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invited review

Molecular chaperones in the kidney: distribution, putative roles, and regulation

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Beck, Franz-X., Wolfgang Neuhofer, and Eva Müller. Molecular chaperones in the kidney: distribution, putative roles, and regulation. *Am J Physiol Renal Physiol* 279: F203–F215, 2000.—Molecular chaperones are intracellular proteins that prevent inappropriate intra- and intermolecular interactions of polypetide chains. A specific group of highly conserved molecular chaperones are the heat shock proteins (HSPs), many of which are constitutively expressed but most of which are inducible by diverse (in some cases specific) stress factors. HSPs, either alone or in cooperation with "partner" chaperones, are involved in cellular processes as disparate as correct folding and assembly of proteins, transport of proteins to specific intracellular locations, protein degradation, and preservation and restructuring of the cytoskeleton. The characteristic distribution of individual HSPs in the kidney, and their response to different challenges, suggests that a number of HSPs may fulfill specific, kidney-related functions. HSP72 and the osmotic stress protein 94 (Osp94) appear to participate in the adaptation of medullary cells to high extracellular salt and urea concentrations; the small HSPs (HSP25/27 and crystallins) may be involved in the function of mesangial cells and podocytes and contribute to the volume-regulatory remodeling of the cytoskeleton in medullary cells during changes in extracellular tonicity. HSP90 contributes critically to the maturation of steroid hormone receptors and may thus be a critical determinant of the aldosterone sensitivity of specific renal epithelial cells. Certain HSPs are also induced in various pathological states of the kidney. The observation that the expression of individual HSPs in specific kidney diseases often displays characteristic time courses and intrarenal distribution patterns supports the idea that HSPs are involved in the recovery but possibly also in the initiation and/or maintenance phases of these disturbances.

heat stress proteins; osmotic stress; ischemia

THE TERM "MOLECULAR CHAPERONES" designates proteins that participate transiently in the folding of other proteins and in the assembly of proteins into oligomeric structures (30, 35, 87). Molecular chaperones are characterized by their ability to bind to nonnative proteins, thus hindering incorrect intra- or intermolecular interactions, facilitating transport of proteins to specific intracellular locations and supporting degradation of proteins (reviewed in Refs. 8, 34, 35, 44, 78, 87). Although many molecular chaperones are expressed constitutively, most of them are induced strongly by physical or metabolic stress. Accordingly, molecular chaperones are essential for cell function under physiological conditions and contribute decisively to the survival of cells subjected to adverse environmental conditions. This explains the remarkable and increasing interest of life scientists in molecular chaperones and the steadily growing number of reports dealing with this issue. A detailed and comprehensive review of all aspects of molecular chaperones is beyond the

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scope of this article, which thus will be restricted to the consideration of a specific class of molecular chaperones, the heat shock proteins (HSPs), in particular those of relevance to the kidney. The term "heat shock protein" stems from the original observation that heat stress greatly enhances the production of a specific class of proteins, subsequently identified as molecular chaperones. HSPs, which are among the most conserved proteins known, are divided into families according to their molecular weight (34, 78, 87). The present classification lists the small HSPs (sHSPs; 16–40 kDa), the HSP40 family, and the HSP60 (together with TriC forming the "chaperonin" family), HSP70, HSP90, and HSP100 families. In the following discussion, the HSPs relevant to kidney function will be discussed in turn.

