Heat shock proteins and human disease

ABSTRACT—At first sight it appears unlikely that a phenomenon which was first observed in 1962 as the appearance of specific puffs in the salivary gland chromosomes of the larvae of the fruit fly *Drosophila buschii* following exposure to elevated temperature [1] should, nearly 30 years later, attract considerable attention from clinicians and scientists interested in such diverse phenomena as autoimmune diabetes and myocardial infarction [2, 3]. The aim of this review is to discuss the information which has been accumulated about the heat shock proteins in the 30 years since their discovery and to indicate how this information has led to studies on their possible role in human disease.

The heat shock proteins (hsps)

The appearance of new puffs in the *Drosophila* salivary gland chromosomes indicates increased activity of specific genes resulting in the synthesis of new proteins. The proteins whose synthesis is induced in this way by elevated temperature were named the 'heat shock proteins' [4, 5]. It was subsequently shown that this response was not confined to *Drosophila* but that all organisms studied, from prokaryotic bacteria such as *E. coli* to mammals including man, respond to higher temperature by switching off the synthesis of most proteins and starting large-scale synthesis of a few hsps.

Moreover, this evolutionary conservation extends not only to the existence of the response in widely different organisms but also to the induced proteins themselves which are very similar to one another in very different organisms. Thus the best characterised hsps—hsp90, hsp70 and hsp65 (each hsp is named according to its mass in kilodaltons)—are induced in response to heat in all organisms studied, from bacteria to man, and are highly conserved between different species, the hsp90 protein, for example, showing 60% amino acid identity with the corresponding yeast protein and 78% with the *Drosophila* protein [6]. The various hsps and their characteristics are listed in Table 1 [4, 5, 7].

Such extensive conservation of the hsps during evolution, which is far greater than that of evidently essential proteins such as actin or myosin, led to the idea that the hsps play some highly critical role in the cellular response to the stress of raising the temperature rather than representing an esoteric phenomenon of no biological significance.

This idea was reinforced by the finding that hsp synthesis is also induced by a wide variety of other stressful stimuli, including infection with different viruses, treatment with ethanol, amino acid analogues, steroid hormones and heavy metals, anoxia and generators of free radicals such as hydrogen peroxide [8]. A wide variety of direct and indirect evidence suggests that they play a critical role in the defence of the cell against damaging agents [5, 9]. Thus, for example, cells subjected to a mild heat shock producing elevated levels of the hsps survive a subsequent heat shock better than untreated cells. Moreover, such cells also exhibit improved survival when exposed to other stressful stimuli such as ethanol or anoxia, while prior treatment with these agents produces enhanced thermotolerance exactly as if hsp synthesis had been induced by elevated temperature.

Clearly, therefore, the enhanced synthesis of all the hsps induced by a particular stress can result in enhanced resistance to a wide variety of stresses. Moreover, experiments in which the synthesis of individual hsps has been artificially reduced or enhanced in yeast and mammals have suggested that hsp90 and hsp70 play a particularly critical role in protecting cells from stress [10–12].

Interestingly, however, the role of the hsps is not likely to be confined to cells under stress. Many of these proteins are also synthesised under normal conditions. For example, hsp90, although accumulating to fivefold higher levels following thermal stress, actually constitutes 1% of total cellular protein in unstressed cells. It is probable, therefore, that the stress proteins fulfil similar functions in both normal and stressed cells, but that these functions are required to a greater degree in stressed cells.

Detailed studies by a number of laboratories [13] have suggested that in most cases this role involves the hsps in interacting with other proteins and affecting their folding and unfolding. Thus in normal cells hsp70 appears to act as a so-called chaperonin protein, preventing inappropriate interactions of proteins which have not reached their final state of assembly into multi-protein complexes (Fig. 1 Ai), while hsp65 aids the transport of other proteins into mitochondria by catalysing their unfolding (Fig. 1 Aii). Similarly, hsp90 is thought to maintain proteins such as the receptors for steroid hormones in an inactive structural form until they change into an active form when treated with steroids (Fig. 1 Aiii).

