COMMENTARY

Clustering of heat-shock factors

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Clusterin is a ubiquitous glycoprotein found in most physiological fluids and tissues. Although not fully understood, the function of clusterin seems to be related to its ability to bind a wide variety of molecules. Since clusterin has been found associated with extracellular protein aggregates, a role as a molecular chaperone has been proposed. In this issue of the *Biochemical Journal*, Le Dréan and colleagues demonstrate an up-regulation of clusterin in neuronal cells exposed to proteotoxic stress that results in unfolded protein accumulation and proteasome impairment, both commonly associated with neurodegenerative diseases. Interestingly, expression of clusterin was found to be regulated by two members of the HSF (heat-shock factor) family, HSF1 and HSF2, which possibly form a trimeric complex on the clusterin promoter. The study proposes clusterin as a player in a cellular defence mechanism against harmful protein accumulation, and highlights the importance of elucidating further the exact role of clusterin and the intriguing interaction between HSF1 and HSF2.

Key words: apolipoprotein J, clusterin, heat-shock factor, proteasome impairment, proteotoxic stress.

Clusterin, also called apolipoprotein J, is a ubiquitously expressed glycoprotein identified more than 20 years ago [1]. However, its function and regulation remain enigmatic. The single-copy clusterin gene is well conserved during evolution, and encodes a 449-amino-acid-long precursor protein. The precursor protein possesses a hydrophobic secretory signal sequence directing it to the endoplasmic reticulum. The translated product is proteolytically cleaved into α- and β-subunits, which are concomitantly linked together by disulphide bonds to form a heterodimer. In addition, maturation of clusterin involves extensive N-linked glycosylation before secretion [2]. Clusterin is an abundant protein in physiological fluids and present in most mammalian organs and tissues. Within a given tissue, however, its expression is limited to particular cell types. In the brain, for example, clusterin is synthesized mainly by glial cells, and the secreted clusterin can be taken up by neurons, particularly upon neuronal stress (e.g. [3]). Apart from the secreted form of clusterin, nuclear truncated forms have been reported that potentially originate from alternative splicing (e.g. [4]).

Clusterin binds a wide range of molecules, including lipids, immunoglobulins, complement components and amyloid proteins, and, accordingly, diverse functions in biological processes, such as cell adhesion, lipid transport, reproduction and cytoprotection, have been proposed. Furthermore, clusterin has been related to numerous severe physiological disturbances, e.g. tissue injuries, atherosclerosis and neurodegenerative diseases [2,5]. An obvious question is whether clusterin truly is as multifunctional as has been suggested, or whether it might play a single primary role that is masked by its capability to bind a wide range of molecules. In support of the latter possibility, clusterin has been reported to function as an extracellular heat-shock protein. Heat-shock proteins are molecular chaperones that protect cells against various forms of stress by binding unfolded or misfolded proteins. Similarly to the small heat-shock proteins, to which clusterin is structurally related, clusterin has been shown to be stress-responsive and to prevent protein aggregation [2,6]. On the other hand, elevated expression of clusterin has paradoxically been suggested to have cytotoxic effects causing cell death. Whatever the function might be, clusterin has been found in various extracellular aggregates and to be up-regulated in numerous neurodegenerative states, including Huntington's and Pick's diseases, multiple sclerosis, scrapie and amyotrophic lateral sclerosis. In Alzheimer's disease, clusterin is associated with amyloid plaques and cerebrovascular deposits. Increased amounts of clusterin mRNA have also been found in acute neuropathies, in epileptic foci in the brain and following neuronal injuries [5,7].

Considering the association of clusterin with cellular stress, protein aggregation and disturbed protein homoeostasis, especially in neurodegenerative diseases, Le Dréan and colleagues [8], in this issue of the *Biochemical Journal*, hypothesized that clusterin would be up-regulated in glial cells in response to proteasome impairment. By disrupting the UPS (ubiquitin–proteasome system) with the proteasome inhibitor MG132 or the amino acid analogue AZC (azetidine-2-carboxylic acid), the authors detected an increase in both clusterin protein and mRNA levels in human glial cells. This finding supports the results of a recent study [9] in which involvement of clusterin in neurodegenerative diseases could be related to failure of the UPS.

