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Review Letter

Heat shock proteins and cell proliferation

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Heat shock proteins (Hsps) carry out a number of essential functions in the cell. These functions could be utilized, in a developmentally regulated manner, to affect proteins necessary for cell growth and proliferation. Data showing Hsp involvement in cell proliferation under non-stress conditions are perhaps not altogether surprising in view of the important roles of Hsps in cell metabolism. In a number of cases stress can facilitate cell proliferation in cells which could otherwise not be amenable to this developmental pathway. It is proposed that Hsps could play an essential helper role in initiating this process.

Numerous articles have been written about the importance of heat shock proteins for the survival of cells under stress conditions. Recently, however, a new and more complex picture has emerged: it seems that Hsps can play a number of essential roles in cellular processes not only under stress conditions but also under non-stress conditions where their synthesis is constitutively or developmentally regulated. Thus, the ability of Hsps to regulate protein folding and assembly [1,2] allows Hsps to transport proteins across cytoplasm and membranes, disrupt protein complexes, stabilize, degrade and regulate the synthesis of proteins and take part in DNA repair [1–7]. For example, DnaK, a heat shock protein found in *E. coli*, has been shown not only to disrupt protein aggregates [6], but also participate in protein export from *E. coli* [8]. Similarly, Hsp60 appears to have a dual function in rat mitochondria: regulating both mitochondrial ATPase activity and the folding and assembly of proteins imported into mitochondria [9]. Some roles of Hsps point to their involvement in cell proliferation. Indeed, it will be argued that Hsps may contribute to the induction of cell proliferation when they appear in cells subsequent to stress imposition.

The link between Hsps and stress-induced cell proliferation might not be as unexpected as first appears. It has long been known that high temperatures can induce synchronous cell division in procaryotic cell suspensions [10]. Similarly, stress treatments such as hormonal manipulations, osmotic shock and high temperatures are used extensively in plant cultures to initiate or release cells to undergo division, differentiation and embryogenesis [11]. The observation, that different stresses such as γ-irradiation and temperature can induce cell proliferation in the same organism [12], suggested that these stresses ultimately affect cell proliferation via a similar mechanism. These stresses are known to induce the synthesis of Hsps. It is proposed that there may be some Hsps which could play an essential helper role in facilitating stress-induced cell proliferation by creating the necessary conditions for this process to take place in cells which are not amenable to this developmental process.

This article considers the relationship between Hsps and cell proliferation, highlights some of the possible roles of Hsps in cell proliferation under non-stress conditions and explores the possible link between Hsps and stress induced cell proliferation.

1. EVIDENCE IMPLICATING Hsps IN CELL PROLIFERATION

Direct evidence is based on data where the function of some constitutively or developmentally expressed Hsps was at least partially established. Thus, when the *E. coli fam* gene, which is likely to be the same as the HS regulatory gene *htpR* coding for σ^{32} and acts upon proteins necessary for cell division, was mutated, mutants had faulty cell division and were not able to induce Hsps after heat stress [13]. Similarly, the *dnaK* gene in *E. coli*, which codes for a protein similar to Hsp70 of *Drosophila*, is essential for λdv DNA replication in vitro [6,14]. In *S. cerevisiae*, one of the eight Hsp70 genes, *SSC1* gene, is essential for vegetative growth since its inactivation prevents cell division from proceeding [15].

There are also many correlative observations supporting the data linking Hsps to cell proliferation. For ex-

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ample, in the mouse, the activation of the embryogenic genome at the two-cell stage is preceded by an appearance of Hsps and a decrease in protein synthesis [16]. In rat cells, *Hsp73* mRNA increases during cell division in vitro [17]. In *Drosophila*, the expression of the hsc4 gene (a member of the *Hsp70* gene family) has been shown to be higher in cells undergoing rapid growth and changes in shape [18]. In HeLa cells, Hsp70 interacts with other cellular proteins in a cell cycle-dependent manner: the synthesis of the proliferation-sensitive human protein IEF14 (corresponding to a 72 kDa Hsp) has been shown to increase during mitosis [19]. Based on studies with [^{35}S]methionine it was estimated that the rate of IEF14 synthesis increased during mitosis compared to G1 or S-phase. Similarly, Hsp70 is inaccesible to antibodies during the G2 phase of the cell cycle in HeLa cells [20]. This was suggested to be a result of Hsp70 interaction with other cellular proteins.