Such a role in maintaining appropriate states of protein folding is not only essential for the survival of normal cells, but is also especially important following

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stress where there is even greater need to inhibit inappropriate interactions of partially denatured, abnormal or foreign proteins produced by various stressful stimuli such as elevated temperature, amino acid analogues or virus infection (Fig. 1 Bi). Similarly the small hsp ubiquitin, which is linked to proteins which are the targets for degradation in normal cells (Fig 1. Aiv), will be required in greater amounts in stressed cells to facilitate the rapid degradation of denatured abnormal or foreign proteins which would otherwise accumulate in the stressed cells (Fig 1. Bii).

It is clear, therefore, that in a wide variety of different organisms, including humans, the hsps play a critical role both in normal function and in the response to stress. What is the possible relevance of these proteins to specific aspects of clinical medicine?

Beneficial effects of the hsps: myocardial protection

In view of the vital importance of the hsps it might be thought that their beneficial effects would be illustrated by the existence of human diseases in which a mutation had affected the gene that encodes one of the hsps, resulting in the failure to produce a functional protein. No such disease has yet been identified, possibly because a mutation of this type would be incompatible with survival. Another way in which the beneficial effect of the hsps may be illustrated is in situations where their overexpression has a positive effect on a particular function, and may therefore be of use in preventing or treating a specific disease in which this function is impaired.

Such an approach has been extensively discussed in relation to the cardiovascular system [3, 9]. The heart, in common with other tissues, synthesises more hsps after stressful stimuli such as heat shock, pressure overload or ischaemia. Most interestingly, Currie *et al* [14] were the first to show that prior hsp synthesis by exposure to heat stress may protect the heart against damage during subsequent periods of hypoxic stress caused by ischaemia, as well as from the oxidative damage which occurs due to free radical generation during subsequent reperfusion. This protective role for the hsps in the heart may involve their interaction with proteins which become unfolded or denatured during hypoxic stress. Alternatively it is possible that after heat shock there is increased activity of the endogenous antioxidant catalase [14] in the heart while, at least in some cell types such as macrophages, hsp synthesis is associated with an inhibition of superoxide anion generation suggesting a direct antioxidant effect [8]. The various mechanisms by which heat shock may protect the heart are summarised in Fig. 2.

Hsp synthesis may also be involved in the protective effect on the heart of the phenomenon known as preconditioning, in which a series of short periods of ischaemia with subsequent reperfusion renders the heart resistant to subsequent longer periods of ischaemia [15]. Such a protective mechanism might act, therefore, via the accumulation of hsps induced by hypoxia during the initial bursts of ischaemia resulting in subsequent protection. However, recent studies have suggested that preconditioning does not require hsp synthesis while heat shock does not precondition the canine myocardium. This suggests that the two protective phenomena of hsp synthesis and preconditioning may be distinct [3, 9].

It might therefore be possible to design therapeutic agents which would induce hsp elevation without the necessity for applying a stressful stimulus. This approach would represent a means of protecting the heart from damage during myocardial infarction and

Table 1 Major eukaryotic hsps

Family	Members	Prokaryotic homologue	Functional role	Comments
Hsp90	Hsp100, Hsp90 Grp94	C62.5, Lon protease (*E. coli*)	Maintenance of proteins such as steroid receptor, Src, in an inactive form until appropriate	*Drosophila* and yeast homologous of hsp 90 are known as hsp83
Hsp70	Grp78 (= Bip) Hsp70, Hsc70, Hsx70	dna K (*E. coli*)	Protein folding and unfolding; assembly of multimeric complexes	Hsx70 only in primates
Hsp60	Hsp60	groEL (*E. coli*) Mycobacterial 65 kd antigen	Protein folding and unfolding; organelle translocation	Major antigen of many bacteria and parasites which infect man
Hsp27	Hsp27, Hsp26 etc.	Mycobacterial 18 kd antigen	Unclear	Very variable in size and number in different organisms
Ubiquitin	Ubiquitin	None	Protein degradation	Also conjugated to histone H2A in the nucleus leading to proteinal role in gene regulation