SHSPS

The members of the sHSP family are more heterogeneous in their molecular weights than are other HSP families, with values ranging from 16 to 40 kDa (78). The 23-kDa lens protein αB-crystallin has been also identified as a member of this HSP family (64). Features common to all sHSPs are, first, that they are phosphorylatable at several sites and appear thus as nonphosphorylated or phosphorylated isoforms (3, 150); second, that they form large multimeric aggregates of ~300–800 kDa (7); and, third, that they share characteristic sequences in the COOH-terminal part, the so-called "α-crystallin domain" (112). The signal transduction pathway leading to phosphorylation of the human HSP27 and murine HSP25 includes p38 MAP kinase, which is the upstream activator of MAPKAP kinase 2/3 (51, 70), which in turn phosphorylates HSP25/27 (130). There is evidence suggesting that phosphorylation of HSP25/27 favors the dissociation of HSP25/27 multimers (59, 114). sHSPs, notably in their multimeric form, act as molecular chaperones (56) and, related to this function, are part of the mechanisms providing protection against the negative effects of diverse stress factors (20). sHSPs seem to participate in the actin polymerization/depolymerization process (HSP25/27) (72), may modulate the assembly of intermediate filament proteins (α-crystallins) (104), and inhibit the aggregation of tubulin (αB-crystallin) (2).

Although the mechanism underlying the molecular chaperone activity of sHSPs is not entirely clear, in vitro studies suggest that sHSPs interact with hydrophobic sites of unfolding proteins that are in the molten globule state, thus preventing their aggregation (28, 73, 77). The observation that sHSPs, probably as large chaperone oligomers, bind unfolding proteins stably in conjunction with the finding that refolding of these bound, nonnative proteins requires ATP, HSP70, and probably additional, as yet undefined, proteins, has led to the hypothesis that sHSPs maintain their substrates in a folding-competent state, to be reactivated by other chaperones once temperature and/or cell metabolism is restored (28, 73). Recently, the crystal structure of HSP16.5 from *Methanococcus jannaschi,* which consists mainly of an α-crystallin domain, has been determined (63). In the crystal structure this HSP forms a sphere of 24 subunits with an outer diameter of 120 and an inner diameter of 65 Å. The interior of the spherical structure appears to be much more hydrophobic than the outside surface.

With respect to the interaction of sHSPs with components of the cytoskeleton, HSP25/27 is supposed to act as an actin-capping protein, which inhibits actin polymerization when bound (85). Phosphorylation probably induces a conformational change of HSP25/27, resulting in dissociation from the barbed ends of the actin filaments, thus freeing them for the addition of actin monomers (9). Several reports have supported the view that the protective function of HSP25/27 is related to this interaction with the actin cytoskeleton, which promotes reorganization of actin filaments and stabilizes stress fibers when cells are exposed to stress, for example, heat, oxidative stress, or cytochalasin D (51, 71). Originally, remodeling of the F-actin network in cells subjected to oxidative stress was assumed to depend on activation of MAPKAP kinase-2/3 and phosphorylation of HSP25/27 (51, 52, 151). Recent evidence, however, suggests that the native size of HSP25/27 oligomers is critical in determining the protective activity, with the large nonphosphorylated HSP25/27 oligomers showing the greatest protective potential against the adverse effects of tumor necrosis factor-α or reactive oxygen species (84, 114). Crystallins may also interact with cytoskeletal components: the in vitro assembly of the intermediate filament proteins vimentin and glial fibrillar acidic protein is inhibited in an ATP-independent manner by α-crystallins (104). This, and the observation that α-crystallin particles bind to intermediate filaments assembled in vitro, is consistent with the notion that α-crystallins participate in modeling of the intermediate filament network. Aggregation of tubulin (into microtubules) is suppressed by αB-crystallin by the formation of large complexes of αB-crystallin and tubulin dimers (2).

In the adult kidney producing a normally concentrated urine, sHSPs display a distribution paralleling the corticopapillary osmotic gradient, with high amounts in the papilla and low amounts in the cortex (61, 96, 106, 120, 142). Immunohistochemical investigations have revealed that, in the cortex, HSP25 is present predominantly in glomerular capillaries and vascular walls but also in mesangial cells and podocytes (61, 96, 125). Although no, or only very weak, staining is detectable in the cytoplasm or nucleus of proximal and distal tubule cells, HSP25 immunoreactivity is clearly visible in the apical domain of proximal tubular cells (4, 120), an intracellular distribution very similar to that of filamentous actin (4). In the outer medulla, vascular walls show intense and the cytoplasmic/nuclear space of all tubules show weak HSP25 immunoreactivity. However, strong positive reactions can be observed in the brush borders of outer medullary proximal straight tubules. In the inner medulla HSP25 is present in collecting duct cells and in the epithelium lining the papillary tip, but is not detected

