Series

What history tells us
II. The discovery of chaperone function

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1. Introduction

Twenty years ago, Hugh Pelham interpreted the effects observed after an overexpression of HSP70 in heat-stressed cells by the ability of this protein to disrupt the complexes of unfolded proteins that form under these conditions (Pelham 1984; Lewis and Pelham 1985). This was one of the important steps which led in less than five years to the emergence of the notion of chaperones.

The history of the discovery of the chaperones was rich and tortuous. Chaperone function was the last of the main cellular functions to emerge from . . . nothing: both the central dogma of molecular biology and the experiments of Christian Anfinsen supported the hypothesis that the folding of proteins and their assembly into macromolecular complexes were spontaneous processes, requiring no assistance.

The conception of chaperone action has evolved since it was initially proposed. This evolution outlines the limits of the notion of function at the macromolecular level. The complex picture of the involvement of chaperones in cell functions can only be understood as the result of the optimisation of organism fitness by natural selection.

2. A complex history

The first use of the notion of chaperones was for a toxin present in the venom of the Australian taipan snake (Fohlman *et al* 1976). The two protein subunits surrounding the active neurotoxic subunit were described as chaperones that increase the specificity of the toxin and protect it against degradation. No extension of the notion of chaperone to other systems was then attempted.

The same is true for Ron Laskey's famous discovery of the chaperone action of nucleoplasmin on histones, allowing their correct assembly into nucleosomes (Laskey *et al* 1978). The role of nucleoplasmin is transient: it is not part of the full nucleosome assembly. Its sole function is to prevent premature, improper interactions between positively charged histones and the negatively charged DNA molecule. The requirement for nucleoplasmin is not absolute, and can be lifted by a very slow *in vitro* decrease in ionic strength during nucleosome assembly.

It was through the study of the heat shock response that the generality of chaperone function emerged. There is a long tradition of research into the effects of heat on organisms, including the possibility of mimicking genetic mutations by phenocopies. The modern history of the cellular heat shock response started with the observation by Ferruccio Ritossa in 1962 that a transient increase in temperature activates the expression of a small group of *Drosophila* genes (Ritossa 1962, 1996). In the following years, it was demonstrated that this response is conserved from bacteria to mammals, as are the different families of induced proteins (heat shock proteins, HSPs). The synthesis of some members of these families, present in the endoplasmic reticulum, could also be increased by glucose starvation. These proteins were not absent from resting cells, but abundant. From the end of the 1970s to the discovery of chaperone function, many observations obscured rather than clarified the picture: overexpression of these proteins in cancer, association of some of them with oncogenic protein kinases as well as steroid hormone receptors, high expression at specific steps of differentiation and development. Hypotheses concerning the role of these proteins in metabolism, or in the control of cytoskeletal structure, were proposed before Hugh Pelham opened the way to chaperone function with his 1985 observations. In 1986, he generalized the picture by including what was known of the behaviour of BiP, a protein interacting with many proteins transiting through the reticulum before their assembly into macromolecular

complexes: he demonstrated that BiP was also a member of the HSP70 family (Munro and Pelham 1986; Pelham 1986).

But it was John Ellis who named the function in 1987 from his observations of a very different experimental system, the assembly of ribulose 1–5 diphosphate carboxylase (Rubisco) – the enzyme responsible for assimilation of CO_2 in chloroplasts (Ellis 1996). A Rubisco-binding protein was discovered in 1980, and its chaperoning of Rubisco progressively demonstrated. The hypothesis that chaperone functions might be general was advanced by John Ellis in *Nature* in July 1987 (Ellis 1987).

3. An unfinished story

The *Nature* article was the beginning of a slow process of conceptual maturation rather than its conclusion. First, because it was the convergence, over the three next years, of very different observations from diverse, unrelated lines of research which pushed chaperone function into the limelight. At the end of the 1987, it was shown by gene sequencing that the Rubisco-binding protein was homologous to GroEL, an abundant protein of *Escherichia coli* required for the growth of many bacteriophages, including *l* (Hemmingsen *et al* 1988). Retrospectively, the role of GroEL and other HSPs in the morphogenesis of bacteriophages could be interpreted as beautiful examples of chaperone function (Georgopoulos *et al* 1973). Two years later, the chaperone function of GroEL – with its associated subunit GroES – was demonstrated *in vitro* (Goloubinoff *et al* 1989). Simultaneously, it was shown that HSPs – HSP70 – were required for the import of proteins into subcellular organelles, in close association with imported proteins on both sides of the membranes delimiting the organelles (Deshaies *et al* 1988; Chirico *et al* 1988). By photo-induced protein cross-linking, GroEL was demonstrated to be associated with nascent polypeptides in an *in vitro E. coli* translation system from (Bochkareva *et al* 1988). Similarly, HSP70 was shown to interact transiently with nascent polypeptides in eukaryotic cells by immunoprecipitation with antibodies targeting HSP70 (Beckmann *et al* 1990). This rapid succession of discoveries was determinant in recognizing the importance of chaperone function.

