Evolution of the α-Crystallin/Small Heat-Shock Protein Family[1]

Wilfried W. de Jong, Jack A. M. Leunissen,† and Christina E. M. Voorter**

*Department of Biochemistry and †CAOS/CAMM Center, University of Nijmegen

The common characteristic of the α-crystallin/small heat-shock protein family is the presence of a conserved homologous sequence of 90–100 residues. Apart from the vertebrate lens proteins—αA- and αB-crystallin—and the ubiquitous group of 15–30-kDa heat-shock proteins, this family also includes two mycobacterial surface antigens and a major egg antigen of *Schistosoma mansoni.* Multiple small heat-shock proteins are especially present in higher plants, where they can be distinguished in at least two classes of cytoplasmic proteins and a chloroplast-located class. The α-crystallins have recently been found in many tissues outside the lens, and αB-crystallin, in particular, behaves in many respects like a small heat-shock protein. The homologous sequences constitute the C-terminal halves of the proteins and probably represent a structural domain with a more variable C-terminal extension. These domains must be responsible for the common structural and functional properties of this protein family. Analysis of the phylogenetic tree and comparison of the biological properties of the various proteins in this family suggest the following scenario for its evolution: The primordial role of the small heat-shock protein family must have been to cope with the destabilizing effects of stressful conditions on cellular integrity. The α-crystallin-like domain appears to be very stable, which makes it suitable both as a surface antigen in parasitic organisms and as a long-living lens protein in vertebrates. It has recently been demonstrated that, like the other heat-shock proteins, the α-crystallins and small heat-shock proteins function as molecular chaperones, preventing undesired protein-protein interactions and assisting in refolding of denatured proteins. Many of the small heat-shock proteins are differentially expressed during normal development, and there is good evidence that they are involved in cytomorphological reorganizations and in degenerative diseases. In conjunction with the stabilizing, thermoprotective role of α-crystallins and small heat-shock proteins, they may also be involved in signal transduction. The reversible phosphorylation of these proteins appears to be important in this respect.

Introduction

Ten years ago Ingolia and Craig (1982) made the exciting discovery that the small heat-shock proteins (hsps) of *Drosophila melanogaster* are evolutionarily related to the lens protein α-crystallin. Whereas α-crystallin has been known for almost a century as a major structural protein of the vertebrate eye lens (Mörner 1894), the small hsps derive their name from the observation that a specific set of proteins is induced in *Drosophila* larvae on exposure to increased temperature (Tissières et al.

1. Key words: α-crystallin, small heat-shock proteins, chaperones, eye lens.

Address for correspondence and reprints: Wilfried W. de Jong, Department of Biochemistry, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

Mol. Biol. Evol. 10(1):103–126. 1993.

0737-4038/93/1001-0007$02.00

1974). The purpose of the present article is to make a plausible reconstruction of the evolutionary history of this intriguing protein family. This will be done by analysis of the ever-increasing set of sequence data and by comparison of the structural and functional properties of the members of this family.

α-Crystallin

α-Crystallin is abundant in the eye lenses of almost all vertebrate species, reaching levels of up to 50% of the water-soluble protein fraction (Bloemendal 1981; Harding and Crabbe 1984; Wistow and Piatigorsky 1988). It is normally isolated as large heteropolymers, composed of two types of related polypeptides, αA and αB, each ~170 residues in length. The αA:αB ratio varies among species, from 1:3 in dogfish to 9:1 in kangaroo. α-Crystallin has a structural function in the lens, contributing to the extremely high protein concentration, up to 50%, in the lens fiber cells, which warrants proper refractive properties and transparency (Tardieu and Delaye 1988). Recently, αA- as well as αB-crystallin have been shown to occur in tissues outside the lens. The highest levels of αB-crystallin are reached in heart, striated muscle, and kidney (Bhat and Nagineni 1989), amounting to 2% of the soluble protein in rat soleus muscle (Kato et al. 1991*b*). αA-Crystallin is present in much smaller amounts, up to 17 ng/mg protein in rat spleen (Kato et al. 1991*a*). Both αA- and αB-crystallin are encoded by single-copy genes, located on chromosomes 21 and 11, respectively, in humans (Quax-Jeuken et al. 1985*b;* Ngo et al. 1989).

Small hsps

The small hsps form one of the four major groups of hsps or stress proteins that share the property that the expression of their genes can be induced by a mild heat shock or other forms of stress (Lindquist and Craig 1988; Morimoto et al. 1990, pp. 1–36). The three groups of larger (60–90 kDa) hsps are all known to be involved as "molecular chaperones" in various aspects of translocation and folding of polypeptides and in the assemblage of protein complexes (Ellis and van der Vies 1991; Gething and Sambrook 1992). In this manner the hsps are thought to contribute to the maintenance and restoration of cellular homeostasis during and after episodes of stress. The small hsps are functionally the least understood, although the evidence is growing that they too are engaged in protein-protein interactions. The small hsps form a diverse family of proteins of 15–30 kDa, all having the tendency to aggregate. In yeast and chicken a single gene for small hsp is present (Susek and Lindquist 1989; Miron et al. 1991), but most species have multiple genes—as in humans, where at least three hsp27 sequences are localized to chromosomes 3, 9, and X (McGuire et al. 1989). The small hsp genes are most numerous and most abundantly expressed in plants (Vierling 1991). The cytoplasmic small hsps in plants belong to at least two different multigene families (Raschke et al. 1988). In addition to the cytoplasmic small hsps, higher plants and algae also synthesize nucleus-encoded small hsps that localize to chloroplasts. In higher plants these chloroplast small hsps are synthesized as precursor proteins in the cytoplasm, which are processed by the removal of 5–6.5 kDa during import in chloroplasts (Vierling et al. 1988; Chen and Vierling 1991).

Distant Relatives

Proteins related to α-crystallin and the small hsps have been found as surface antigens in parasitic eukaryotes and bacteria. The blood parasite *Schistosoma mansoni* has a major egg antigen, p40, which shows a duplicated region homologous with

α-crystallin/small hsps (Nene et al. 1986; de Jong et al. 1988). In *Mycobacterium leprae* an 18-kDa immunodominant antigen belongs to the α-crystallin family (Nerland et al. 1988), and in *M. tuberculosis* a related, but quite distinct, 14-kDa protein is exposed outside the cell wall (Verbon et al. 1992).

Sequence Comparisons

All published sequences of the small hsps that are available from the data bases were obtained and aligned with a selection of αA- and αB-crystallins and with the related surface antigens of *Schistosoma* and *Mycobacterium.* The members of the α-crystallin/small-hsp family vary in length, from 143 residues for *Caenorhabditis elegans* hsp16 and hsp17 to 242 for *Petunia* chloroplast hsp21, while p40 of *S. mansoni,* with its duplicate homologous domain, has a length of 354 residues. Conspicuous similarity among all the members of the family is limited to the C-terminal parts of the polypeptides, and only this region could be aligned satisfactorily, as shown in figure 1. Certain residues and regions are conserved throughout the evolution of the family. Consensus sequences (e.g., see Linquist and Craig 1988; Grimm et al. 1989) become obviously more degenerate with the expanding membership of the family. The necessary introduction of gaps disrupts the consistency of the alignment, and their positioning is sometimes ambiguous.

The characteristic features of the sequence alignment can be related to the model for the tertiary structure of α-crystallin subunits, proposed on the basis of intron positions and intrachain sequence similarities (Wistow 1985). This model proposes a two-domain structure, each domain consisting of two similar structural motifs. A C-terminal arm of variable length is supposed to extend from the compact two-domain structure. The main region (the conserved region of the α-crystallin/small-hsp family, approximately from position 1 up to 100 in figure 1, corresponds to the putative C-terminal structural domain of the α-crystallin subunit and, by inference, of the small hsps as well. This region begins at the position corresponding to the first intron in the αA- and αB-crystallin genes and to the single intron in the small-hsp genes of *C. elegans.* The conserved residues D/E 7, G 39, D 54, P 75, and G 93 have been suggested to be of structural importance (Wistow 1985). The conservation of a short sequence motif K/R-X-I/V-P/Q/E/D-I/V (positions 116–120) in the proposed C-terminal extension of the polypeptides is separated from the main region by a region of variable length and sequence.

The N-terminal regions, preceding the α-crystallin-like domains, vary in length, from 31 residues in the *M. leprae* 18K antigen to 143 in *Petunia* hsp21. No sequence similarity in the N-terminal region throughout all members of the family can be detected: thus their absence. However, the N-terminal halves of the αA- and αB-crystallins are as similar in sequence as are their C-terminal halves, and also the mammalian and chicken small hsps display great similarities with the α-crystallins in this region (Hickey et al. 1986; Miron et al. 1991). Some similarity can still be detected between the N-terminal sequences of α-crystallin and *Drosophila* small hsps, and plant class I small hsps share with higher-vertebrate hsps27 a similar sequence P-F-S-L-(X_{2-5})-W-D/E-P-F-K/R-D-(X_{0-1})-Y/F-P near their N-termini (de Jong et al. 1988, and present extended data set). No traces of similarity are, however, detectable between the N-terminal sequences of the other small hsps. It has therefore been proposed that exon shuffling might have been involved in the evolution of these genes (van den Heuvel et al. 1985). Alternatively, all members of the family may have originated from the

