CMLS, Cell. mol. life sci. 53 (1997) 212–213
1420-682X/97/020212-02 $ 1.50 + 0.20/0

CMLS Cellular and Molecular Life Sciences

Conclusion

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We hope that readers who, as did most of us in the near or more distant past, encountered the heat shock proteins (HSPs) and chaperones on their research pathway will have found in this multi-author review the information necessary to apprehend the specific or general functions of these proteins during differentiation and development and the mechanisms regulating their expression.

We do not want to add a long conclusion to the wealth of data reported in the different articles. As readers will have discovered by themselves, despite this abundance we still lack firm conclusions in many areas. The mechanisms regulating the constitutive expression of HSPs and chaperones are diverse, and the role (even the existence) of additional heat shock transcription factors (HSFs) still remains obscure. The differential response of the different tissues of an embryo or an adult organism to stress is well established and has been described in *Drosophila* and in *Xenopus*, in the zebrafish and mouse, but the mechanisms involved and the physiological significance of these variations remain largely unknown.

The major achievements during recent years concern the functions of the HSPs. The role of HSPs in protein folding (and degradation) have been well described in vitro and are widely confirmed by in vivo studies. The roles of the different members of the HSP70 family in the different cell compartments, and that of HSP60 in the mitochondria, are now well established. However, these HSPs, which participate in normal protein-folding pathways and address proteins towards the different cell compartments, are probably not the most important for developmental biologists. These pathways are so fundamental that the variations in the expression of their components will only reflect general variations in protein synthesis. The same is probably also true for the proteins participating in secretion, such as GRP78, GRP94, calnexin, calreticulin or HSP47. We already have some data on their expression during embryogenesis (see D. Walsh et al. and P. H. Krone et al. in this review) which strongly suggest that their abundance in the different cells is more or less directly linked with the secretory activity of these cells.

However, the functions of other HSPs are probably more diverse than a 'general chaperoning function'. They may participate in chaperoning a specific group of proteins (such as HSP90 or small HSP/α-crystallins). It remains possible that the general chaperone function demonstrated in vitro for these proteins is partially artefactual and that their real function lies elsewhere (see the different articles for some clues on these potential functions). Moreover, some HSP families, such as HSP/HSC70, are so complex that some of their members probably have very specialized functions that are quite distinct from the generalized functions of the others. Even a pleiotropic chaperone such as HSC70 supports more specialized functions such as clathrin uncoating. These specific functions rely on the interaction with specific cochaperones [1, 4]. The main limits to our understanding the developmental role of HSPs and chaperones lie in the still scarce information that we have on what these specific functions might be.

Despite this limitation in our present knowledge, however, this multi-author review bears witness to a significant change in this domain of research, by which future directions can clearly be defined. We now observe a transition between the mundane idea that HSPs participate in cell differentiation and development to the precise hypothesis that they play a crucial role in some specific developmental processes. Despite the bias represented by the choice of contributors to this review, readers will realize that the data, collected in different organisms, point to the same developmental processes in which HSPs seem to be crucially involved: gametogenesis and early development, or the formation of the nervous system, central or peripheral. Other processes are also potential candidates for further study: muscle (and heart) formation and bone morphogenesis. This list is far from being exhaustive, but it clearly suggests that the efforts have to be strengthened and focussed in these directions.

In the last major review on HSPs and development, J. J. Heikkila placed his hopes in the advent of the new technology of gene disruption by homologous recombination to determine the precise role of HSPs in development [3]. Progress in this direction has been slower than one might have hoped, and the fact that the major HSPs are encoded by multiple genes makes this approach all the more difficult. Moreover, probably most specialists in this field were convinced that the disruption of these genes would have dramatic effects on development, due to the ubiquitous role of the encoded proteins. Disruption of the unique HSF in *Drosophila*

blocks the development of this organism (C. Wu, unpublished results), although it appears that this blockade is not due to a modification of HSP gene expression. Yet the result obtained with the *hsp70.2* gene has shown the value of such an approach. The corresponding protein, which is a minor member of the HSP70 family, is specifically expressed during spermatogenesis in pachytene spermatocytes and round spermatids. The disruption of the corresponding gene leads to a complete block of meiosis in male germ cells [2].

In cases where gene disruption would be impossible, due to the pleiotropic action of the corresponding proteins, other approaches to inactivate the gene in specific tissues or during specific ex vivo differentiations still remain possible: use of antisense oligonucleotides, expression in vivo of antisense constructions, or conditional and/or tissue-specific homologous recombination. Gene disruption will also be indirectly very useful in assessing the role of HSP gene expression. In the mouse, but also in the zebrafish, many mutations have been characterized which affect precise developmental steps. These mutations are valuable tools in investigating the specific steps at which HSPs are expressed, the mechanisms controlling their expression, and also their function(s) in these differentiation pathways.

Specialists in heat shock have not yet fully exploited the powers of the molecular tools now accessible to developmental biologists to assess the role of HSPs in differentiation and development. There is no doubt that the results of such studies will constitute the scope of a future review.

1 Cyr D. M., Langer T. and Douglas M. G. (1994) DnaJ-like proteins: molecular chaperones and specific regulators of Hsp70. TIBS **19:** 176–181

2 Dix D. J., Allen J. W., Collins B. W., Mori C., Nakamura N., Poorman-Allen P. et al. (1996) Targeted gene disruption of Hsp70.2 results in failed meiosis, germ cell apoptosis and male infertility. Proc. Natl. Acad. Sci. USA **93:** 3264–3268

3 Heikkila J. J. (1993) Heat shock gene expression and development. II. An overview of mammalian and avian developmental systems. Dev. Genet. **14:** 87–91

4 Ungewickell E., Ungewickell H., Holstein S. E. H., Lindner R., Prasad K., Barouch W. et al. (1995) Role of auxilin in uncoating clathrin-coated vesicles. Nature **378:** 632–635

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