Chandra, H.S. (1999) An apparent excess of sex- and reproduction-related genes on the human X chromosome. *Proc. R. Soc. London Ser. B* 266, 203–209
13 Wu, C.I. and Davis, A.W. (1993) Evolution of postmating reproductive isolation: the composite nature of Haldane's rule and its genetic bases. *Am. Nat.* 142, 187–212
14 Turelli, M. and Orr, H.A. (1995) The dominance theory of Haldane's rule. *Genetics* 140, 389–402
15 Fisher, R.A. (1930) *The Genetical Theory of Natural Selection*, Clarendon Press
16 Lande, R. and Arnold, S.J. (1985) Evolution of mating preference and sexual dimorphism. *J. Theor. Biol.* 117, 651–664
17 Miller, G. (2000) *The Mating Mind: How Sexual Choice Shaped the Evolution of Human Nature,* Heinemann/Doubleday
18 Baechner, D. *et al.* (1993) Enhanced expression of the murine FMR1 gene during germ cell proliferation suggests a special function in both the male and the female gonad. *Hum. Mol. Genet.* 2, 2043–2050
19 Coyne, J.A. (1992) Genetics and speciation. *Nature* 355, 511–515
20 Duboule, D. and Wilkins, A.S. (1998) The evolution of 'bricolage'. *Trends Genet.* 14, 54–59
21 Hedges, L.V. and Nowell, A. (1995) Sex differences in mental test scores, variability, and numbers of high-scoring individuals. *Science* 269, 41–45
22 Phillips, P.C. (1999) From complex traits to complex alleles. *Trends Genet.* 15, 6–8
23 Gécz, J. *et al.* (1996) Gene structure and subcellular localization of FMR2, a member of a new family of putative transcription activators. *Nat. Genet.* 13, 105–108
24 D'Adamo, P. *et al.* (1998) Mutations in GDI1 are responsible for X-linked non-specific mental retardation. *Nat. Genet.* 19, 134–139
25 Billuart, P. *et al.* (1998) Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. *Nature* 392, 923–926
26 Allen, K.M. *et al.* (1998) PAK3 mutation in nonsyndromic X-linked mental retardation. *Nat. Genet.* 20, 25–30
27 Trivier, E. *et al.* (1996) Mutations in the kinase Rsk-2 associated with Coffin–Lowry syndrome. *Nature* 384, 567–570
28 Carrié, A. (1999) A new member of the IL-1 receptor family highly expressed in hippocampus and involved in X-linked mental retardation. *Nat. Genet.* 23, 25–31
29 Zemni, R. *et al.* (2000) A new gene involved in X-linked mental retardation identified by analysis of an X;2 balanced translocation. *Nat. Genet.* 24, 167–171
30 Kutsche, K. *et al.* (2000) Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. *Nat. Genet.* 26, 247–250
31 Yntema, H.G. *et al.* (1999) A novel risosomal S6-kinase (RSK4; RPS6KA6) is commonly deleted in patients with complex X-linked mental retardation. *Genomics* 62, 332–343
32 Chelly, J. (1999) Breakthroughs in molecular and cellular mechanisms underlying X-linked mental retardation. *Hum. Mol. Genet.* 8, 1833–1838
33 Gécz, J. and Mulley, J. (2000) Genes for cognitive function: developments on the X. *Genome Res.* 10, 157–163
34 Toniolo, D. and D'Adamo, P. (2000) X-linked non-specific mental retardation. *Curr. Opin. Genet. Dev.* 10, 280–285

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 genome[1] (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 diseases[2], 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 Lindquist[6] 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* prion[8] [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 evolution[9].

'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

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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 malfolded 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 yeast[8] 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 mortality[10]. 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[11]. 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 chaperones[2]. 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 amounts[16]. 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 diseases[2] 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 protein[20], 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 molecule[21]. 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 errors[24]. Thus,