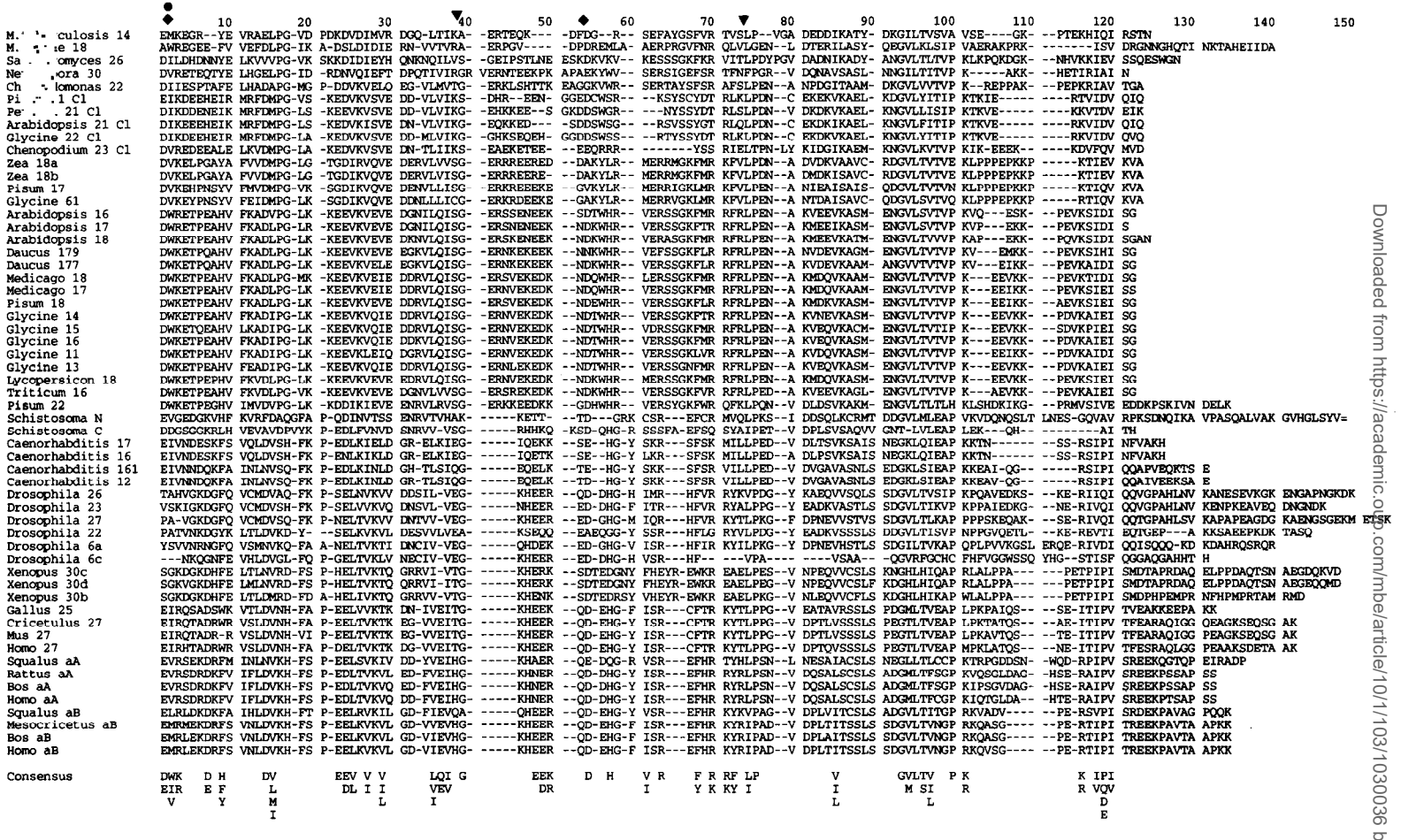

FIG. 1.—Alignment of the homologous C-terminal regions of small hsps, α-crystallins, the *Schistosoma mansoni* egg antigen p40, and the mycobacterial 14K and 18K surface antigens. An initial alignment was made with the multiple alignment program PileUp from the GCG package (Devereux et al. 1984) and was manually improved using the SALE editor (Leunissen et al. 1990*a*). All published small-hsp sequences that could be obtained from the NBRF, SwissProt, and EMBL data bases are included. Only eight representatives from the many known αA- and αB-crystallin sequences are shown. Sequences are designated by genus name and, for the small hsps, the number as used in the data bases. These numbers can be different from those used in the literature. *Schistosoma*-N and -C = N- and C-terminal copies, respectively, of the duplicated α-crystallin-like region in the egg protein p40. aA and aB = αA- and αB-crystallin sequences, respectively. The positions corresponding to the introns in the genes for *Caenorhabditis elegans* small hsps (●), *Homo sapiens* hsp27 (▼), and αA- and αB-crystallins (♦) are indicated. The *Neurospora crassa* hsp30 sequence has an insertion of 64 residues between positions 44 and 45 in the alignment. The region from about positions 2–50 represents the first structural motif of the proposed C-terminal globular domain, positions 51–100 the second motif, and from position 101 onward the extending C-terminal arm. The "consensus" residues emphasize at which positions the character of amino acid side chains has clearly been conserved throughout the disparate sequences in the family. Visual inspection rather than a clear-cut quantitative criterion was used to designate these consensus residues, because they are influenced by the biased sampling in the data set and also depend on the partially subjective positioning of gaps. The sources of sequences are *Mycobacterium tuberculosis* 14K (corresponding to positions 45–144 of the original sequence; Verbon et al. 1992), *Mycobacterium leprae* 18K (32–148; Booth et al. 1988), *Saccharomyces cerevisiae* hsp26 (95–213; Susek and Lindquist 1989), *N. crassa* hsp30 (59–228; Plesofsky-Vig and Brambl 1990), *Chlamydomonas reinhardtii* hsp22 (50–157; Grimm et al. 1989), *Pisum sativum* hsp21 (134–232; Vierling et al. 1988), *Petunia hybrida* hsp21 (144–242; Chen and Vierling 1991), *Arabidopsis thaliana* hsp21 (131–228; Chen and Vierling 1991), *Glycine max* hsp22 (81–181; Vierling et al. 1988), *Chenopodium rubrum* hsp23 (108–204; Knack and Kloppstech 1989), *Zea mays* hsp18a (58–164; Goping et al. 1991), *Z. mays* hsp18b (56–161; Goping et al. 1991), *Pisum sativum* hsp17 (46–152; Lauzon et al. 1990), *Glycine max* hsp61 (53–159; Raschke et al. 1988), *A. thaliana* hsp16 (52–156; Takahashi and Komeda 1989), *A. thaliana* hsp17 (53–156; Helm and Vierling 1989), *A. thaliana* hsp18 (55–161; Takahashi and Komeda 1989), *Daucus carota* hsp179 (55–159; Darwish et al. 1991), *Daucus carota* hsp177 (53–157; Darwish et al. 1991), *Medicago sativa* hsp18 (54–158; Gyoergyey et al. 1991), *Medicago sativa* hsp17 (39–143; Gyoergyey et al. 1991), *Pisum sativum* hsp18 (54–158; Lauzon et al. 1990), *Glycine max* hsp14 (50–154; Czarnecka et al. 1985), *Glycine max* hsp15 (50–154; Nagao et al. 1985), *Glycine max* hsp16 (57–161; Raschke et al. 1988), *Glycine max* hsp11 (49–153; Schoeffl et al. 1984), *Glycine max* hsp13 (49–153; Nagao et al. 1985), *Lycopersicon esculentum* hsp18 (50–154; Fray et al. 1990), *Triticum aestivum* hsp16 (47–151; McElwain and Spiker 1989), *Pisum sativum* hsp22 (78–197; Lauzon et al. 1990), *Schistosoma mansoni* p40-N (133–260; Nene et al. 1986), *Schistosoma mansoni* p40-C (261–354; Nene et al. 1986), *Caenorhabditis elegans* hsp17 (46–143; Russnak and Candido 1985), *Caenorhabditis elegans* hsp16 (46–143; Jones et al. 1986), *Caenorhabditis elegans* hsp16-1 (42–145; Russnak and Candido 1985), *Caenorhabditis elegans* hsp12 (42–145; Jones et al. 1986), *Drosophila melanogaster* hsp26 (81–208; Southgate et al. 1983), *Drosophila melanogaster* hsp23 (63–186; Southgate et al. 1983), *Drosophila melanogaster* hsp27 (82–213; Southgate et al. 1983), *Drosophila melanogaster* hsp22 (56–174; Southgate et al. 1983), *Drosophila melanogaster* hsp6a (119–238; Ayme and Tissières 1985), *Drosophila melanogaster* hsp6c (73–169; Pauli and Tonka 1987), *Xenopus laevis* hsp30c (86–213; Krone et al. 1992), *X. laevis* hsp30d (88–215; Krone et al. 1992), *X. laevis* hsp30b (80–212; Krone et al. 1992), *Gallus gallus* hsp25 (85–193; Miron et al. 1991), *Cricetulus longicaudatus* hsp27 (95–213; Lavoie et al. 1990), *Mus musculus* hsp27 (91–208; Gaestel et al. 1989), *H. sapiens* hsp27 (87–199; Hickey et al. 1986, corrected by Carper et al. 1990), *Squalus acanthius* αA (63–177; de Jong et al. 1988), *Rattus norvegicus* αA* (63–173; de Jong et al. 1988), *Bos taurus* αA (63–173; van der Ouderaa et al. 1973), *H. sapiens* αA (63–173; de Jong et al. 1975), *Squalus acanthias* αB (69–177; de Jong et al. 1988), *Mesocricetus auratus* αB* (67–175; Quax-Jeuken et al. 1985*a*), *B. taurus* αB (67–175; van der Ouderaa et al. 1974), and *H. sapiens* αB (67–175; Dubin et al. 1990). The higher-plant small hsps are grouped in the chloroplast-localized hsps (from *Pisum sativum* 21 to *Chenopodium rubrum* 23, indicated by "C1"), the class II hsps (from *Z. mays* 18a to *Glycine max* 61), and the class I hsps (from *A. thaliana* 16 to *Pisum sativum* 22).

same primordial gene, with considerably more changes having occurred in the N-terminal parts, making homology undetectable.

One group of plant cytoplasmic small hsps, designated as class I by Vierling (1991), has well-conserved N-terminal sequences, with *Pisum* hsp22 somewhat more divergent. The other group of plant cytoplasmic small hsps (class II) are less conserved in this region and are also completely distinct from the corresponding part in the class I proteins. The first 40–50 residues of plant chloroplast small hsps have the characteristics typical of chloroplast transit peptides, including a high content of serine and basic residues and no acidic amino acids (Chen and Vierling 1991). These transit sequences are very different among species. The N-terminal regions of the plant chloroplast small hsps also contain a consensus region of 28 residues, rich in methionine (Chen and Vierling 1991). The chloroplast small hsp of *Chlamydomonas* and the surface-exposed 14-kDa antigen of *M. tuberculosis* have no transit sequences and are apparently translocated without processing (Grimm et al. 1989; Verbon et al. 1992).

Not only for the N-termini, but even stronger for the C-terminal parts, it appears that similarity between the small hsps is generally much greater within organisms than between species. This can be explained by gene duplications after species divergence but can in part also be attributed to gene conversion events between closely linked hsp genes.

Tracing the Genealogy

The main region, including residues 1–101 in figure 1, was used to construct phylogenetic trees. A distance matrix for the 57 sequences was calculated, using an unpublished program, on the basis of minimum substitution distances (Fitch and Margoliash 1967); gaps were excluded in the pairwise comparisons. Phylogenetic trees were obtained with the programs NEIGHBOR (Saitou and Nei 1987) and FITCH (Fitch and Margoliash 1967; Prager and Wilson 1978). The program FITCH was used with exclusion of negative branch lengths and with global rearrangements. The program NEIGHBOR, of which the resulting tree is shown in figure 2, yielded no negative branches. The topology obtained with the program FITCH was essentially identical—apart from the divergence of the deepest branches, including the prokaryotes, *Saccharomyces, Neurospora, Chlamydomonas, Schistosoma,* and *Caenorhabditis*. Consistent features are the following: As noticed by Verbon et al. (1992), the α-crystallin-related antigens from the two species of *Mycobacterium* are extremely divergent. The proteins are in no case depicted as sister groups. It is unlikely that *M. leprae* and *M. tuberculosis* diverged before the advent of their higher-vertebrate hosts. If it is assumed that the respective 18-kDa and 14-kDa antigens are the products of orthologous genes, then the most likely explanation for the large sequence difference would be a highly increased rate of evolutionary change. This might indeed be useful for bacterial surface antigens intended to evade the host's immune response. The same explanation probably applies to the extreme divergence between the duplicated α-crystallin-like sequences in the egg antigen p40 of the blood parasite *Schistosoma mansoni*. In the FITCH tree based on the total data set, the N- and C-terminal domains of p40 are not even positioned as sister groups. Additional sequences of prokaryotic and lower-eukaryotic α-crystallin-related proteins are needed to resolve this difference.