in interstitial fibroblasts. The expression pattern of αB-crystallin also parallels the corticopapillary osmotic gradient. The cellular distribution, however, differs from that of HSP25 (54, 55). Although in the cortex αB-crystallin is not detectable, in the outer medulla the proximal tubules and thin descending limbs of the loop of Henle exhibit strong immunoreactivity for αB-crystallin. In the inner medulla, collecting duct cells and cells of the Henle's loop stain strongly. This pattern of αB-crystallin expression is not seen in the embryonic kidney but is acquired during the first 2 wk after birth (54), a period when the urine-concentrating ability is developing as well.

sHSPs seem to have functional significance already in the developing kidney. Iwaki et al. (54) observed that the prominent elongation of Henle's loop during the first days of life is accompanied by increased αB-crystallin expression. The authors interpreted this as suggesting an involvement of this sHSP in the acquisition of tubule function.

In the adult kidney sHSPs may play distinct roles in the three kidney zones. For the medulla, several investigators have hypothesized that the strong expression of sHSPs is related to the osmotic stress generated by the hypertonic environment in this kidney zone. In vitro investigations on renal [Madin-Darby canine kidney (MDCK; 99)] and nonrenal [lens epithelial (26), glial (60)] cells have revealed that exposure to high NaCl concentrations evokes enhanced synthesis of sHSPs and that levels stay elevated as long as cells are exposed to osmotic stress. On the other hand, in vivo investigations have shown that increasing papillary osmolality by vasopressin administration for up to 6 h has no influence on sHSP mRNA levels (145), whereas water restriction for 2 days slightly increases papillary sHSP mRNA (83). Long-term changes (3-wk diuresis; 5-day antidiuresis) in papillary osmolality also have no influence on sHSP mRNA nor on the already high protein levels (95). In view of the fact that even after chronic diuresis the inner medulla is still hyperosmolar, it is conceivable that this elevated osmolality suffices to induce sHSPs significantly. Interestingly, both increases and decreases in osmolality activate the kinase [MAPKAP kinase-2/3 (42, 134)] responsible for sHSP phosphorylation, resulting in strong phosphorylation of sHSPs in vitro and in vivo (94, 95). Taken together, these data show that changes in osmolality but also the continued exposure to a hypertonic environment may influence expression, synthesis, and phosphorylation of sHSPs.

It must be conceded, however, that the functional significance of these effects is not yet clear. Because hyperosmolality effectively induces sHSP synthesis and phosphorylation only when generated by the exogenous addition of relatively membrane-impermeant substances [NaCl (99)] but not by membrane-permeant substances [urea (99, 148)], it seems that it is not hyperosmolality per se but rather hypertonicity that is the actual trigger for these effects. Exposure to hyper- or hypotonicity is accompanied by cell shrinkage or swelling, respectively, processes requiring a pronounced remodeling of the actin cytoskeleton. The fact that sHSPs are major components of this process (see above) and the observations that sHSPs colocalize with the actin-cytoskeleton when cells are exposed to hypertonic stress (74, 98) support the view that sHSPs are involved in the adaptation to osmotic stress via their participation in cytoskeleton modulation. In view of this, the high amounts of sHSP in the renal medulla might be necessary to enable these cells to adapt to the frequently changing environmental osmolality caused by the variable hydration status of an animal. However, the observation that levels of sHSPs remain elevated also in adapted cells and do not greatly change even when the intensity of the osmotic stress is altered, points to (a) further role(s) of sHSPs in cells growing in an hypertonic environment, e.g., papillary cells. The finding that sHSPs not only participate in the modulation of the cytoskeleton but also trap aggregation-prone proteins in a refoldable state until reactivation by additional molecular chaperones (see above) supports this view. Hence, depending on the availability of such additional factors, sHSPs may or may not confer enhanced resistance to hypertonic stress. The requirement of such additional factors is exemplified by the observation that αB-crystallin-expressing glial cells, but not NIH/3T3 fibroblasts displaying high levels of HSP27 and αB-crystallin, have increased tolerance to elevated salt concentrations (60).