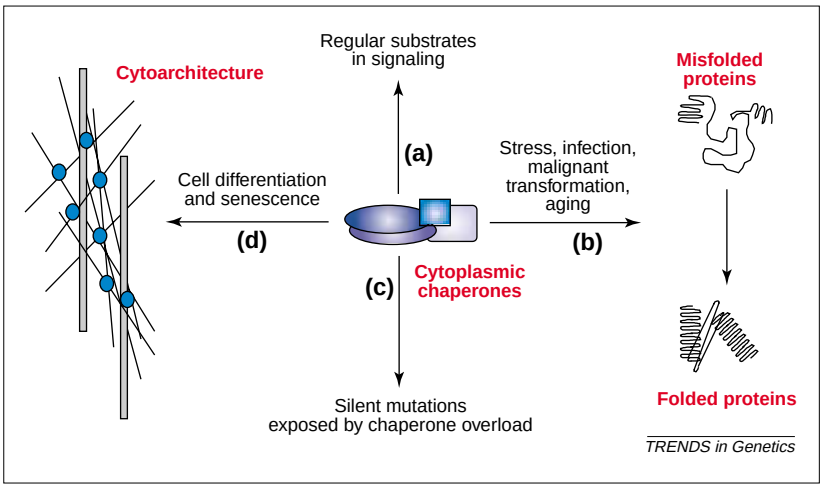

Fig. 1. Hypothetical mechanisms competing for chaperone occupancy. (a) Cytoplasmic chaperones of eukaryotic cells participate in the maintenance of the conformation of some selected protein substrates. Most of these unstable proteins are parts of various signalling cascades[3,4]. (b) In case of environmental 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 traps[6]. (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 exposed[6]. Exposure of silent mutations probably occurs if damaged proteins become gradually more abundant; for example, during the ageing process[11]. 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 variation[25].

(7) Obviously, numerous other factors influencing the conformational homeostasis of proteins, and the development of multigene 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 homeostasis[26], 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 rate[27].

(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 proteins[29]) 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 mutants[30] 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 variants[33].

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 multigene diseases. Finally, inhibition of Hsp90 chaperone function by specific inhibitors, such as geldanamycin[6] should cause an accelerated development of polygenic diseases in animal models.

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Detection and analysis of silent mutations

Mutant proteins linked to polygenic diseases could have altered folding or differences in chaperone binding capacity[32]. Also, the model predicts that, in developed countries, there are more chaperone-corrected, silent, nonsynonymous mutations in 'fold critical' regions of proteins than in less developed countries where modern medical practices have been less used. Whether this difference is already large enough to detect is an open question. Identified genes of polygenic diseases could have a larger variation (polymorphism) in subjects from developed countries compared with those from less developed countries, or primates.

The ratio between the nonsynonymous mutation rate (K_a) and the synonymous mutation rate (K_s) should be higher in developed than in less developed countries (if the small number of generations from the advent of modern medicine has allowed the development of a measurable difference). Similarly in animal models, K_a/K_s should increase if chaperone occupancy is increased, and vice versa should decrease if chaperone occupancy is decreased.

Conclusions

In summary, medical efforts of the past two centuries potentially increased the occurrence of multigenic, 'civilization diseases'; first, by increasing the number of silent mutations in the genome, and second, by allowing survival to the age when the gradual overload of the buffering capacity of chaperones exposes these mutations phenotypically. The delicate balance between the amounts of chaperones and their potential targets (i.e. chaperone occupancy) emerges as an integrator of cellular, organismal and population responses (Fig. 1)[37,38]. The present hypothesis emphasizes the need for efficient ways to enhance chaperone capacity in ageing subjects[35,36], and calls for the identification and future 'repair' of chaperone-buffered, phenotypically silent mutations.