The branching order of the prokaryotic proteins, the small hsps of the two fungi *Saccharomyces* and *Neurospora,* and the chloroplast-located hsp22 of *Chlamydomonas* cannot reliably be resolved on the basis of the present sequence data. In no case, however, did the *Chlamydomonas* hsp22 group with the chloroplast small hsps of the

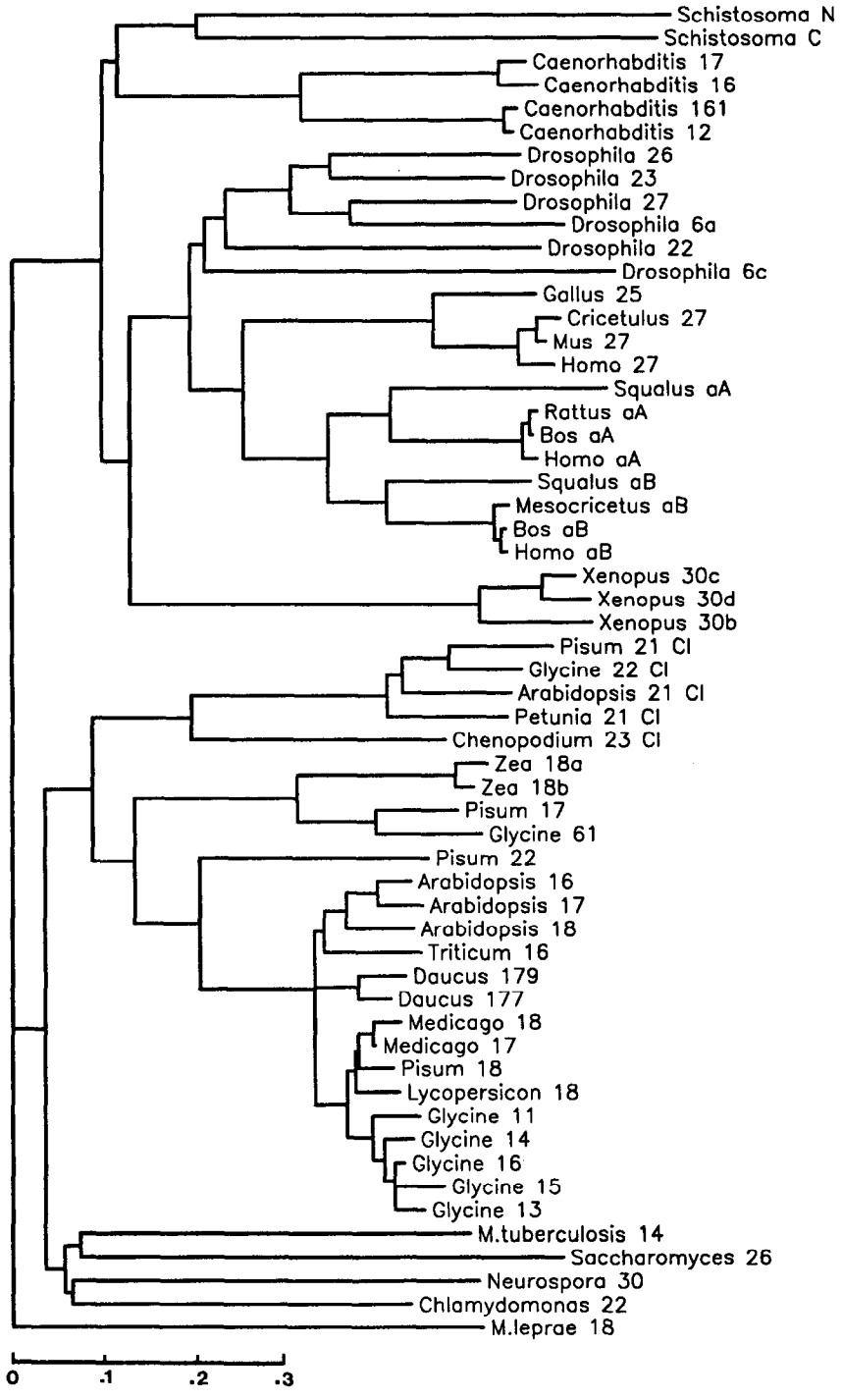

FIG. 2.—Phylogenetic tree of small hsps, α-crystallins, and antigens of *Mycobacterium* and *Schistosoma*, based on the homologous amino acid sequences presented as positions 1–101 in fig. 1. This tree was constructed using the neighbor-joining method (Saitou and Nei 1987). Branch lengths are proportional to evolutionary distance. The scale for branch lengths is in minimum number of nucleotide differences/amino acid residue.

higher plants. The latter consistently diverge, from the base of the lineage to the cytoplasmic plant small hsps. This would be in accordance with the endosymbiotic prokaryotic origin of chloroplasts, although the chloroplast small hsps are encoded by nuclear genes. The plant cytoplasmic small hsps divided at an early stage—before the separation of monocots and dicots—into two major groups, designated as "class I" and "class II" by Vierling (1991). Both classes can be present in the same species, as in *Pisum* and in *Glycine. Pisum* hsp22 is a divergent member of class II. Repeated further duplications have occurred in both classes, resulting in the two closely related *Zea* hsps in class II and in the multiple closely related small hsps of *Glycine, Arabidopsis, Daucus,* and *Medicago* in class I.

In light of the branch lengths in figure 2, and when the root is placed near the divergence of prokaryotes, fungi, and higher eukaryotes, it appears that the rates of change have been lower in the class I cytoplasmic hsps of plants than in most other branches. This suggests that the functional constraints in this group of hsps may be different from those in the others.

While the plant chloroplast and cytoplasmic small hsps consistently emerge as a monophyletic group from the prokaryotic and lower-eukaryotic cluster, the animal proteins display an even stronger monophyly. *Schistosoma* p40 and *Caenorhabditis* form sister groups on a very short common stem. The *Caenorhabditis* small-hsp genes duplicated at an early stage, followed by a more recent, further duplication, resulting in two pairs of very closely linked genes, of which one pair is present in two identical, inverted copies (Candido et al. 1989). It is remarkable that the *Drosophila* small hsps appear more closely related to those of chicken and mammals than do the *Xenopus* small hsps, as was also observed in an earlier analysis based on far fewer sequences (de Jong et al. 1988). An explanation might be that the *Xenopus* small hsps are paralogues of the *Drosophila* and higher-vertebrate small hsps. This is a reasonable assumption, in light of the multigene nature of the family, which probably already existed at the time of divergence of the animal phyla. The six very divergent *Drosophila* small hsps consistently appear to be monophyletic, but whether this reflects genuine duplications of one original gene in this lineage or, rather, is caused by conversion events among the closely linked genes at locus 67B (Ingolia and Craig 1982; Southgate et al. 1983) is difficult to decide. The branching order of the *Drosophila* small hsps was consistently found to be the same in the different tree constructions. The three *Xenopus* sequences are relatively similar and all very distant from the other animal small hsps. Also, four additional incomplete *Xenopus* hsp sequences appear to belong to this same monophyletic group (Krone et al. 1992). The chicken and mammalian small hsps clearly form the sister group of the monophyletic αA- and αB-crystallins. This is also evident from the considerable similarity shared by these proteins in their N-terminal domains (Hickey et al. 1986; de Jong et al. 1988). Because α-crystallins are present from lamprey to man, the divergence of the ancestral α-crystallin gene from the small hsp genes must have occurred before the earliest vertebrate radiation. That *Xenopus* small hsps branched off long before this time is further evidence that these are paralogously related with the chicken and mammalian small hsps. It will be interesting to see whether orthologues of the higher-vertebrate small hsps will still be discovered in *Xenopus* or whether these have disappeared altogether. Similarly, one wonders whether disparate paralogous small-hsp genes have been retained in higher vertebrates, as have the class I and class II genes in plants.

Common Features of the α-Crystallin/Small-hsp Family

To place the genealogy of the α-crystallin/small-hsp family in the proper context, it is important to compare the known functional and structural properties of these proteins. Unfortunately, a conspicuous feature still shared by α-crystallin and the small hsps is the lack of information about their three-dimensional structures and their actual biological functions. However, those pieces of information that are available about their structures and properties demonstrate many similarities (summarized in table 1).

Table 1
Comparison of α-Crystallins and Small hsps

	αA-Crystallin	αB-Crystallin	Small hsps
Gene structure	Single copy; two introns	Single copy; two introns	Usually multigene; usually intronless
Stress inducible	No	Yes	Yes
Hormone induction	...	Dexamethasone	Estrogen in mammals; ecdysteroids in *Drosophila*
Constitutive expression	Very high in eye lens; very low in spleen and thymus	Very high in eye lens; low in heart, muscle, kidney, and brain	Absent in certain species; developmentally regulated in others
Increased expression in disease	...	In certain tumors and degenerative diseases	In certain tumors
Subunit lengths (residues)	170–177	175–177	143–242
Secondary structure	Mainly β-sheet	Mainly β-sheet	Mainly β-sheet
Normal aggregate size	Average 800 kDa	Average 800 kDa	400–800 kDa
Effect of stress	...	Increased aggregate size; redistribution from cytoplasm to nuclear region	Increased aggregate size; redistribution from cytoplasm to nuclear region
Structural stability	High thermostability	High thermostability	Very stable in vivo
Association with RNA	...	...	In tomato; not in animals
Association with membranes	Yes	No?	Plant chloroplast hsps
Association with cytoskeleton	Yes	Yes; with intermediate filaments in ubiquitinated inclusion bodies	Chicken hsp25 inhibits actin polymerization
Molecular chaperone	Yes	Yes	Mammalian hsps
Serine phosphorylation	At single site Arg-x-x-Ser motif	At three sites	At two or three sites in Arg-x-x-Ser motifs
Glycosylation (O-GlcNac)	Yes	Yes	...
Substrate for transglutaminase	No	At C-terminal lysine	At C-terminal lysine
Providing thermotolerance	...	...	Mammalian and *Drosophila* hsps
Involvement in cytomorphological rearrangements	...	During development and degenerative processes	In embryogenesis and teratogenesis
Role in signal transduction	...	...	During platelet and endothelial cell activation
Protease inhibitory activity	Yes	Yes	Mammalian hsp27

NOTE.—References to the various features are given in the text. Ellipses indicate that information was not available.

Gene Structure, Expression, and Regulation

The small hsps, like the larger hsps, are generally encoded by intronless genes. Only in the six genes for the *Caenorhabditis* small hsps and in the gene for human hsp27 are there, respectively, one and two introns present, at different positions in the two species (as indicated in fig. 1). The αA- and αB-crystallin genes contain two introns at homologous positions, of which the first one coincides precisely with the intron in the *C. elegans* genes. The expression of the α-crystallins as abundant lens proteins imposes specific demands on the regulation of their genes (Piatigorsky and Zelenka 1992).

The 5′ flanking regions of all analyzed small-hsp genes and of the αB-crystallin gene contain one or more heat-shock elements. As a consequence, these genes—but not the αA gene—can be induced by elevated temperatures and various other types of stress (Morimoto et al. 1990, pp. 1–36; Klemenz et al. 1991*b*; DasGupta et al. 1992). There is no evidence for increased synthesis of the 14K antigen of *Mycobacterium tuberculosis* on heat shock (Verbon et al. 1992). The mammalian small hsps are transcriptionally induced by estrogen and may also interact with the estrogen receptor (Hayward et al. 1990; Mendelsohn et al. 1991). This resembles the induction of hsp27 in *Drosophila* by ecdysteroids (Morimoto et al. 1990, pp. 361–378). Dexamethasone induction of v-mos and Ha-ras oncogenes in NIH3T3 cells results in the accumulation of large amounts of αB-crystallin (Klemenz et al. 1991*a*, 1991*b*). Both hsp27 and αB-crystallin are expressed in nononcogenic—but not in oncogenic—adenovirus-transformed cells (Zantema et al. 1989).

In rat osteoblasts and human lymphocytes, the expression of hsp27 is highest at the peak of cellular proliferation and at the onset of growth arrest (Shakoori et al. 1992; Spector et al. 1992). However, hsp27-negative cell lines demonstrated that the expression of hsp27 is not essential for cell growth (Spector et al. 1992).