The expression levels of clusterin vary markedly, showing low levels under normal conditions in most cells and strong up-regulation in response to various stresses. Thus the expression of clusterin must be carefully regulated. The components and regulatory mechanisms are poorly characterized and the promoter shows relatively little sequence homology among species. However, the clusterin promoter contains a highly conserved 14-bp element recognized by HSF1 (heat-shock factor 1). This element was named CLE (clusterin element), and it differs by only a single base from the classical HSE (heat-shock element). The fact that the clusterin gene contains the CLE that upon heat stress is occupied by HSF1 indicates that clusterin might function as a molecular chaperone [6]. Le Dréan and colleagues [8] explored further the regulation of clusterin and found that the CLE is important for clusterin up-regulation during proteasomal insult. The element was shown to be absolutely necessary, and, most interestingly, it serves as a binding site for both HSF1 and HSF2.

In vertebrates, four members of the HSF family have been characterized that all share the ability to bind to HSEs in the promoters of their target genes. The best known genes activated

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by HSFs code for a set of heat-shock proteins involved in the heat-shock response, a cellular defence mechanism that protects the cell from the deleterious effects of stress [10]. The finding by Le Dréan and colleagues [8] that both HSF1 and HSF2 bind to the clusterin promoter upon proteotoxic stress is exciting, since HSF1 and HSF2 were originally thought to have profoundly distinct roles. HSF1 is considered the *bona fide* stress-responsive factor that, upon elevated temperatures or other forms of protein-damaging stress, is converted from a monomer into a trimer, acquires DNA-binding activity and induces transcription of heat-shock genes. Furthermore, no other factor is able to compensate for HSF1 in the heat-shock response. HSF2, on the other hand, has been thought to be refractory to classical stressors, and rather to be involved in development- and differentiation-related processes [10]. However, several reports have indicated that the roles of HSF1 and HSF2 might not be so distinct after all. For example, HSF1-knockout mice display some developmental defects, suggesting that HSF1 also has a role in development [10]. Likewise, HSF2 has been shown to respond to heat by altered localization and solubility [11,12]. Interestingly, evidence for an interaction between HSF1 and HSF2 has recently been presented. In addition to HSF1 and HSF2 physically interacting through their trimerization domains [12,13], both factors can bind to the same target genes [14].

The present study by Le Dréan and colleagues [8] agrees with the above-mentioned results concerning the interaction between HSF1 and HSF2. Interestingly, the study expands further the concept of an interaction between the two factors to also apply to proteasome impairment and clusterin transcription. Binding of both HSF1 and HSF2 to the CLE was demonstrated through supershift EMSA and chromatin immunoprecipitation. Using gel filtration, the two factors were found in the same fraction, and the complex had a mass similar to that of HSF1 homotrimers, implying that heterotrimers could be formed. Although the study does not provide definitive evidence that a heterotrimer does indeed bind to the clusterin promoter, the structure of the CLE suggests that this is the case, since it matches the minimal binding site for only one trimer of HSFs. Apparent questions for future studies are the stoichiometry between HSF1 and HSF2 in a possible complex and how the factors interact with each other. In this respect, generation of structural models of HSF complex formation in vertebrates could provide important knowledge. Why, then, would the HSFs form heterotrimers? Interdependency of DNA-binding activities among different transcription factors offers an efficient way to control gene expression in a cell- and stimulus-specific manner. By interacting with different partners in distinct situations, HSFs might execute differential gene regulation. Given the slightly dissimilar binding preferences of HSF1 and HSF2 [10], the composition of the HSE in the target gene could determine what kind of HSF complex is formed. An exciting cross-talk between the two HSF members HSF1 and the avian-specific HSF3 has been reported, although no physical interaction was detected. In avian cells, HSF1 and HSF3 are both stress-responsive, albeit to different degrees. Interestingly, absence of HSF3 affected the heat-shock response and hampered the formation of HSF1 trimers, indicating that HSF3 directly influences HSF1 activity [15]. Another example of cross-talk between HSF members is found in mouse lens cells, where HSF1 and HSF4 have opposite effects on the expression of fibroblast growth factors, HSF1 functioning as an activator and HSF4 as a repressor [16]. It would be important to establish whether similar interdependencies apply to HSF1 and HSF2 in human cells, where the factors perhaps could act competitively or by one factor regulating the other.