References

1 The international SNP map working group. (2001) A map of human sequence variation containing 1.4 million single nucleotide polymorphisms. *Nature* 409, 928–933
2 Aridor, M. and Balch, W.E. (1999) Integration of endoplasmic reticulum signalling in health and disease. *Nat. Med.* 5, 745–751
3 Hartl, F-U. (1996) Molecular chaperones in cellular protein folding. *Nature* 381, 571–580
4 Bukau, B. and Horwich, A.L. (1998) The Hsp70 and Hsp60 chaperone machines. *Cell* 92, 351–366
5 Csermely, P. (1999) The 'chaperone-percolator' model: a possible molecular mechanism of Anfinsen-cage type chaperone action. *BioEssays* 21, 959–965
6 Rutherford, S.L. and Lindquist, S. (1998) Hsp90 as a capacitor for morphological evolution. *Nature* 396, 336–342
7 Roberts, S.P. and Feder, M.E. (1999) Natural hyperthermia and expression of the heat shock protein Hsp70 affect developmental abnormalities in *Drosophila melanogaster*. *Oecologia* 121, 323–329
8 True, H.L. and Lindquist, S.L. (2000) A yeast prion provides a mechanism for genetic variation and phenotypic diversity. *Nature* 407, 477–483
9 Chow, K-C. (2000) Hsp70 (DnaK) - an evolution facilitator? *Trends Genet.* 16, 484–485
10 Crow, J.F. (2000) The origins, patterns and implications of human spontaneous mutation. *Nat. Rev. Genet.* 1, 40–47
11 Soti, Cs. and Csermely, P. (2000) Molecular chaperones and the aging process. *Biogerontology* 1, 225–233
12 Trosko, J.E. and Chang, C.C. (1980) An integrative hypothesis linking cancer, diabetes and atherosclerosis: the role of mutations and epigenetic changes. *Med. Hypotheses* 6, 455–468
13 Andreassi, M.G. *et al.* (2000) Genetic instability and atherosclerosis: can somatic mutations account for the development of cardiovascular diseases? *Environ. Mol. Mutagen.* 35, 265–269
14 Croute, F. *et al.* (2000) Pattern of stress protein expression in human lung cell line A549 after short- or long-term exposure to cadmium. *Environ. Health Perspect.* 108, 55–60
15 Yahav, S. *et al.* (1997) Effect of acquisition of improved thermotolerance on the induction of heat shock proteins in broiler chicken. *Poult. Sci.* 76, 1428–1434
16 Feder, J.H. *et al.* (1992) The consequences of expressing hsp70 in *Drosophila* cells at normal temperatures. *Genes Dev.* 6, 1402–1413
17 Ulmasov, K.A. *et al.* (1992) Heat shock proteins and thermoresistance in lizards. *Proc. Natl. Acad. Sci. U. S. A.* 89, 1666–1670
18 Warrick, J.M. *et al.* (1999) Suppression of polyglutamine-mediated neurodegeneration in *Drosophila* by the molecular chaperone Hsp70. *Nat. Genet.* 23, 425–428
19 Kazemi-Esfarjani, P. and Benzer, S. (2000) Genetic suppression of polyglutamine toxicity in *Drosophila*. *Science* 287, 1837–1840
20 Bullock, A.N. *et al.* (1997) Thermodynamic stability of wild-type and mutant p53 core domain. *Proc. Natl. Acad. Sci. U. S. A.* 94, 14338–14342
21 Lascu, I. *et al.* (1997) A point mutation of human nucleoside diphosphate kinase found in aggressive neuroblastoma affects protein folding. *J. Biol. Chem.* 272, 15599–15602
22 Alon, U. *et al.* (1999) Robustness in bacterial chemotaxis. *Nature* 397, 168–171
23 Wagner, A. (2000) Robustness against mutations in genetic networks of yeast. *Nat. Genet.* 24, 355–361
24 Caporale, L.H. (ed., 1999) Molecular strategies in biological evolution. *Ann. New York Acad. Sci.* 870
25 Szafraniec, K. *et al.* (2001) Environmental stress and mutational load in diploid strains of the yeast *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1107–1112
26 Satyal, S.H. *et al.* (2000) Polyglutamine aggregates alter protein folding homeostasis in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. U. S. A.* 97, 5750–5755
27 Madden, C.R. *et al.* (2000) Expression of hepatitis B virus X protein does not alter the accumulation of spontaneous mutations in transgenic mice. *J. Virol.* 74, 5266–5272
28 Gregersen, N. *et al.* (2000) Defective folding and rapid degradation of mutant proteins is a common disease mechanism in genetic disorders. *J. Inher. Metab. Dis.* 23, 441–447
29 Burrows, J.A.J. *et al.* (2000) Chemical chaperones mediate increased secretion of mutant a1-antitrypsin (a1-AT) Z: a potential pharmacological strategy for prevention of liver injury and emphysema in a1-AT deficiency. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1796–1801
30 Otterson, G.A. *et al.* (1999) Temperature-sensitive RB mutations linked to incomplete penetrance of familial retinoblastoma in 12 families. *Am. J. Hum. Genet.* 65, 1040–1046
31 Mushegian, A.R. *et al.* (1997) Positionally cloned human disease genes: patterns of evolutionary conservation and functional motifs. *Proc. Natl. Acad. Sci. U. S. A.* 94, 5831–5836
32 Rubin, G.M. *et al.* (2000) Comparative genomics of the eukaryotes. *Science* 287, 2204–2215
33 Rutherford, S.L. (2000) From genotype to phenotype: buffering mechanisms and the storage of genetic information. *BioEssays* 22, 1095–1105
34 Welch, W.J. and Brown, C.R. (1996) Influence of molecular and chemical chaperones on protein folding. *Cell Stress Chaperones* 1, 109–115
35 Vígh, L. *et al.* (1997) Bimoclomol: A nontoxic, hydroxylamine derivative with stress protein-inducing activity and cytoprotective effects. *Nature Med.* 3, 1150–1154
36 Krobitsch, S. and Lindquist, S. (2000) Aggregation of huntingtin in yeast varies with the length of the polyglutamine expansion and the expression of chaperone proteins. *Proc. Natl. Acad. Sci. U. S. A.* 97, 1589–1594
37 Pratt, W.B. *et al.* (1999) A model for the cytoplasmic trafficking of signalling proteins involving the hsp90-binding immunophilins and p50cdc37. *Cell Signal.* 11, 839–851
38 Csermely, P. (2001) A nonconventional role of molecular chaperones: involvement in the cytoarchitecture. *News Physiol. Sci.* 15, 123–126