The above examples serve to illustrate not only that a positive correlation exists between the presence of Hsps and cell proliferation but also that some Hsps are necessary for this process to occur.

2. POSSIBLE ROLES OF Hsps IN CELL PROLIFERATION

So far, the best-studied roles of Hsps relate to their interaction with other proteins. Most evidence to date for the role of Hsps in cell proliferation is consequently based on such data. The evidence refers to constitutively or developmentally regulated Hsps and may be categorized as follows.

(1) Hsps may be involved in cell proliferation by interacting with proteins needed for the proliferation process. Specifically, some Hsps could affect cell proliferation by disrupting hydrophobic aggregates in the presence of ATP [1]. For example, Hsps DnaK and DnaJ are required for DNA replication: in the case of *E. coli* λdv DNA, the release of λP from hydrophobic preribosomal complex by DnaK and DnaJ in the presence of ATP is sufficient to initiate λdv DNA replication [6]. Association between Hsps and other proteins might also be needed to regulate transport of proteins required for cell proliferation across membranes. Thus, the *SSA1* gene product in yeast (the gene belongs to the same Hs70 family as *SSC1*) has been shown to be involved in the importation of proteins across membranes into the endoplasmic reticulum and mitochondria by changing the conformation of the precursor protein, the process being reversible by ATP [4,5]. The possibility also exists that, for example, the Hsp90 and Hsp70 families may affect cell proliferation by interacting with oncogene products such as src and p53 proteins [21,22].

(2) Hsps may be involved in modifying the activity of steroid (hormone) receptors and consequently steroid action. In most instances, this appears to involve Hsp90 and Hsp70 families [23–27]. The steroid receptors appear to become capable of associating with targeted DNA sequences only after these Hsps dissociate from the receptor complex, suggesting that such Hsps may inhibit receptor activity. The association of Hsp90 family with steroid receptors may have evolved early in steroid-response systems [28].

(3) Ubiquitin may be involved in cell proliferation through its interaction with CDC gene products [29,30]. Ubiquitin and at least some of the ubiquitin-conjugating enzymes are heat shock inducible [31,32]; indeed, the Hsp70 family of proteins and ubiquitin may be functionally interrelated [7]. The *CDC34* START gene product is required to transfer ubiquitins to an appropriate substrate. Mutations in *CDC34* are defective in the G1-S phase of cell cycle [33,34]. Thus, progression to S-phase of the cell cycle is dependent on the conjugation of ubiquitin to target proteins by the *CDC34* enzyme. It is noteworthy that ubiquitin has also been shown to interact with histones; ubiquitin is removed from histone H2A and H2B during metaphase but reappears during anaphase [35]. This type of interaction might be important for the progression of the cell cycle and/or DNA transcription [7,29,36–38].

3. IMPLICATIONS FOR STRESS-INDUCED CELL PROLIFERATION

As has already been discussed, Hsps can in some cases affect cell proliferation and, therefore, appear to be necessary for this process. There are thus possibly conditions when lack of certain Hsps might limit the proliferation of cells otherwise primed to proliferate. These cells would only execute the proliferation program when the limiting Hsps are synthesized, for example, under stress conditions. However, the involvement of Hsps in stress-induced cell proliferation may be more complex. Thus some of the functions of Hsps, as listed in the introduction, may be important under stress conditions to facilitate induction of cell proliferation. It is proposed that during the initial phases of stress-induced cell proliferation, stress and perhaps Hsps might modify levels and stability of certain proteins important for cell proliferation. Three possible modes of action will be considered.

(1) Hsps can disrupt and influence folding of proteins [1–7]. It is envisaged that Hsps may consequently, after they appear in cells subsequent to stress imposition, inactivate repressor(s) which would normally inhibit induction of cell proliferation; perhaps by disrupting such proteins, preventing binding of the repressor to the DNA target by changing the protein conformation or interacting with proteins modulating the repressor activity. Hsps would thus facilitate induction of a cell proliferation pathway by influencing the regulatory repressor protein. There is already an exam-

ple where a stress-activated protein can switch the developmental pathway of a cell by affecting a repressor protein: λ lysogen can be induced to a lytic (reproductive) cycle by irradiation of its bacterial host in a process mediated by RecA. When activated under stress conditions RecA exhibits a protease-like activity (it has a different function under non-stress conditions) and cleaves a repressor, which is required to sustain λ lysogen in a dormant cycle, thus triggering activation of genes leading to a lytic cycle of the phage [39]. Indeed, some Hsps could have a protease-like function or influence the proteolytic pathway: Hsp27 has a serine protease-like active site [40], at least one *E. coli* heat shock gene product has a protease activity [41] and mutations in, for example, *dnaK* and *dnaJ* heat shock genes resulted in defective proteolysis of polypeptide fragments [42].