The description of different steps in chaperone action and the structural characterization of the various families of chaperones at the molecular level revealed how these extraordinary ATP-driven nano-machines assist protein folding and assembly. It demonstrated once more the remarkable explanatory power of structural biology. High-molecular-weight chaperonins such as the GroEL-GroES complex, with their huge cavity – named the Anfinsen cage – inside which proteins fold, were distinguished from simpler low-molecular-weight chaperones such as HSP70. Both kinds of chaperones recognize areas or sequences of hydrophobic amino acids.

In parallel with this molecular description, the significance of chaperone function shifted. The part played by chaperones in protein assembly-disassembly revealed by early studies became secondary, the mere consequence of the primary role that chaperones have in protein folding (an evolution already clear in Ellis and Hemingsen 1989). This was the first step towards a second transformation of chaperone function: its involvement in the more general process of quality control of proteins (Hurtley and Helenius 1989). This shift was due to two kinds of observations: that chaperones are not ‘folders’, but that their unique role is to prevent proteins from entering folding dead-ends; and that chaperones cooperate with proteolytic systems to eliminate stably misfolded proteins.

4. A heretical discovery?

Was the discovery of chaperones heretical, a blow to the dogma of molecular biology? The *credo* that protein folding is not assisted is rather recent in the history of molecular biology, the consequence of the main hypotheses proposed by Francis Crick in 1957, and of the experiments performed by Christian Anfinsen at the beginning of the 1960s. Before then, the dominant idea was that proteins were ‘moulded’ on protein-forming centres.

The dogma is not violated since chaperones do not orient the folding process but only prevent parasitic reactions such as protein aggregation. The hypothesis that chaperones could have an active role, a steric action on their protein targets, was not totally rejected by John Ellis – although it is hard to reconcile with the general action of chaperones – and still haunts the dreams of many biologists. It has been proposed that the highly specific characteristics of prions might be explained by a self-chaperoning activity of these proteins (Liautard 1991). Some examples supporting the hypothesis of a steric action of chaperones can be given, but most data do not support a role of active folders. The role of chaperones appears to be passive, limited to the prevention of dead-end folding. Even the less ambitious possibility that chaperonins are able to unfold proteins, and therefore to extract them from folding dead-ends, lacks unambiguous experimental support.

This does not prevent many from considering chaperones as magic components that could shed light on fields of research where explanations are still lacking: immunology – with the puzzling development of self-immune diseases, control of cell death, control of differentiation and development, mechanisms of ageing. One family of HSPs (HSP90) has even been considered as a capacitor for evolution, masking mutations which would reveal themselves in stressful conditions and open new path-

ways to the adaptation of organisms (Rutherford and Lindquist 1998). This persistent search for 'extra' functions for chaperones probably stems from the initial observations of the as yet unnamed chaperones at the beginning of the 1980s, before 'mundane' chaperone function was discovered.

5. A Darwinian discovery

Some of these additional functions for chaperones may in the future turn out to be physiologically significant. In any case, the discovery of chaperones should persuade us to revise our all too frequently naive vision of molecular functions.

In a brilliant article, Peter Lawrence made fun of biologists who overestimate the importance of the genes on which they work, and refuse to admit that they may be trivial ones. He particularly criticized the tendency to consider a gene as "major developmental gene" simply because its inactivation leads to disruption of the developmental process (Lawrence 2001). Such genes can be necessary to development without controlling it. Lawrence's remarks are a welcome reminder to biologists to be more cautious. In reality it is not so easy to distinguish genes that play a major part in controlling development from those that are bit players. What has been retained by natural selection is what increases fitness, not functions as such.

During evolution, organisms have tinkered (Jacob 1977) with their genomes to improve the reliability of their protein structures. One of the means used was the invention of chaperones. Another was certainly the modification of protein sequences, which explains the erratic observations made on chaperones during the last two decades. Some proteins always need chaperones to fold correctly, while others never do. A third category of proteins requires the help of chaperones in just some cases, probably in adverse physico-chemical conditions or when they have taken the wrong folding pathway. Various cell compartments and different organisms have adopted distinct chaperoning strategies. Different chaperone machines with partially redundant specificity co-exist in the same compartment. Chaperonins are important in bacteria, apparently have a more limited role in the cytosol of eukaryotic cells, and are absent in the endoplasmic reticulum. Does this matter? What counts is the reliability of the folding process. A wonderful example of this tinkering action of evolution is the synthesis by bacteriophages of protein subunits able to replace the GroES subunits. These phage subunits increase the size of the Anfinsen cage of the chaperonin, which is then better adapted to the large capsid protein (Hunt *et al* 1997).