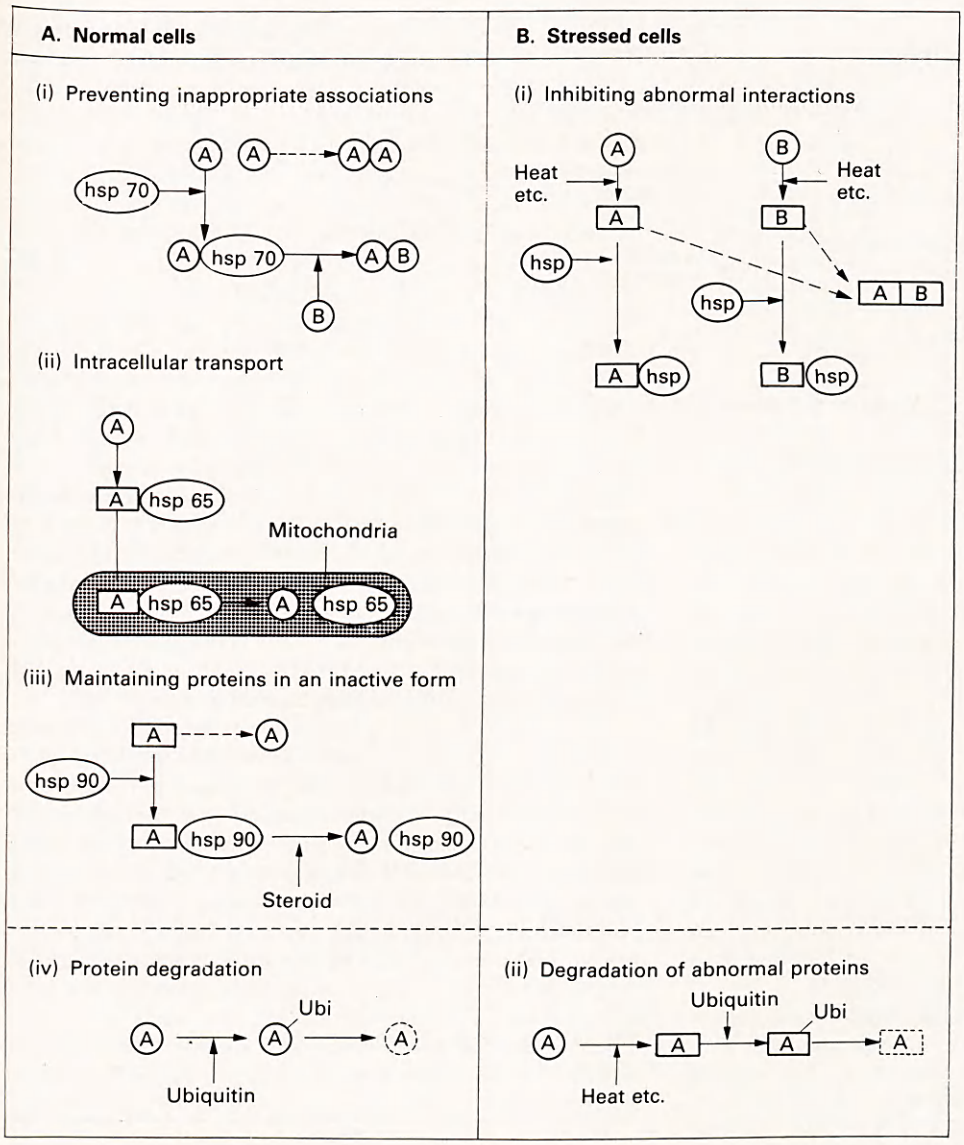


Fig. 1. *Actions of the hsps in normal and stressed cells.*

ischaemia by enhancing its own endogenous protective procedures. As such it represents a significant potential advance over attempts to protect the ischaemic myocardium by exogenous pharmacological intervention which have had little success.

A similar role for the hsps in protecting tissue during episodes of hypoxia and reperfusion has also been suggested to occur in inflamed joints [16, 17] in which repeated cycles of hypoxia and reperfusion occur during movement of inflamed joints. It is of interest that the antirheumatic drug auranofin induces the synthesis of the 32 kd hsp, and it has been speculated [17] that several antirheumatic therapies, such as ultrasound and temperature alterations, may be effective precisely because they induce hsp synthesis.