Developmental regulation of the constitutive expression of both αB-crystallin and the small hsps has been well documented. In rat tissues αB-crystallin increases until adult levels are reached several weeks after birth (Kato et al. 1991*b*). Small hsps of various lower organisms are induced during normal growth at particular stages in development, as in yeast (Rossi and Lindquist 1989) and *Drosophila* (Arrigo and Pauli 1988). In yeast, hsp26 is strongly induced during ascospore development (Kurtz et al. 1986). A common feature of these developmental inductions is that only a subset of the small hsps are produced. The small hsps of *C. elegans* and of soybean are never expressed constitutively (Nagao et al. 1985; Stringham et al. 1992). The inducibility of small hsps in *Xenopus* only appears at the tadpole stage and varies considerably in different adult tissues (Bienz 1984).

Cellular Functions and Role in Disease

The overexpression of small hsps in mammalian cells leads to the acquisition of thermotolerance, the transient ability to survive otherwise lethal heat exposure after a mild heat shock (Landry et al. 1989; Lee et al. 1992). Thermal resistance is also acquired when *Drosophila* hsp27 is expressed in rodent cells (Rollet et al. 1992). The level of hsp27 in mammalian cells correlates positively not only with thermotolerance but also with survival after treatment with various anticancer drugs (Huot et al. 1991). Elevated levels of hsp27, especially the phosphorylated isoforms, are associated with increased resistance of human breast cancer cells to doxorubicin (Ciocca et al. 1992). Susek and Lindquist (1989) failed to uncover a function for the unique small hsp in

yeast. Deletion of the gene had no detectable effect on growth at high temperature, acquisition of thermotolerance, spore development, or germination. However, Bentley et al. (1992) observed a slightly elevated thermotolerance of yeast cells that overexpressed the small hsp.

hsp27 of *Drosophila* is abundant during embryogenesis and reaches maximal levels in late pupae, suggesting that it has a role in cellular rearrangements (Arrigo and Pauli 1988). Similarly, αB-crystallin, in conjunction with protein ubiquitination, appears to have a role in cells undergoing major cytomorphological reorganizations in early chicken embryogenesis (Scotting et al. 1991). Moreover, small hsps are involved in retinoic acid–induced teratogenesis in mouse embryos (Anson et al. 1991). Also, the presence of αB-crystallin in intracellular inclusions in degenerative disorders of brain and liver is associated with extensive cytoskeletal and organellar rearrangements (Mayer et al. 1991). Thus members of this protein family are involved in mechanisms that change the internal architecture of cells during normal development or during disease progression.

Increased expression of αB-crystallin appears to be part of the repertoire of reactive processes of astrocytes and oligodendrocytes in the central nervous system (Iwaki et al. 1992). Increased expression of αB is also observed in the benign tumors associated with tuberous sclerosis and results from disordered cell migration and abnormal cell differentiation (Iwaki and Tateishi 1991). Among neuroectodermal neoplasms, αB-crystallin is mainly expressed by astrocytic tumors (Iwaki et al. 1991). αB-crystallin is a major component of ubiquitinated inclusion bodies in human degenerative diseases (Lowe et al. 1992). It occurs in cortical Lewy bodies, in astrocytic Rosenthal fibers, and in hepatic Mallory bodies. In these inclusions αB-crystallin is tightly associated with intermediate filaments and ubiquitin (Tomokane et al. 1991). To a variable extent, αB-crystallin is also present, together with ubiquitin and glial fibrillar acidic protein, in eosinophilic granular bodies in astrocytomas (Murayama et al. 1992).

hsp27 is overexpressed in human breast carcinomas and correlates well with the level of steroid hormone receptors and with a shorter disease-free survival period (Thor et al. 1991). In contrast, the expression of hsp27 in malignant fibrous histiocytoma is associated with a more favorable prognosis (Têtu et al. 1992). Zantema et al. (1989) demonstrated an inverse relation between levels of hsp27 and tumor induction in syngeneic immunocompetent rats. hsp27 is also expressed in certain primary brain tumors (Kato et al. 1992*a*). Other functional aspects appear to be related to the reversible phosphorylation of the proteins and will be mentioned below.

Structural Properties and Stability

The common functional features of α-crystallins and small hsps must obviously relate to the structural characteristics shared by these proteins. Spectroscopic evaluation reveals primarily β-sheet conformation, with <5% α-helix structure, for both α-crystallin (see Walsh et al. 1991) and for mouse hsp27 (J. Horwitz, K. Merck, and W. W. de Jong, unpublished data). Secondary-structure predictions of some small hsps suggest the presence of amphiphilic α-helices with high hydrophobic moment, mostly at the N-termini and sometimes at the C-terminus (Plesofsky-Vig and Brambl 1990). These helices may serve for insertion into membranes or to promote specific interactions among proteins. Also, the methionine-rich consensus region in chloroplast small hsps is predicted to form an amphipathic α-helix and may serve for substrate recognition (Chen and Vierling 1991). Similarities of the hydropathy plots of the homologous region of α-crystallin and the small hsps have often been noticed (see de Jong et al.

1988; Lindquist and Craig 1988). Especially well conserved is a region of pronounced hydrophilicity around positions 41–55 in the alignment. This region corresponds to the connecting peptide between the putative motifs of the carboxy-terminal domain.

There is no direct information about the tertiary structures of α-crystallin or the small hsps, but indirect evidence supports the above-mentioned two-domain model of Wistow (1985) (van den Oetelaar and Hoenders 1989; Merck et al. 1992). Wistow (1985) also suggested that the C-terminal structural domain might be shared by α-crystallins and small hsps, because of its thermodynamic stability. α-Crystallins are indeed exceptionally thermostable (Walsh et al. 1991), with extremely long half-lives in the lens, and the small hsps also are reported to be very stable in vivo (Landry et al. 1991).

Aggregate Formation

Both α-crystallins and small hsps form large aggregates. For α-crystallin an average molecular mass of 800 kDa is often observed, but complexes ranging in size from 280 to >10,000 kDa have also been isolated (Spector et al. 1971; Clauwaert et al. 1989). Aggregate size of α-crystallin depends on protein concentration and can reach values of >50,000 kDa at protein concentrations >15% (Koenig et al. 1992). The presence of monomeric γ-crystallins decreases the size of α-crystallin aggregates (Mach et al. 1990). A mass of 180 kDa has been reported for chicken small hsp (Collier et al. 1988), but values of 400–800 kDa are more usually observed for animal small hsps (Arrigo et al. 1988). Mixed aggregates of αB-crystallin and small hsp naturally occur in adenovirus-transformed cells (Zantema et al. 1992) and in human pectoral muscle (Kato et al. 1992*b*). The complex of hsp-αB-crystallin falls apart on stress, accompanied by a change in conformation of αB-crystallin (Zantema et al. 1992). In the electron microscope, both α-crystallin and small hsps appear, under normal conditions, as 10–18-nm globular, sometimes torus-like or hollow-core, particles (Arrigo and Pauli 1988; Nover et al. 1989; Longoni et al. 1990; Behlke et al. 1991).

The quaternary structure of α-crystallin is influenced by factors such as pH, ionic strength, temperature, and calcium ion concentration (Siezen et al. 1980). This structure is a matter of much controversy. Experimental data support quite distinct models. Three-layered spherical models have been proposed by Bindels et al. (1979) and Tardieu et al. (1986), whereas Augusteyn and Koretz (1987) suggested a micellar structure. Some biophysical properties of α-crystallin can be better explained by the micelle hypothesis (Radlick and Koretz 1992). Walsh et al. (1991) recently proposed a structure that reconciles both models, to a certain extent. For mouse hsp27 a spherelike structure composed of about 32 monomers, arranged in hexagonal packing, has been proposed (Behlke et al. 1991). Because α-crystallin and small hsps share the ability to form large aggregates, it is usually suggested that this property is caused by the common C-terminal region (Arrigo et al. 1988; Hockertz et al. 1991). However, such a concept is difficult to reconcile with the micelle model, where the N-terminal region is thought to be responsible for interaction. Also, recent reaggregation experiments with the recombinant second domain of αA do not support a role for this region in the aggregation behavior of α-crystallin (Merck et al. 1992).

The size of the α-crystallin aggregates in the lens increases on aging (Harding 1991). It appears that αB-crystallin in the heart forms larger aggregates on stress, i.e., during ischemia (Chiesi et al. 1990). Heat shock increases the aggregate size of the small hsps in mammalian cells (Arrigo et al. 1988), in chicken embryo fibroblasts (Collier et al. 1988), and in tomato cell cultures (Nover et al. 1989), which assume

sizes ≤2 MDa and form stress granules. After heat shock and other forms of stress the small hsps redistribute from the cytoplasm, toward the perinuclear region or into the nucleus (Collier and Schlesinger 1986; Arrigo et al. 1988). Similarly, αB-crystallin is in the cytoplasm of mammalian cells under normal conditions and, at higher temperature, becomes associated with the nucleus (Klemenz et al. 1991*b;* Inaguma et al. 1992; Voorter et al. 1992). In all cases, redistribution back to the cytoplasm occurs during recovery. In yeast the intracellular location of hsp26 depends not simply on the effect of heat stress but also on the physiological state of the cell (Rossi and Lindquist 1989).

In heat-shocked tomato cell cultures, the heat-shock granules are tightly associated with a specific subset of mRNAs (Nover et al. 1989). It is proposed that this association might explain the striking conservation of untranslated mRNAs during heat shock. In mammalian systems, however, previously suggested associations between small hsps and mRNA have probably been due to copurification of hsp complexes and prosomes. Also, the chicken stress granules do not contain significant amounts of RNA (Collier et al. 1988).

Interactions with Membranes and Proteins

Native α-crystallin has been shown to associate specifically with lens membranes, which apparently requires the presence of the major membrane protein MIP26 (Mulders et al. 1985; Liang and Li 1992). This high-affinity binding of α-crystallin is lost on aging of the aggregates (Ifeanyi and Takemoto 1989; Mulders et al. 1989). A strong noncovalent association also exists between α-crystallin and the lens-fiber cytoskeleton, notably the intermediate filaments (Fitzgerald and Graham 1991). Cardiac αB-crystallin displays specific interaction with actin (Chiesi et al. 1990), and in rat myocytes αB has the tendency to associate with desmin (Longoni et al. 1990).

Plant chloroplast small hsps are largely unbound in the stroma, under normal conditions, but become associated with the grana region of the thylakoid membranes ≤15 min after heat shock (Grimm et al. 1989; Adamska and Kloppstech 1991). Heat-induced alteration of these membranes is required for the binding. A short hydrophobic region close to the C-terminus of *Chlamydomonas* hsp22 might be involved in its binding as a thylakoid extrinsic protein (Schuster et al. 1988; Grimm et al. 1989). hsp25 from turkey and chicken smooth muscle has been shown to inhibit the polymerization of actin (Miron et al. 1991). This inhibitory activity is lost on aggregation of hsp25.

Stabilizing and protective interactions with other proteins may be crucial in the functioning of α-crystallins and small hsps. Strong evidence for chaperon-like properties of α-crystallin and mouse hsp27 has recently been obtained. Both proteins protect against heat-induced protein aggregation and enable the proper refolding of chemically denatured proteins (Horwitz et al., accepted; Merck et al., accepted; W. W. de Jong, unpublished data; U. Jakob, M. Gaestel, and J. Buchner, personal communication).