And the extraordinary ability of chaperones to bind transiently to hydrophobic surfaces has been exploited during evolution each time such a function could increase fitness: the role of HSP70 in the disassembly of clathrin cages is one such example (Ungewickell 1985), but many others probably remain to be discovered.

It may even be that some proteins have acquired a steric chaperone activity, i.e. are able to orient the structure of their target proteins towards 'unnatural' conformational states. Why not? Cells and organisms are not constrained by the principles we build, if they have any opportunity to increase their fitness by violating them.

The tinkering action of evolution also prevents the simple use of chaperones in biotechnology. Chaperones are adapted to function in certain very specific physico-chemical conditions, and with particular targets. It is a vain hope – already shown to be misplaced – that overexpressing them will constitute a universal way to increase the production of active proteins.

References

Beckmann R P, Mizzen L A and Welch W J 1990 Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly; *Science* **248** 850–854

Bochkareva E S, Lissin N M and Girshovich A S 1988 Transient association of newly synthesized unfolded proteins with the heat-shock GroEL protein; *Nature* (*London*) **336** 254–257

Chirico W J, Waters M G and Blobel G 1988 70 K heat shock related proteins stimulate protein translocation into microsomes; *Nature* (*London*) **332** 805–810

Deshaies R J, Koch B D, Werner-Washburne M, Craig E A and Schekman R 1988 A subfamily of stress proteins facilitates translocation of secretory and mitochondrial precursor polypeptides; *Nature* (*London*) **332** 800–805

Ellis J 1987 Proteins as molecular chaperones; *Nature* (*London*) **328** 378–379

Ellis R J 1996 Discovery of molecular chaperones; *Cell Stress Chap*. **1** 155–160

Ellis R J and Hemmingsen S M 1989 Molecular chaperones: proteins essential for the biogenesis of some macromolecular structures; *Trends Biochem. Sci*. **14** 339–342

Fohlman J, Eaker D, Karlsson E and Thesleff S 1976 Taipoxin, an extremely potent presynaptic neurotoxin from the venom of the Australian snake taipan; *Eur. J. Biochem*. **68** 457–469

Georgopoulos C P, Hendrix R W, Casjens S R and Kaiser A D 1973 Host participation in bacteriophage *l* head assembly; *J. Mol. Biol*. **76** 45–60

Goloubinoff P, Christeller J T, Gatenby A A and Lorimer G C 1989 Reconstitution of active dimeric ribulose bisphosphate carboxylase from an unfolded state depends on two chaperonin proteins and Mg-ATP; *Nature* (*London*) **342** 884–889

Hemmingsen S M, Woolford C, van der Vies S M, Tilly K, Dennis D T, Georgopoulos C P, Hendrix R W and Ellis R J 1988 Homologous plant and bacterial proteins chaperone oligomeric protein assembly; *Nature* (*London*) **333** 330–334

Hunt J F, van der Vies S M, Henry L and Deisenhofer J 1997 Structural adaptations in the specialized bacteriophage T4 co-chaperonin Gp31 expand the size of the Anfinsen cage; *Cell* **90** 361–371

Hurtley S M and Helenius A 1989 Protein oligomerization in the endoplasmic reticulum; *Annu. Rev. Cell Biol*. **5** 277–307

Jacob F 1977 Evolution and tinkering; *Science* **196** 1161–1166

Laskey R A, Honda B M, Mills A D and Finch J T 1978 Nucleosomes are assembled by an acidic protein that binds histones and transfers them to DNA; *Nature* (*London*) **275** 416–420

Lawrence P A 2001 Science or alchemy?; *Nat. Rev. Genet.* **2** 139–141

Lewis M J and Pelham H R B 1985 Involvement of ATP in the nuclear and nucleolar functions of the 70 kd heat shock protein; *EMBO J.* **4** 3137–3143

Liautard J P 1991 Are prions misfolded molecular chaperones?; *FEBS Lett.* **294** 155–157

Munro S and Pelham H R B 1986 An hsp70-like protein in the ER: Identity with the 78 kd glucose-regulated protein and immunoglobulin heavy chain binding protein; *Cell* **46** 291–300

Pelham H R B 1984 Hsp70 accelerates the recovery of nucleolar morphology after heat shock; *EMBO J.* **3** 3095–3100

Pelham H R B 1986 Speculations on the functions of the major heat shock and glucose-regulated proteins; *Cell* **46** 959–961

Ritossa F 1962 A new puffing pattern induced by temperature shock and DNP in *Drosophila; Experientia* **18** 571–573

Ritossa F 1996 Reflections: Discovery of the heat shock response; *Cell Stress Chap.* **1** 97–98

Rutherford S L and Lindquist S 1998 Hsp90 as a capacitor for morphological evolution; *Nature* (*London*) **396** 336–342

Ungewickell E 1985 The 70-kd mammalian heat shock proteins are structurally and functionally related to the uncoating protein that releases clathrin triskelia from coated vesicles; *EMBO J.* **4** 3385–3391

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