Deleterious effects of the hsps: autoimmune disease

Paradoxically, however, the hsps have also been postulated to be involved in a deleterious manner in the aetiology of rheumatoid arthritis and of other autoimmune diseases.

As discussed above, the hsps are highly conserved in evolution and homologues of the human hsps are present in bacteria and other organisms such as parasitic protozoa which infect man. Such exogenous hsps constitute the major target of the human immune response to these pathogens, and antibodies and T cells against the appropriate exogenous hsps have been detected in individuals infected with organisms as different as the *Mycobacteria* and the protozoan para-

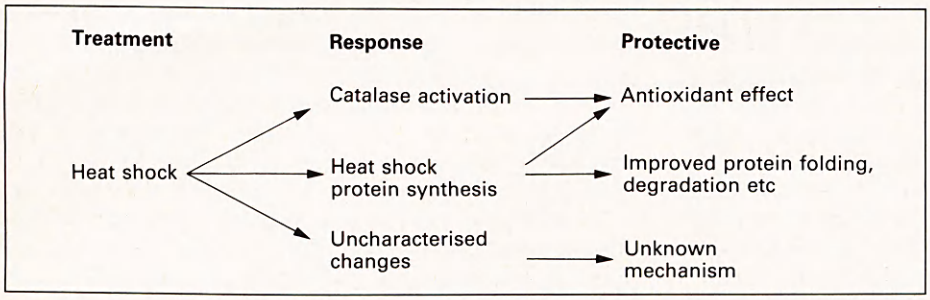


Fig. 2. *Potential mechanisms by which heat shock produces myocardial protection.*

sites *Plasmodium falciparum* and *Schistosoma mansoni* [18, 19].

Such antibodies and T cells directed against bacterial or protozoan hsps may have the potential to react also with the closely related endogenous human hsps resulting in autoimmune disease. It is unlikely, however, that an immune response to invading micro-organisms is sufficient in itself to provoke autoimmune disease since T cells directed against the hsps can be detected in both normal individuals and those infected with *Mycobacteria* who exhibit no evidence of autoimmune disease. It is more likely that, following the initial priming of the immune system by exposure to exogenous hsps, some subsequent event involving the endogenous human hsps is required to trigger the autoimmune response. Such an event could involve either the enhanced expression of the human hsps or their expression on the cell surface, both of which have been observed in patients with autoimmune disease [20, 21] and which can be brought about by a variety of stimuli, such as viral infection [22].

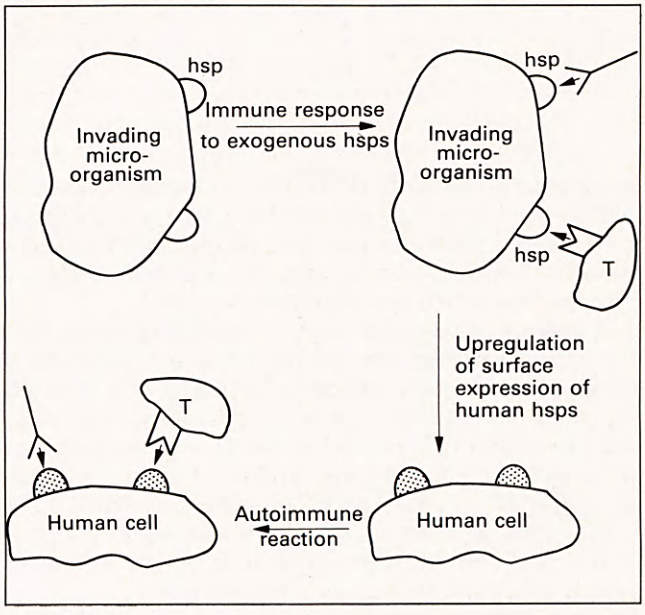
In this model, therefore, initial exposure to exogenous HSPs is followed by a second event, such as viral infection, which results in upregulation of the human hsps and/or their surface localisation. This, in turn, results in antibodies and T cells primed against the bacterial or protozoan proteins reacting with the human proteins, leading to autoimmune disease (Fig. 3).