Phosphorylation

A common feature of α-crystallin and small hsps is their phosphorylation on specific serine residues. In αA-crystallin a single serine, at position 122 (76 in fig. 1), and in αB-crystallin, two or three serines at positions 19, 45, and 59 in the N-terminal domain are phosphorylated (Voorter et al. 1986, 1989; Chiesa et al. 1987). It appears that these phosphorylations are catalyzed by a cAMP-dependent protein kinase. Ser-122 in αA is indeed in a consensus sequence for such a kinase, but the substrate serines

in αB are not preceded by known recognition signals. In mature lens-fiber cells these phosphorylations are essentially irreversible, but in the lens epithelium the phosphate group may be removed by a calcium/calmodulin-dependent phosphatase (Chiesa and Spector 1989). A maximum of 30% of the αA and αB chains becomes phosphorylated in the bovine lens. In other species phosphorylation occurs to a different extent and often is even absent, e.g., in chicken. Also, in other tissues αB-crystallin can be partially phosphorylated, as in mouse heart (C. E. M. Voorter, unpublished data) and in Alexander disease brain (Mann et al. 1991). The reversible phosphorylation of α-crystallin suggests that it may be subject to metabolic control, but the significance of this modification is completely unknown.

Both human and mouse hsp27 have a major phosphorylation site at Ser-82 (human sequence), just N-terminal of the α-crystallin-like domain (Gaestel et al. 1991; Landry et al. 1992). A second site is present at Ser-15 in mouse hsp27 and probably in human hsp27 too. Human has a third major phosphorylation site at Ser-78, which is replaced by asparagine in rodents. These serines are all located, as in αA-crystallin, in the sequence Arg-X-X-Ser, which is the recognition motif for multifunctional calmodulin-dependent protein kinase II and ribosomal protein S6 kinase II. hsp27 kinase activity is not effected by inhibitors of kinase A, kinase C, or caseine kinase II (for overview, see Landry et al. 1992).

Increased phosphorylation of the mammalian small hsps occurs in response to a number of mitogenic stimuli, most notably serum, growth factors, and tumor promoters (Arrigo and Welch 1987; Saklatvala et al. 1991). Also, calcium ionophores and chelators, cadmium, arsenite, cycloheximide, and phorbol esters induce phosphorylation at normal growing temperature (Regazzi et al. 1988; Landry et al. 1991). The rapid phosphorylation of the small hsps by these different agents is probably achieved by modulating the activities of protein kinases or phosphoprotein phosphatases.

Activation of platelets by thrombin leads to rapid phosphorylation of hsp27, suggesting a role for hsp27 in signal transduction during platelet activation (Mendelsohn et al. 1991). Likewise, thrombin, histamine, and other activators of endothelial cell function enhance the phosphorylation of hsp27 in these cells (Santell et al. 1992). In Sertoli cells from rat testes, phosphorylation of hsp27 is increased on treatment with germ cells, suggesting a role in paracrine signaling (Pittenger 1992). It has indeed been proposed that the thermoprotective function of hsp27 might be an extension of a normal function of this protein in maintaining signal transduction (Landry et al. 1992). hsp27 is a major phosphoprotein in smooth muscle and appears to be involved in sustained contraction induced by protein kinase C or by the neuropeptide bombesin (Bitar et al. 1991). It would play a role in the orientation or activation of the contractile machinery necessary to maintain a sustained contraction. Phosphorylation of hsp27 has been suggested to be a possible signal for development of thermotolerance (Landry et al. 1991). In HeLa cells there is, within minutes after heat shock, a shift from the unphosphorylated to the phosphorylated isoforms of hsp27, concomitant with the rapid translocation of the small hsps from cytosol to nucleus (Guesdon and Saklatvala 1991; Landry et al. 1992). The heat-induced phosphorylation of hsp27 is reduced in thermotolerant cells, as is the redistribution from cytoplasm to nucleus (Landry et al. 1991). That phosphorylation occurs at unrelated sites in αA, αB, and small hsps and that different kinases may be involved suggests that the functional significance of this modification may also be different.

Other Modifications and Properties

Both αA- and αB-crystallin are modified in vivo with O-linked N-acetyl-glucosamine (Roquemore et al. 1992). A single O-GlcNac substitution is present on αA, most of the carbohydrate being attached to the C-terminal extension at the serine at position 121 in figure 1. It has been postulated that O-GlcNac is a regulatory modification analogous to phosphorylation or, alternatively, that it may be involved in the organization of multiprotein complexes.

Both αB-crystallin and mouse hsp27, but not αA-crystallin, are posttranslationally modified by transglutaminase. The C-terminal lysine residues of αB and hsp27 are amine donors for transglutaminase and can be cross-linked to exposed glutamines in other proteins (Groenen et al. 1992; Merck et al., accepted). Intracellular calcium, which activates transglutaminase, is increased under certain pathological and stressful conditions and thus may favor covalent cross-linking of αB and small hsps to other cellular proteins.

A remarkable property shared by α-crystallin and the small hsps is their protease-inhibitory activity. One mole of α-crystallin (800 kDa) is able to bind 13–19 mol of elastase (Orthwerth and Olesen 1992). A similar elastase-inhibitor activity is present in murine hsp27 (Merck et al., accepted). Whether α-crystallin and small hsps function in vivo as protease inhibitors remains to be investigated. Such a function would, however, be useful to prevent endogenous proteases from too readily attacking partially or transiently denatured proteins during stress.

Evolutionary Scenario

What is the common denominator in a protein family whose members have functions as diverse as being major surface antigens in bacteria, protecting against light-induced damage of the photosynthetic machinery in plants (Schuster et al. 1988; Grimm et al. 1989), inhibiting the polymerization of actin in chicken gizzard (Miron et al. 1991), and providing stability and transparency for the eye lens?

The Common α-Crystallin Domain

Structurally, all members of the family are characterized by the presence of a common protein domain of ~90 residues (positions 1–100 in fig. 1), followed by an extension, of variable length, containing a small conserved consensus sequence around position 120. The common domain is preceded by an N-terminal region varying in length, from 31 residues in *Mycobacterium leprae* to 142 residues in *Petunia* hsp21. The α-crystallin-like domain is duplicated in *Schistosoma* p40. These duplicated domains are not directly in tandem but have a sequence of ~45 residues between them (*Schistosoma*-N positions 101–148 in fig. 1), possibly corresponding to the C-terminal extension of the protein before duplication occurred. In *Neurospora* hsp30 the domain is interrupted by an insertion of 64 residues, at position 44 in figure 1, a region where alignment is difficult, and in close proximity to the second intron of the α-crystallins. All this evidence would confirm Wistow's (1985) proposal that this region represents a structural domain, N-terminally demarcated by the intron positions in α-crystallin and *Caenorhabditis* hsp genes and C-terminally by a variable extension starting around position 100. This domain would in turn be composed of two similar structural motifs connected by a variable and hydrophilic loop around positions 40–60.

If it is assumed that this domain and its C-terminal arm present a primordial, nuclear structure of the α-crystallin/small-hsp family, then the variable N-terminal

regions of the members of the family can be explained in two ways. A tandem duplication of the primordial domain would account for the structural similarities between the N- and C-terminal domains of the α-crystallins (Wistow 1985) and probably of the higher-vertebrate small hsps as well. The duplication of the primordial α-crystallin-like domain may have occurred at the very beginning of the evolution of the family, the traces of this event being obliterated in most members of the family by subsequent mutations. Alternatively, exon shuffling may in some cases have been involved in bringing about the great variation in N-terminal regions.

Evolving Functions

It seems logical to assume that the conserved C-terminal domain and extension are responsible for the common structural and functional properties of the α-crystallin/small-hsp family. The N-terminal domain may either be functionally less important or be involved in specific functions for a particular group of proteins, such as the methionine-rich region in chloroplast small hsps. Little is known about the structural and functional properties of the surface antigens in *Mycobacterium* and *Schistosoma*. Moreover, their likely major function—i.e., to elude the host's immune system—is an evolutionarily recent acquisition and tells us little about the properties of the ancestral α-crystallin-like hsps. Structural stability, however, seems to be a useful attribute for surface antigens, and this may indeed be one of the crucial properties of the family. Similarity between epitopes in the conserved structurally stable domains of parasitic surface antigens and the host's small hsps would be an obvious advantage in evading immune reactions. This same interrelation between common epitopes and the host's immune response applies even more strongly for the 65-kDa hsps of bacteria and vertebrates, and its possible involvement in autoimmune disease, notably rheumatoid arthritis, continues to be a subject of much debate (Res et al. 1991). There are currently no indications of a relation between α-crystallin-related surface antigens of parasites and autoimmune reactions.

To trace the primordial function of the α-crystallin-like hsps it would be important to recover related proteins from free-living bacteria. However, it is more than likely that this function, as for the larger hsps, will be to chaperone other proteins during normal development and especially under conditions of stress. This is obvious from (*a*) the information given above about the involvement of α-crystallin/small hsps in cytomorphological rearrangements, (*b*) interactions with cytoskeletal elements and membranes, and, most recently, (*c*) the direct demonstration that α-crystallin and mouse hsp27 stabilize protein-protein interactions and assist in the refolding of denatured proteins. The recruitment of α-crystallin as a major lens protein now becomes understandable. Not only does its intrinsic structural stability makes it suitable for residing life long, without turnover, in the lens; but, by preventing undesirable protein interactions and restoring unfolded proteins, α-crystallin would contribute to the maintenance of lens transparency and integrity. In fact, the constitutively high level of α-crystallin in the lens cells would make them permanently stress tolerant.

Logically, the functioning of α-crystallin as a stress protein in various tissues must have preceded its recruitment as an abundant lens protein. Therefore, the divergence of the α-crystallin ancestor from the small hsps—and probably also the subsequent duplication into the original αA- and αB-crystallin genes—must have preceded the origin of the vertebrate eye. This is indeed obvious from the ubiquitous presence of αA- and αB-crystallins in vertebrate lenses. The required high expression of αA and αB in the lens may have been facilitated by the easy inducibility that is characteristic

of the hsps. The presence of the α-crystallins in the lens thus resembles the recruitment of a variety of metabolic enzymes to function as abundant structural lens proteins (Piatigorsky and Wistow 1991). Several of these "enzyme-crystallins" appear to be structurally stable and can be induced under conditions of stress (de Jong et al. 1989; C. E. M. Voorter, unpublished data). Whether the dual function of α-crystallins—as (1) stress proteins in and outside the lens and (2) structural lens proteins—imposes additional evolutionary constraints is yet uncertain. In this respect, however, both αA- and αB-crystallin have avoided changes in charge during evolution (Leunissen et al. 1990*b*). This might be useful in the lens environment, where changes in surface charges could easily disturb the proper tight packing of the crystallins.

LITERATURE CITED

ADAMSKA, I., and K. KLOPPSTECH. 1991. Evidence for the localization of the nuclear-coded 22-kDa heat-shock protein in a subfraction of thylakoid membranes. Eur. J. Biochem. **198**: 375–381.

ANSON, J. F., J. B. LABORDE, J. L. PIPKIN, W. G. HINSON, D. K. HANSER, D. M. SHEEHAN, and J. F. YOUNG. 1991. Target tissue specificity of retinoic acid-induced stress proteins and malformations in mice. Teratology **44**:19–28.

ARRIGO, A.-P., and D. PAULI. 1988. Characterization of HSP27 and three immunologically related polypeptides during *Drosophila* development. Exp. Cell Res. **175**:169–183.

ARRIGO, A.-P., J. P. SUHAN, and W. J. WELCH. 1988. Dynamic changes in the structure and intracellular locale of the mammalian low-molecular-weight heat shock protein. Mol. Cell. Biol. **8**:5059–5071.

ARRIGO, A.-P., and W. J. WELCH. 1987. Characterization and purification of the small 28,000-Dalton mammalian heat shock protein. J. Biol. Chem. **262**:15359–15369.