In the cortex, pronounced staining for HSP25 is detectable in glomerular mesangial cells and podocytes (61, 96, 125). Both cell types contain well-developed contractile systems and respond to vasoactive substances by contraction. The observations that incubation with monoclonal antibodies against HSP27 blocks bombesin-induced smooth muscle contraction (11) and that thrombin-stimulated contraction of smooth muscle cells is accompanied by increased sHSP phosphorylation (14) point to involvement of sHSPs in the contraction mechanism. That sHSPs exert this function also in the above two renal cells is further suggested by the finding that inhibition of sHSP phosphorylation prevents angiotensin II-induced contraction of mesangial cells (92). A role for HSP25/27 also in the constriction-dilatation cycle of intrarenal arterial vessels, which are also highly positive for this sHSP (96), may be inferred from these observations. The intense staining for HSP25 of the brush-border region of proximal straight tubules may reflect the contribution of this protein to the assembly-disassembly process of actin filaments in this cell domain (4, 120).

sHSPs seem to have functional significance also in the diseased kidney. Takashi et al. (133) have detected elevated contents of both HSP25/27 and αB-crystallin in chemically induced renal cell tumors. In mesenchymal tumors the tissue content only of HSP25/27 was higher whereas that of αB-crystallin was significantly lower than in normal kidney samples. The diagnostic, prognostic, and/or therapeutic implications of these findings are as yet unclear. In an experimental model of nephrotic syndrome induced by puromycin aminonucleoside, retraction, and effacement of glomerular

epithelial cell foot processes were associated with enhanced expression of glomerular HSP25 protein and increased glomerular HSP25 phosphorylation (125). The authors surmised that these increases in HSP25 expression and phosphorylation, by modulating actin polymerization-depolymerization, may play an important role in the development of the observed changes in foot process architecture.

During renal ischemia and the initial reperfusion period HSP25 moves from a major cytoplasmic, soluble protein fraction into an insoluble fraction (4, 121). The detergent-insoluble fraction contains cytoskeletal components, nuclei, and aggregated proteins. This redistribution, noted in all kidney zones (121), is gradually reversed during the reflow period. In the cortex and outer medulla, expression of HSP25 increases significantly during the initial 24 h of reperfusion but normalizes after 14 days (4, 121). In the inner medulla, however, soluble HSP25 is reduced throughout this period (121). Studies on intracellular HSP25 localization by using immunofluorescence microscopy revealed an initial and transient disappearance of HSP25 from its subapical location and the brush borders (4, 120) and concomitant dispersion throughout the cytoplasm, with small punctate accumulations similar to fragmented, DNase I-reactive actin (4). During later reflow, the cytoplasmic localization of DNase-reactive actin is clearly different from that of HSP25, a portion of which, together with F-actin, begins to reaccumulate in the proximal tubule brush-border region. Given that cytoskeletal disruption is integral to ischemic renal injury, HSP25 is thought to interact with actin, a function described in detail above, during ischemia and the early reperfusion period, thus participating in the ischemia-induced restructuring of the cytoskeleton (4). In addition, the finding that ischemia induces HSP25 in cortical and outer medullary blood vessels, presumably in endothelial cells, suggests similar functions in nontubular cells (120). The ischemia-induced increase in detergent-insoluble HSP25 and its specific intracellular distribution during later reflow periods may also be related to the property of sHSPs of sequestering nonnative proteins in an ATP-independent manner for subsequent reactivation by ATP-dependent chaperoning systems (see above).