Although other factors apart from the hsps are involved in the aetiology and pathogenesis of autoimmune disease, evidence exists for such a model and suggests a critical role for hsps in these diseases. Autoantibodies and T cells reacting with the human hsps have been detected in individuals with autoimmune disease, antibodies to hsp90 and ubiquitin being particularly prevalent in systemic lupus erythematosus, while similar autoantibodies to hsp65 and hsp70 are present in rheumatoid arthritis [19, 21]. These findings support a role for autoreactivity to the hsps in the pathogenesis of autoimmune diseases.

More direct evidence for a specific role of the hsps in the aetiology of autoimmune disease is available in the case of adjuvant arthritis which can be induced in rats by a single injection of an extract of heat-killed *Mycobacterium tuberculosis* in Freund's complete adjuvant. Injected rats developed T cells specifically reacting with the mycobacterial hsp65 protein contained in the extract; such T cells can induce the disease when transferred to irradiated rats which have never been exposed to *M. tuberculosis* [18, 19, 21]. Moreover, if hsp65 is administered to these animals in oil rather than adjuvant, they can be protected against the arthritogenic effect of subsequent injections of mycobacterial extract in adjuvant. This raises the interesting possibility of using hsp65 in a vaccine for human diseases such as rheumatoid arthritis.

Similarly, in human autoimmune disease T cells and antibodies reacting with bacterial hsp65 or other hsps may be produced in response to a bacterial infection and then produce autoimmune disease by cross-reacting with an endogenous protein. The endogenous human hsps represent the most likely potential targets for such autoimmune cross-reaction in view of their extensive homology to the bacterial hsps. It should be noted, however, that T cells or antibodies to bacterial

Fig. 3. *Reactivity of antibodies and T cells induced by exposure to the hsps of micro-organisms with the corresponding human hsps.*



hsps may react in some cases with apparently unrelated human proteins on the basis of a shared epitope, and that in these cases such proteins, rather than the endogenous hsps, may therefore be the autoimmune target. For example, in the case of adjuvant arthritis the critical epitope on the mycobacterial hsp65 protein is more like the link protein of the cartilage proteoglycan than the corresponding endogenous rat hsp [23].

A remarkably similar pattern is also present in autoimmune diabetes. Thus in the NOD/Lr mouse, the genetically determined development of autoimmune diabetes is accompanied by the production of antibodies and T cells reactive with hsp65. Moreover, such T cells can promote the premature development of diabetes in young NOD/Lr mice, while injection of hsp65 in a non-immunogenic form can reduce the severity of subsequent disease [24]. Hence in this case, as in the arthritic diseases, the autoimmune target is likely to be a molecule which cross-reacts at the immunological level with hsp65. However, in human insulin-dependent diabetes, this target does not appear to be hsp65 itself as originally suggested [25], but has now been shown to be the human glutamic acid decarboxylase protein [26].

It is clear, therefore, that the production of antibodies and T cells reactive with hsps either genetically or following bacterial infection plays a critical role in the aetiology of autoimmune disease. Subsequently, the disease itself appears to involve the autoimmune reaction of these reagents either with the natural endogenous hsps whose expression has been elevated and/or relocalised within the cell, or with other cellular proteins with which the hsps share an epitope. The observation that the course of diseases as diverse as adjuvant arthritis and diabetes can be altered by injection of purified hsps offers hope for an effective vaccination against human diseases such as rheumatoid arthritis and insulin-dependent diabetes.

Conclusions

The hsps have made the transition from the esoteric phenomenon of 30 years ago to a central role, both as targets in autoimmune diseases as diverse as arthritis and diabetes and in protecting tissues such as the heart and inflamed joints from stress-induced damage. It is likely that as further tissues and diseases are studied, new roles for the hsps will be discovered. For example, it has recently been shown that hsp synthesis occurs at sites of brain damage [27], and that the hsp ubiquitin is present at high levels in the plaques characteristic of Alzheimer's disease [28]. It is probable, therefore, that the clinical interest in these proteins may only just be beginning and that the future will open further insights into their central role in numerous biological processes in both health and disease.

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