AUGUSTEYN, R. C., and J. F. KORETZ. 1987. A possible structure for α-crystallin. FEBS Lett. **222**:1–5.

AYME, A., and A. TISSIÈRES. 1985. Locus 67B of *Drosophila melanogaster* contains seven, not four, closely related heat shock genes. EMBO J. **4**:2949–2954.

BEHLKE, J., G. LUTSCH, M. GAESTEL, and H. BIELKA. 1991. Supramolecular structure of the recombinant murine small heat shock protein hsp25. FEBS Lett. **288**:119–122.

BENTLEY, N. J., I. T. FITCH, and M. F. TUITE. 1992. The small heat-shock protein hsp26 of *Saccharomyces cerevisiae* assembles into a high molecular weight aggregate. Yeast **8**:95–106.

BHAT, S. P., and C. N. NAGINENI. 1989. αB subunit of lens-specific protein α-crystallin is present in other ocular and non-ocular tissues. Biochem. Biophys. Res. Commun. **158**:319–325.

BIENZ, M. 1984. Developmental control of the heat shock response in *Xenopus*. Proc. Natl. Acad. Sci. USA **81**:3138–3142.

BINDELS, J. G., R. J. SIEZEN, and H. J. HOENDERS. 1979. A model for the architecture of α-crystallin. Ophthalmic Res. **11**:441–452.

BITAR, K. N., M. S. KAMINSKI, N. HAILAT, K. B. CEASE, and J. R. STRAHLER. 1991. HSP27 is a mediator of sustained smooth muscle contraction in response to bombesin. Biochem. Biophys. Res. Commun. **181**:1192–1200.

BLOEMENDAL, H., ed. 1981. Molecular and cellular biology of the eye lens. John Wiley & Sons, New York.

BOOTH, R. J., D. P. HARRIS, J. M. LOVE, and J. D. WATSON. 1988. Antigenic proteins of *Mycobacterium leprae:* complete sequence of the gene for the 18-kDa protein. J. Immunol. **140**:597–601.

CANDIDO, E. P. M., D. JONES, D. K. DIXON, R. W. GRAHAM, R. H. RUSSNAK, and R. J. KAY. 1989. Structure, organization, and expression of the 16-kDa heat shock gene family of *Caenorhabditis elegans*. Genome **31**:690–697.

CARPER, S. W., T. A. ROCHELEAU, and F. K. STORM. 1990. DNA sequence of a human heat shock protein HSP27. Nucleic Acids Res. **18**:6457.

CHEN, Q., and E. VIERLING. 1991. Analysis of conserved domains identifies a unique structural feature of a chloroplast heat shock protein. Mol. Gen. Genet. **226**:425–431.

CHIESA, R., M. A. GAWINOWICZ-KOLKS, N. J. KLEIMAN, and A. SPECTOR. 1987. The phosphorylation sites of the B2 chain of bovine α-crystallin. Biochem. Biophys. Res. Commun. **144**:1340–1347.

CHIESA, R., and A. SPECTOR. 1989. The dephosphorylation of lens α-crystallin A chain. Biochem. Biophys. Res. Commun. **162**:1494–1501.

CHIESI, M., S. LONGONI, and U. LIMBRUNO. 1990. Cardiac alpha-crystallin. III. Involvement during heart ischemia. Mol. Cell. Biochem. **97**:129–136.

CIOCCA, D. R., S. A. W. FUQUA, S. LOCKLIM, D. O. TOFT, W. J. WELCH, and W. L. MCGUIRE. 1992. Response of human breast cancer cells to heat shock and chemotherapeutic drugs. Cancer Res. **52**:3648–3654.

CLAUWAERT, J., H. D. ELLERTON, J. F. KORETZ, K. THOMSON, and R. C. AUGUSTEYN. 1989. The effect of temperature on the renaturation of α-crystallin. Curr. Eye Res. **8**:397–403.

COLLIER, N. C., J. HEUSER, M. A. LEVY, and M. J. SCHLESINGER. 1988. Ultrastructural and biochemical analysis of the stress granule in chicken embryo fibroblasts. J. Cell Biol. **106**: 1131–1139.

COLLIER, N. C., and M. J. SCHLESINGER. 1986. The dynamic state of heat shock proteins in chicken embryo fibroblasts. J. Cell Biol. **103**:1495–1507.

CZARNECKA, E., W. B. GURLEY, R. T. NAGAO, L. A. MOSQUERA, and J. L. KEY. 1985. DNA sequence and transcription mapping of a soybean gene encoding a small heat shock protein. Proc. Natl. Acad. Sci. USA **82**:3726–3730.

DARWISH, K., L. WANG, C. H. HWANG, N. APUYA, and J. L. ZIMMERMAN. 1991. Cloning and characterization of genes encoding low molecular weight heat shock proteins from carrot. Plant Mol. Biol. **16**:729–731.

DASGUPTA, S., T. C. HOHMAN, and D. CARPER. 1992. Hypertonic stress induces αB-crystallin expression. Exp. Eye Res. **54**:461–470.

DE JONG, W. W., W. HENDRIKS, J. W. M. MULDERS, and H. BLOEMENDAL. 1989. Evolution of eye lens crystallins: the stress connection. Trends Biochem. Sci. **14**:365–368.

DE JONG, W. W., J. A. M. LEUNISSEN, P. J. M. LEENEN, A. ZWEERS, and M. VERSTEEG. 1988. Dogfish α-crystallin sequences: comparison with small heat shock proteins and *Schistosoma* egg antigen. J. Biol. Chem. **263**:5141–5149.

DE JONG, W. W., E. C. TERWINDT, and H. BLOEMENDAL. 1975. The amino acid sequence of the A chain of human α-crystallin. FEBS Lett. **58**:310–313.

DEVEREUX, D., W. A. HAEBERLI, and O. SMITHIES. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **12**:387–395.

DUBIN, R. A., A. H. ALLY, S. CHUNG, and J. PIATIGORSKY. 1990. Human αB-crystallin gene and preferential promoter function in lens. Genomics **7**:594–601.

ELLIS, R. J., and S. M. VAN DER VIES. 1991. Molecular chaperones. Annu. Rev. Biochem. **60**: 321–347.

FITCH, W. M., and E. MARGOLIASH. 1967. Construction of phylogenetic trees. Science **155**: 279–284.

FITZGERALD, P. G., and D. GRAHAM. 1991. Ultrastructural localization of alpha-crystallin to the bovine lens fiber cell cytoskeleton. Curr. Eye Res. **10**:417–436.

FRAY, R. G., G. W. LYCETT, and D. GRIERSON. 1990. Nucleotide sequence of a heat-shock and ripening-related cDNA from tomato. Nucleic Acids Res. **18**:7148.

GAESTEL, M., B. GROSS, R. BENNDORF, M. STRAUSS, W.-H. SCHUNK, R. KRAFT, A. OTTO, H. BOEHM, J. STAHL, H. DRABSCH, and H. BIELKA. 1989. Molecular cloning, sequencing and expression in *Escherichia coli* of the 25-kDa growth-related protein of Ehrlich ascites tumor and its homology to mammalian stress proteins. Eur. J. Biochem. **179**:209–213.

GAESTEL, M., W. SCHRODER, R. BENNDORF, C. LIPPMANN, K. BUCHNER, F. HUCHO, V. A.

ERDMANN, and H. BIELKA. 1991. Identification of the phosphorylation sites of the murine small heat shock protein hsp25. J. Biol. Chem. **266**:14721–14724.

GETHING, M.-J., and J. SAMBROOK. 1992. Protein folding in the cell. Nature **355**:33–45.

GOPING, I. S., J. R. H. FRAPPIER, D. B. WALDEN, and B. G. ATKINSON. 1991. Sequence, identification and characterization of cDNAs encoding two different members of the 18 kDa heat shock family of *Zea mays L.* Plant Mol. Biol. **16**:699–711.

GRIMM, B., D. ISH-SHALOM, D. EVEN, H. GLACZINSKI, P. OTTERSBACH, K. KLOPPSTECH, and I. OHAD. 1989. The nuclear-coded chloroplast 22-kDa heat-shock protein of *Chlamydomonas.* Eur. J. Biochem. **182**:539–546.

GROENEN, P. J. T. A., H. BLOEMENDAL, and W. W. DE JONG. 1992. The carboxy-terminal lysine of αB-crystallin is an amine-donor substrate for tissue transglutaminase. Eur. J. Biochem. **205**:671–674.

GUESDON, F., and J. SAKLATVALA. 1991. Identification of a cytoplasmic protein kinase regulated by IL-1 that phosphorylates the small heat shock protein, HSP27. J. Immunol. **147**:3402–3407.

GYOERGYEY, J., A. GARTNER, K. NEMETH, Z. MAGYAR, H. HIRT, E. HEBERLE-BORS, and D. DUDITS. 1991. Alfalfa heat shock genes are differentially expressed during somatic embryogenesis. Plant Mol. Biol. **16**:999–1007.

HARDING, J. 1991. Cataract: biochemistry, epidemiology and pharmacology. Chapman & Hall, London and New York.

HARDING, J. J., and M. J. C. CRABBE. 1984. The lens: development, proteins, metabolism and cataract. Pp. 207–492 *in* H. DAVSON, ed. The eye. Academic Press, New York.

HAYWARD, J. R., A. I. COFFER, and R. J. B. KING. 1990. Immunoaffinity purification and characterisation of p29—an estrogen receptor related protein. J. Steroid Biochem. Mol. Biol. **37**:513–519.

HELM, K. W., and E. VIERLING. 1989. An *Arabidopsis* cDNA clone encoding a low molecular weight heat shock protein. Nucleic Acids Res. **17**:7995.

HICKEY, E., S. E. BRANDON, R. POTTER, G. STEIN, J. STEIN, and L. A. WEBER. 1986. Sequence and organization of genes encoding the human 27 kDa heat shock protein. Nucleic Acids Res. **14**:4127–4145.

HOCKERTZ, M. K., I. CLARK-LEWIS, and E. P. M. CANDIDO. 1991. Studies of the small heat shock proteins of *Caenorhabditis elegans* using anti-peptide antibodies. FEBS Lett. **280**:375–378.

HORWITZ, J., Q.-L. HUANG, and L.-L. DING. Alpha-crystallin can function as a molecular chaperone. Proc. Natl. Acad. Sci. USA (accepted).

HUOT, J., G. ROY, H. LAMBERT, P. CHRÉTIEN, and J. LANDRY. 1991. Increased survival after treatments with anticancer agents of Chinese hamster cells expressing the human Mr 27,000 heat shock protein. Cancer Res. **51**:5245–5252.

IFEANYI, F., and L. J. TAKEMOTO. 1989. Differential binding of α-crystallins to bovine lens membranes. Exp. Eye Res. **49**:143–147.

INAGUMA, Y., H. SHINOHARA, S. GOTO, and K. KATO. 1992. Translocation of αB-crystallin by heat shock in rat glioma (GA-1) cells. Biochem. Biophys. Res. Commun. **182**:844–850.

INGOLIA, T. D., and E. A. CRAIG. 1982. Four small heat shock proteins are related to each other and to mammalian α-crystallin. Proc. Natl. Acad. Sci. USA **79**:2360–2364.

IWAKI, T., A. IWAKI, M. MIYAZONO, and J. E. GOLDMAN. 1991. Preferential expression of αB-crystallin in astrocytic elements of neuroectodermal tumors. Cancer **68**:2230–2240.

IWAKI, T., and J. TATEISHI. 1991. Immunohistochemical demonstration of alphaB-crystallin in hamartomas of tuberous sclerosis. Am. J. Pathol. **139**:1303–1308.