(2) Preferential synthesis of Hsps under stress conditions may contribute to the inhibition of protein synthesis [43] and consequently increase the levels of proteins involved in cell proliferation by inhibiting the synthesis of enzymes required to degrade or inactivate proliferation-specific proteins. For example, three observations support the notion of oncogene product stabilization due to inhibition of protein synthesis: (i) a number of external stressful stimuli can increase the expression of c-*fos*, c-*myc* and the *Hsp70* family of genes [44–47]; (ii) protein synthesis is suppressed during heat stress [48,49]; and (iii) application of protein inhibitors (for example, cycloheximide and anisomycin) causes the accumulation of protooncogene c-*fos* mRNA and protein and of c-*myc* protein, protein synthesis being needed for the degradation rather than the synthesis of c-*myc* and c-*fos* mRNA and proteins [50–54]. Interestingly, it has been possible to induce cell division in a temperature-sensitive division-defective *E. coli* mutant subsequent to the addition of protein synthesis inhibitors [55].

(3) The reduced turnover of the cell proliferation proteins may also be due their stabilization by Hsps, or by cytoplasmic heat shock granules (containing subsets of Hsps) which have been suggested to serve as mRNA storage sites and to protect or stabilize proteins or mRNA under stress conditions [1,33,48,49]. Indeed, some Hsps may bind reversibly to proteins without triggering proteolysis and during recovery from stress treatment alter protein conformation to its native functionally active state [1,7,36,56]. With regard to mRNA, morphological analysis of the heat shock granules during the recovery from stress indicated reactivation of the bound mRNA making it available for protein synthesis [49].

It is not clear how the cell proliferation process becomes independent of the initial stress treatment. It is interesting, in view of the increased levels and stabilization of c-*myc* protein under stress conditions, that the c-*myc* protein has been reported to be capable of activating the *Hsp70* promoter [57], perhaps pointing to a mechanism by which regulation of some *Hsp* genes might become independent of the initial stress treatment and fulfill the cell proliferation roles as described in the previous section. In this case, c-*myc* would be the stress-sensitive trigger. The idea of the control of stress-induced Hsps by proteins involved in cell proliferation is consistent with observations for constitutively or developmentally expressed Hsps. Regulation of their expression can be achieved by a number of proteins involved in cell proliferation, including hormones [21,58,59]. Indeed, some *Hsp* genes are activated by a number of factors. Thus, the promoter of constitutively expressed *Hsp70* gene contains 4 response elements, each recognized by a different protein [60]. It is also possible that some *Hsp* genes and genes important in cell proliferation could be activated under certain conditions by a common factor, as can be illustrated from promoter data comparisons. Thus, for example, yeast *CDC25* [61] and *Drosophila Hsp22* [62] share 76% homology over 42 bases (with one base missing) in the promoter regions, including a possible heat shock element. Moreover, the *CDC25* gene and *Xenopus Hsp70* [63] share a significant 79% homology over 19 bases in the promoter regions. *CDC25*, which controls entry into mitosis in yeast is also a homologue to a *Drosophila* gene required for embryogenic cell cycles [64].

The role of Hsps in stress-induced cell poliferation might be coincidental in that Hsps synthesis is initiated in response to stress to contain stress damage. Their action in the cell would, however, facilitate cell proliferation under certain conditions such as the correct phase of the cell cycle and stage of development. Alternatively, there might be a causal relationship. Under adverse conditions cell proliferation (including sporulation) can improve the chances of an organism to pass its genetic material to future generations and thus increase the likelihood of its survival. In multicellular organisms, cell proliferation can also help with containing or recovering from, for example, physical injury. Since certain Hsps are evolutionarily highly conserv [65], it is tempting to propose that the stress response mechanism, and consequently Hsps, might have played a role in the evolution of the cell proliferation mechanism in eucaryotes to serve as an additional mechanism to deal with stress.

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