The study by Le Dréan and colleagues [8] demonstrates an intimate relationship between HSF1 and HSF2 in the context of clusterin regulation. It will be crucial to elucidate further the interaction between the two factors, thereby shedding light on the regulation of their target genes. Although the role of clusterin in extracellular aggregates such as those found in neurodegenerative diseases is still a matter of debate, there are strong indications for a protective function, perhaps as a molecular chaperone. Therefore revealing the role of clusterin in proteotoxic stress, as well as its transcriptional regulation, is of most importance.

REFERENCES

1 Blaschuk, O., Burdzy, K. and Fritz, I. B. (1983) Purification and characterization of a cell-aggregating factor (clusterin), the major glycoprotein in ram rete testis fluid. J. Biol. Chem. **258**, 7714–7720

2 Wilson, M. R. and Easterbrook-Smith, S. B. (2000) Clusterin is a secreted mammalian chaperone. Trends Biochem. Sci. **25**, 95–98

3 Michel, D., Moyse, E., Trembleau, A., Jourdan, F. and Brun, G. (1997) Clusterin/ApoJ expression is associated with neuronal apoptosis in the olfactory mucosa of the adult mouse. J. Cell Sci. **110**, 1635–1645

4 Reddy, K. B., Jin, G., Karode, M. C., Harmony, J. A. and Howe, P. H. (1996) Transforming growth factor β (TGF β)-induced nuclear localization of apolipoprotein J/clusterin in epithelial cells. Biochemistry **35**, 6157–6163

5 Rosenberg, M. E. and Silkensen, J. (1995) Clusterin: physiologic and pathophysiologic considerations. Int. J. Biochem. Cell Biol. **27**, 633–645

6 Michel, D., Chatelain, G., North, S. and Brun, G. (1997) Stress-induced transcription of the clusterin/apoJ gene. Biochem. J. **328**, 45–50

7 Calero, M., Rostagno, A., Matsubara, E., Zlokovic, B., Frangione, B. and Ghiso, J. (2000) Apolipoprotein J (clusterin) and Alzheimer's disease. Microsc. Res. Tech. **50**, 305–315

8 Loison, F., Debure, L., Nizard, P., Le Goff, P., Michel, D. and Le Dréan, Y. (2005) Up-regulation of the clusterin gene after proteotoxic stress. Implication of HSF1/HSF2 heterocomplexes. Biochem. J. **395**, 223–231

9 Carreras, I., Garrett-Young, R., Ullman, M. D., Eisenhauer, P. B., Fine, R. E., Wells, J. M. and Conn, K. J. (2005) Upregulation of clusterin/apolipoprotein J in lactacystin-treated SH-SY5Y cells. J. Neurosci. Res. **79**, 495–502

10 Pirkkala, L., Nykänen, P. and Sistonen, L. (2001) Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J. **15**, 1118–1131

11 Mathew, A., Mathur, S. K., Jolly, C., Fox, S. G., Kim, S. and Morimoto, R. I. (2001) Stress-specific activation and repression of heat shock factors 1 and 2. Mol. Cell. Biol. **21**, 7163–7171

12 Alastalo, T.-P., Hellesuo, M., Sandqvist, A., Hietakangas, V., Kallio, M. and Sistonen, L. (2003) Formation of nuclear stress granules involves HSF2 and coincides with the nucleolar localization of Hsp70. J. Cell Sci. **116**, 3557–3570

13 He, H., Soncin, F., Grammatikakis, N., Li, Y., Siganou, A., Gong, J., Brown, S. A., Kingston, R. E. and Calderwood, S. K. (2003) Elevated expression of heat shock factor (HSF) 2A stimulates HSF1-induced transcription during stress. J. Biol. Chem. **278**, 35465–35475

14 Trinklein, N. D., Chen, W. C., Kingston, R. E. and Myers, R. M. (2004) Transcriptional regulation and binding of heat shock factor 1 and heat shock factor 2 to 32 human heat shock genes during thermal stress and differentiation. Cell Stress Chaperones **9**, 21–28

15 Tanabe, M., Kawazoe, Y., Takeda, S., Morimoto, R. I., Nagata, K. and Nakai, A. (1998) Disruption of the HSF3 gene results in the severe reduction of heat shock gene expression and loss of thermotolerance. EMBO J. **17**, 1750–1758

16 Fujimoto, M., Izu, H., Seki, K., Fukuda, K., Nishida, T., Yamada, S., Kato, K., Yonemura, S., Inouye, S. and Nakai, A. (2004) HSF4 is required for normal cell growth and differentiation during mouse lens development. EMBO J. **23**, 4297–4306

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