IWAKI, T., T. WISNIEWSKI, A. IWAKI, E. CORBIN, N. TOMOKANE, J. TATEISHI, and J. E. GOLDMAN. 1992. Accumulation of αB-crystallin in central nervous system glia and neurons in pathological conditions. Am. J. Pathol. **140**:345–356.

JONES, D., R. H. RUSSNAK, R. J. KAY, and E. P. M. CANDIDO. 1986. Structure, expression

and evolution of a heat shock gene locus in *Caenorhabditis elegans* that is flanked by repetitive elements. J. Biol. Chem. **261**:12006–12015.

KATO, M., E. HERZ, S. KATO, and A. HIRANO. 1992*a*. Expression of stress-response (heat-shock) protein 27 in human brain tumors. Acta Neuropathol. **83**:420–422.

KATO, K., H. SHINOHARA, S. GOTO, Y. INAGUMA, R. MORISHITA, and T. ASANO. 1992*b*. Copurification of small heat shock protein with αB crystallin from human skeletal muscle. J. Biol. Chem. **267**:7718–7725.

KATO, K., H. SHINOHARA, N. KUROBE, S. GOTO, Y. INAGUMA, and K. OHSHIMA. 1991*a*. Immunoreactive αA-crystallin in rat non-lenticular tissues detected with a sensitive immunoassay method. Biochim. Biophys. Acta **1080**:173–180.

KATO, K., H. SHINOHARA, N. KUROBE, Y. INAGUMA, K. SHIMIZU, and K. OHSHIMA. 1991*b*. Tissue distribution and developmental profiles of immunoreactive αB-crystallin in the rat determined with a sensitive immunoassay system. Biochim. Biophys. Acta **1074**:201–208.

KLEMENZ, R., E. FRÖHLI, A. AOYAMA, S. HOFFMANN, R. J. SIMPSON, R. L. MORITZ, and R. SCHÄFER. 1991*a*. αB crystallin accumulation is a specific response to Ha-*ras* and v-*mos* oncogene expression in mouse NIH3T3 fibroblasts. Mol. Cell. Biol. **11**:803–812.

KLEMENZ, R., E. FRÖHLI, R. H. STEIGER, R. SCHÄFER, and A. AOYAMA. 1991*b*. αB-crystallin is a small heat shock protein. Proc. Natl. Acad. Sci. USA **88**:3652–3656.

KNACK, G., and K. KLOPPSTECH. 1989. cDNA sequence of a heat-inducible protein of *Chenopodium* sharing little homology with other heat-shock proteins. Nucleic Acids Res. **17**:5380.

KOENIG, S. H., R. D. BROWN III, M. SPILLER, B. CHAKRABARTI, and A. PANDE. 1992. Intermolecular protein interactions in solutions of calf lens α-crystallin. Biophys. J. **61**:776–785.

KRONE, P. H., A. SNOW, A. ALI, J. J. PASTERNAK, and J. J. HEIKKILA. 1992. Comparison of regulatory and structural regions of the *Xenopus laevis* small heat-shock protein-encoding gene family. Gene **110**:159–166.

KURTZ, S., J. ROSSI, L. PETKO, and S. LINDQUIST. 1986. An ancient developmental induction: heat-shock proteins induced in sporulation and oogenesis. Science **231**:1154–1157.

LANDRY, J., P. CHRÉTIEN, H. LAMBERT, E. HICKEY, and L. A. WEBER. 1989. Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. J. Cell Biol. **109**:7–15.

LANDRY, J., P. CHRÉTIEN, A. LASZLO, and H. LAMBERT. 1991. Phosphorylation of HSP27 during development and decay of thermotolerance in Chinese hamster cells. J. Cell. Physiol. **147**:93–101.

LANDRY, J., H. LAMBERT, M. ZHOU, J. N. LAVOIE, E. HICKEY, L. A. WEBER, and C. W. ANDERSON. 1992. Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. J. Biol. Chem. **267**:794–803.

LAUZON, L. M., K. HELM, and E. VIERLING. 1990. A cDNA clone form *Pisum sativum* encoding a low molecular weight heat shock protein. Nucleic Acids Res. **18**:4274.

LAVOIE, J., P. CHRÉTIEN, and J. LANDRY. 1990. Sequence of the Chinese hamster small heat shock protein HSP27. Nucleic Acids Res. **18**:1637.

LEE, Y. J., Z.-Z. HOU, L. CURETTY, and P. M. CORRY. 1992. Expression, synthesis, and phosphorylation of HSP28 family during development and decay of thermotolerance in CHO plateau-phase cells. J. Cell. Physiol. **150**:441–446.

LEUNISSEN, J. A. M., H. M. TEN VERGERT, and C. W. G. VAN GELDER. 1990*a*. CAMMSA User manual. CAOS/CAMM Center, University of Nijmegen, Nijmegen.

LEUNISSEN, J. A. M., H. W. VAN DEN HOOVEN, and W. W. DE JONG. 1990*b*. Extreme differences in charge changes during protein evolution. J. Mol. Evol. **31**:33–39.

LIANG, J. J. N., and X.-Y. LI. 1992. Spectroscopic studies on the interaction of calf lens membranes with crystallins. Exp. Eye Res. **54**:719–724.

LINDQUIST, S., and E. A. CRAIG. 1988. The heat shock proteins. Annu. Rev. Genet. **22**:631–677.

LONGONI, S., S. LATTONEN, G. BULLOCK, and M. CHIESI. 1990. Cardiac alpha-crystallin. II Intracellular localization. Mol. Cell. Biochem. **97**:121–128.

LOWE, J., H. MCDERMOTT, I. PIKE, I. SPENDLOVE, M. LANDON, and R. J. MAYER. 1992. αB crystallin expression in non-lenticular tissues and selective presence in ubiquitinated inclusion bodies in human disease. J. Pathol. **166**:61–68.

MCELWAIN, E. F., and S. SPIKER. 1989. A wheat cDNA clone which is homologous to the 17 kd heat shock protein gene family of soybean. Nucleic Acids Res. **17**:1764.

MCGUIRE, S. E., S. A. W. FUQUA, S. L. NAYLOR, D. A. HELIN-DAVIS, and W. L. MCGUIRE. 1989. Chromosomal assignments of human 27-kDa heat shock protein gene family. Somat. Cell Mol. Genet. **15**:167–171.

MACH, H., P. A. TRAUTMAN, J. A. THOMSON, R. V. LEWIS, and C. R. MIDDAUGH. 1990. Inhibition of α-crystallin aggregation by γ-crystallin. J. Biol. Chem. **265**:4844–4848.

MANN, E., M. J. MCDERMOTT, J. GOLDMAN, R. CHIESA, and A. SPECTOR. 1991. Phosphorylation of α-crystallin B in Alexander's disease brain. FEBS Lett. **294**:133–136.

MAYER, R. J., J. ARNOLD, L. LASZLO, M. LANDON, and J. LOWE. 1991. Ubiquitin in health and disease. Biochim. Biophys. Acta **1089**:141–157.

MENDELSOHN, M. E., Y. ZHU, and S. O'NEILL. 1991. The 29-kDa proteins phosphorylated in thrombin-activated human platelets are forms of the estrogen receptor-related 27-kDa heat shock protein. Proc. Natl. Acad. Sci. USA **88**:11212–11216.

MERCK, K. B., W. A. DE HAARD-HOEKMAN, B. B. OUDE ESSINK, H. BLOEMENDAL, and W. W. DE JONG. 1992. Expression and aggregation of recombinant αA-crystallin and its two domains. Biochim. Biophys. Acta **1130**:267–276.

MERCK, K. B., P. J. T. A. GROENEN, C. E. M. VOORTER, W. A. DE HAARD-HOEKMAN, J. HORWITZ, H. BLOEMENDAL, and W. W. DE JONG. Structural and functional similarities of bovine α-crystallin and mouse small heat-shock protein: a family of chaperones. J. Biol. Chem. (accepted).

MIRON, T., K. VANCOMPERNOLLE, J. VANDEKERCKHOVE, M. WILCHEK, and B. GEIGER. 1991. A 25-kD inhibitor of actin polymerization is a low molecular weight mass heat shock protein. J. Cell Biol. **114**:255–261.

MORIMOTO, R. I., A. TISSIÈRES, and C. GEORGOPOULOS, eds. 1990. Stress proteins in biology and medicine. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

MÖRNER, C. T. 1894. Untersuchung der Proteinsubstanzen in den leichtbrechenden Medien des Auges. Hoppe Seylers Z. Physiol. Chem. **18**:61–106.

MULDERS, J. W. M., J. STOKKERMANS, J. A. M. LEUNISSEN, E. L. BENEDETTI, H. BLOEMENDAL, and W. W. DE JONG. 1985. Interaction of α-crystallin with lens plasma membranes: affinity for MP26. Eur. J. Biochem. **152**:721–728.

MULDERS, J. W. M., E. WOJCIK, H. BLOEMENDAL, and W. W. DE JONG. 1989. Loss of high-affinity membrane binding of bovine nuclear α-crystallin. Exp. Eye Res. **49**:149–152.

MURAYAMA, S., T. W. BOULDIN, and K. SUZUKI. 1992. Immunocytochemical and ultrastructural studies of eosinophilic granular bodies in astrocytic tumors. Acta Neuropathol. **83**: 408–414.

NAGAO, R. T., E. CZARNECKA, W. B. GURLEY, F. SCHOEFFL, and J. L. KEY. 1985. Genes for low-molecular-weight heat shock proteins of soybeans: sequence analysis of a multigene family. Mol. Cell. Biol. **5**:3417–3428.

NENE, V., D. W. DUNNE, K. S. JOHNSON, D. W. TAYLOR, and J. S. CORDINGLEY. 1986. Sequence and expression of a major egg antigen from *Schistosoma mansoni*: homologies to heat shock proteins and α-crystallins. Mol. Biochem. Parasitol. **21**:179–188.

NERLAND, A. H., A. S. MUSTAFA, D. SWEETSER, T. GODAL, and R. A. YOUNG. 1988. A protein antigen of *Mycobacterium leprae* is related to a family of small heat shock proteins. J. Bacteriol. **170**:5919–5921.

NGO, J. T., I. KLISAK, R. A. DUBIN, J. PIATIGORSKY, T. MOHANDAS, R. S. SPARKES, and B. BATEMAN. 1989. Assignment of the αB-crystallin gene to human chromosome 11. Genomics **5**:665–669.

NOVER, L., K.-L. SCHARF, and D. NEUMANN. 1989. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. Mol. Cell. Biol. **9:** 1298–1308.

ORTWERTH, B. J., and P. R. OLESEN. 1992. Characterization of the elastase inhibitor properties of α-crystallin and the water-insoluble fraction from bovine lens. Exp. Eye Res. **54:**103–111.

PAULI, D., and C. H. TONKA. 1987. A *Drosophila* heat shock gene from locus 67B is expressed during embryogenesis and pupation. J. Mol. Biol. **198:**235–240.

PIATIGORSKY, J., and G. WISTOW. 1991. The recruitment of crystallins: new functions precede gene duplication. Science **252:**1078–1079.

PIATIGORSKY, J., and P. S. ZELENKA. 1992. Transcriptional regulation of crystallin genes. Adv. Dev. Biochem. **1:**211–256.

PITTENGER, G. L., R. R. GILMONT, and M. J. WELSH. 1992. The low molecular weight heat shock protein (hsp27) in rat Sertoli cells: evidence for identity of hsp27 with a germ cell-responsive phosphoprotein. Endocrinology **130:**3207–3215.

PLESOFSKY-VIG, N., and R. BRAMBL. 1990. Gene sequence and analysis of hsp30, a small heat shock protein of *Neurospora crassa* which associates with mitochondria. J. Biol. Chem. **265:** 15432–15440.

PRAGER, E. M., and A. C. WILSON. 1978. Construction of phylogenetic trees for proteins and nucleic acids: empirical evaluation of alternative matrix methods. J. Mol. Evol. **11:**129–142.

QUAX-JEUKEN, Y., W. QUAX, G. VAN RENS, P. M. KHAN, and H. BLOEMENDAL. 1985*a*. Complete structure of the αB-crystallin gene: conservation of the exon/intron distribution in the two nonlinked α-crystallin genes. Proc. Natl. Acad. Sci. USA **82:**5819–5823.

QUAX-JEUKEN, Y., W. QUAX, G. L. N. VAN RENS, P. MEERA KHAN, and H. BLOEMENDAL. 1985*b*. Assignment of the human αA-crystallin gene (CRYA1) to chromosome 21. Cytogenet. Cell Genet. **40:**727–728.

RADLICK, L. W., and J. F. KORETZ. 1992. Biophysical characterization of α-crystallin aggregates: validation of the micelle hypothesis. Biochim. Biophys. Acta **1120:**193–200.

RASCHKE, E., G. BAUMANN, and F. SCHÖFFL. 1988. Nucleotide sequence analysis of soybean small heat shock protein genes belonging to two different multigene families. J. Mol. Biol. **199:**549–557.

REGAZZI, R., U. EPPENBERGER, and D. FABBRO. 1988. The 27,000 Daltons stress proteins are phosphorylated by protein kinase C during the tumor promoter-mediated growth inhibition of human mammary carcinoma cells. Biochem. Biophys. Res. Commun. **152:**62–68.

RES, P. C. M., J. E. R. THOLE, and R. R. P. DE VRIES. 1991. Heat shock proteins in immunopathology. Curr. Opin. Immunol. **3:**924–929.

ROLLET, E., J. N. LAVOIE, J. LANDRY, and R. M. TANGUAY. 1992. Expression of Drosophila's 27 kDa heat shock protein into rodent cells confers thermal resistance. Biochem. Biophys. Res. Commun. **185:**116–120.

ROQUEMORE, E. P., A. DELL, H. R. MORRIS, M. PANICO, A. J. REASON, L.-A. SAVOY, G. J. WISTOW, J. S. ZIGLER JR., B. J. EARLES, and G. W. HART. 1992. Vertebrate lens α-crystallins are modified by *O*-linked *N*-acetylglucosamine. J. Biol. Chem. **267:**555–563.

ROSSI, J. M., and S. LINDQUIST. 1989. The intracellular location of yeast heat-shock protein 26 varies with metabolism. J. Cell Biol. **108:**425–439.

RUSSNAK, R. H., and E. P. M. CANDIDO. 1985. Locus encoding a family of small heat shock genes in *Caenorhabditis elegans.* Mol. Cell. Biol. **5:**1268–1278.

SAITOU, N., and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4:**406–425.

SAKLATVALA, J., P. KAUR, and F. GUESDON. 1991. Phosphorylation of the small heat-shock protein is regulated by interleukin 1, tumor necrosis factor, growth factors, bradykinin and ATP. Biochem. J. **277:**635–642.

SANTELL, L., N. S. BARTFELD, and E. G. LEVIN. 1992. Identification of a protein transiently phosphorylated by activators of endothelial cell function as the heat-shock protein HSP27. Biochem. J. **284:**705–710.

SCHOEFFL, F., E. RASCHKE, and R. T. NAGAO. 1984. The DNA sequence analysis of soybean heat-shock genes and identification of possible regulatory promoter elements. EMBO J. **3**: 2491–2497.

SCHUSTER, G., D. EVEN, K. KLOPPSTECH, and I. OHAD. 1988. Evidence for protection by heat-shock proteins against photoinhibition during heat-shock. EMBO J. **7**:1–6.

SCOTTING, P., H. MCDERMOTT, and R. J. MAYER. 1991. Ubiquitin-protein conjugates and αB crystallin are selectively present in cells undergoing major cytomorphological reorganisation in early chicken embryos. FEBS Lett. **285**:75–79.

SHAKOORI, A. R., A. M. OBERDORF, T. A. OWEN, L. A. WEBER, E. HICKEY, J. L. STEIN, J. B. LIAN, and G. S. STEIN. 1992. Expression of heat shock genes during differentiation of mammalian osteoblasts and promyelocytic leukemia cells. J. Cell. Biochem. **48**:277–287.

SIEZEN, R. J., J. G. BINDELS, and H. J. HOENDERS. 1980. The quaternary structure of bovine α-crystallin. Eur. J. Biochem. **111**:435–444.

SOUTHGATE, R., A. AYME, and R. VOELLMY. 1983. Nucleotide sequence analysis of the *Drosophila* small heat shock gene cluster at locus 67B. J. Mol. Biol. **165**:35–57.

SPECTOR, A., L.-K. LI, R. C. AUGUSTEYN, A. SCHNEIDER, and T. FREUND. 1971. α-Crystallin: the isolation and characterization of distinct macromolecular fractions. Biochem. J. **124**: 337–343.

SPECTOR, N. L., W. SAMSON, C. RYAN, J. GRIBBEN, W. URBA, W. J. WELCH, and L. M. NADLER. 1992. Growth arrest of human lymphocytes-B is accompanied by induction of the low molecular weight mammalian heat shock protein (hsp28). J. Immunol. **148**:1668–1673.

STRINGHAM, E. G., D. K. DIXON, D. JONES, and E. P. M. CANDIDO. 1992. Temporal and spatial expression patterns of the small heat shock (hsp-16) genes in transgenic *Caenorhabditis elegans.* Mol. Biol. Cell **3**:221–233.

SUSEK, R. E., and S. L. LINDQUIST. 1989. HSP26 of *Saccharomyces cerevisiae* is related to the superfamily of small heat shock proteins but is without a demonstrable function. Mol. Cell. Biol. **9**:5265–5271.

TAKAHASHI, T., and Y. KOMEDA. 1989. Characterization of two genes encoding small heat-shock proteins in *Arabidopsis thaliana.* Mol. Gen. Genet. **219**:365–372.

TARDIEU, A., and M. DELAYE. 1988. Eye lens proteins and transparency: from light transmission theory to solution X-ray structural analysis. Annu. Rev. Biophys. Chem. **17**:47–70.

TARDIEU, A., D. LAPORTE, P. LICINIO, B. KROP, and M. DELAYE. 1986. Calf lens α-crystallin quaternary structure: a three-layer tetrahedral model. J. Mol. Biol. **192**:711–724.

TÊTU, B., B. LACASSE, H.-L. BOUCHARD, R. LAGACÉ, J. HUOT, and J. LANDRY. 1992. Prognostic influence of HSP-27 expression in malignant fibrous histiocytoma. Cancer Res. **52**:2325–2328.

THOR, A., C. BENZ, D. MOORE II, E. GOLDMAN, S. EDGERTON, J. LANDRY, L. SCHWARTZ, B. MAYALL, E. HICKEY, and L. A. WEBER. 1991. Stress response protein (srp-27) determination in primary human breast carcinoma. J. Natl. Cancer Inst. **83**:170–178.

TISSIÈRES, A., H. K. MITCHELL, and V. M. TRACY. 1974. Protein synthesis in salivary glands of *Drosophila melanogaster:* relation to chromosome puffs. J. Mol. Biol. **84**:389–398.

TOMOKANE, N., T. IWAKI, J. TATEISHI, A. IWAKI, and J. E. GOLDMAN. 1991. Rosenthal fibers share epitopes with αB-crystallin, glial fibrillary acidic protein, and ubiquitin, but not with vimentin. Am. J. Pathol. **138**:875–885.

VAN DEN HEUVEL, R., W. HENDRIKS, W. QUAX, and H. BLOEMENDAL. 1985. Complete structure of the αA-crystallin gene: reflection of an evolutionary history by means of exon shuffling. J. Mol. Biol. **185**:273–284.

VAN DEN OETELAAR, P. J. M., and H. J. HOENDERS. 1989. Folding-unfolding and aggregation-dissociation of bovine α-crystallin subunits: evidence for unfolding intermediates of the αA subunits. Biochim. Biophys. Acta **995**:91–96.

VAN DER OUDERAA, F. J., W. W. DE JONG, and H. BLOEMENDAL. 1973. The amino-acid sequence of the αA2 chain of bovine α-crystallin. Eur. J. Biochem. **39**:207–222.

VAN DER OUDERAA, G. J., W. W. DE JONG, A. HILDERINK, and H. BLOEMENDAL. 1974. The amino acid sequence of the αB2 chain of bovine α-crystallin. Eur. J. Biochem. **49**:157–168.

VERBON, A., R. A. HARTSKEERL, A. SCHUITEMA, A. H. J. KOLK, D. B. YOUNG, and R. LATHIGRA. 1992. The 14,000-molecular-weight antigen of *Mycobacterium tuberculosis* is related to the alpha-crystallin family of low-molecular-weight heat shock proteins. J. Bacteriol. **174**: 1352–1359.

VIERLING, E. 1991. The roles of heat shock proteins in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. **42**:579–620.

VIERLING, E., R. T. NAGAO, A. E. DEROCHER, and L. M. HARRIS. 1988. A heat shock protein localizes to chloroplasts is a member of an eukaryotic superfamily of heat shock proteins. EMBO J. **7**:575–581.

VOORTER, C. E. M., W. A. DE HAARD-HOEKMAN, E. S. ROERSMA, H. E. MEYER, H. BLOEMENDAL, and W. W. DE JONG. 1989. The in vivo phosphorylation sites of bovine αB-crystallin. FEBS Lett. **259**:50–52.

VOORTER, C. E. M., J. W. M. MULDERS, H. BLOEMENDAL, and W. W. DE JONG. 1986. Some aspects of the phosphorylation of α-crystallin A. Eur. J. Biochem. **160**:203–210.

VOORTER, C. E. M., L. WINTJES, H. BLOEMENDAL, and W. W. DE JONG. 1992. Relocalization of αB-crystallin by heat shock in ovarian carcinoma cells. FEBS Lett. **309**:111–114.

WALSH, M. T., A. C. SEN, and B. CHAKRABARTI. 1991. Micellar subunit assembly in a three-layer model of oligomeric α-crystallin. J. Biol. Chem. **266**:20079–20084.

WISTOW, G. 1985. Domain structure and evolution in α-crystallins and small heat-shock proteins. FEBS Lett. **181**:1–6.

WISTOW, G. J., and J. PIATIGORSKY. 1988. Lens crystallins: the evolution and expression of proteins for a highly specialized tissue. Annu. Rev. Biochem. **57**:479–504.

ZANTEMA, A., E. DE JONG, R. LARDENOIJE, and A. J. VAN DER EB. 1989. The expression of heat shock protein hsp27 and a complexed 22-kilodalton protein is inversely correlated with oncogenicity of adenovirus-transformed cells. J. Virol. **63**:3368–3375.

ZANTEMA, A., M. VERLAAN-DE VRIES, D. MAASDAM, S. BOL, and A. VAN DER EB. 1992. Heat shock protein 27 and αB-crystallin can form a complex, which dissociates by heat shock. J. Biol. Chem. **267**:12936–12941.

MASATOSHI NEI, reviewing editor

Received June 12, 1992; revision received August 12, 1992

Accepted August 13, 1992