The cellular triggers responsible for induction of sHSPs are largely unknown. Transcriptional activation is believed to play a major role, because the regulatory heat shock element (HSE), which consists of three inverted repeats of the sequence nnGAAn, has been found in the s*hsp* genes in all species examined so far (36, 39, 49, 64). The observation that heat-shock transcription factor (HSF), which in its trimeric form binds to the HSE, is activated by hypertonic stress (1) supports the view that this mode of transcriptional regulation may also apply to the epithelial cells of the renal medulla in vivo. This, however, does not explain the constitutively high HSP25 levels of nonepithelial cells in the cortex (mesangial cells, podocytes, vascular structures), structures that are not exposed to elevated environmental tonicity (96, 125). In addition, not all cell types respond to hypertonic stress by enhanced expression of HSP25/27 (47, 60, 93) but rather by induction of αB-crystallin (47, 60). Interestingly, the 5′ flanking regions of sHSPs contain an estrogen-responsive, half-palindromic motif, potential target sequences for the transcription factors Sp1 and *NFκB*, and sequences resembling those observed in an osmoregulated *Escherichia coli* gene (*osm*B) (26, 36, 39, 46, 49). Hence, sHSPs are probably regulated by several independent pathways that adjust the expression of HSP25/27 and crystallins in a cell type- and condition-specific manner.

HSP60

HSP60 is a molecular chaperone that, together with the TCP-1 ring complex TRiC, belongs to the chaperonin family (for review, see Refs. 34, 44, 50). Fourteen HSP60 monomers form a large structure, similar to the bacterial homolog GroEL, consisting of two stacked rings (heptamers) enclosing a central cavity that accommodates up to two molecules of polypeptides (29, 34, 44). This chamber, in combination with HSP10 heptamers, provides a secluded and protected compartment that favors the folding of partially folded intermediates and prevents incorrect association with neighboring, aggregation-prone polypeptides. The operation of the HSP60-HSP10-chaperonin system is coupled to the binding and hydrolysis of ATP. In eukaryotic cells, HSP60 is localized primarily in the matrix space of mitochondria (48, 126) where it, in cooperation with mitochondrial HSP70, assists in the folding and assembly of imported proteins (103).

In the normal kidney, HSP60 content is lower in the inner medulla than in the cortex or outer medulla (95, 96, 143). This intrarenal distribution parallels the abundance of mitochondria in the various kidney zones (111). Immunohistochemical studies have shown high signal intensities in proximal tubule cells and moderate intensities in distal tubule cells. Positive reactions have been observed in podocytes but not in Bowman's capsule (48, 96). In the outer medulla, conspicuous staining is seen in thick ascending limbs of the loop of Henle, whereas collecting ducts display only weak immunoreactivity. Positive staining in the inner medulla is restricted to the cells of the terminal collecting duct. No positive reactions were observed in vascular structures in any of the kidney zones (96). HSP60 is induced by heat stress (58), but medullary HSP60 content is barely affected by changes in medullary osmolarity (95, 143, 145). Hence, HSP60 may assist in the refolding and/or elimination of mitochondrial proteins damaged by heat stress but probably does not play an important role in the adaptation to hypertonic stress. In $HgCl_2$-induced, nephrotoxic acute renal failure, enhanced expression of HSP60 is noted initially in medullary collecting duct cells and in the transitional epithelial cells of the pelvis and calyces. Later, in parallel with the appearance of tubular necrosis, HSP60 immuoreactivity increases in all tubular structures of the cortex but is most pronounced in the pars recta of the proximal

tubule, coinciding with the degree of histological damage (48). Because mercury promotes protein denaturation and aggregation, it is tempting to speculate that enhanced expression of HSP60 in $HgCl_2$-induced acute renal failure accelerates the reconfiguration of "disordered" proteins.

THE HSP70 FAMILY

The HSP70 family includes molecular chaperones that are found in most compartments of the eukaryotic cell and are both constitutively expressed and induced by stress. This family of HSPs assists in the correct folding of newly synthesized proteins, in the refolding of partially denatured or misfolded proteins, and in the degradation of irreparably damaged proteins (8, 44, 45). They may interact with cytoskeletal structures and participate in the translocation of proteins across membranes into organelles and in the disassembly of protein aggregates (44, 75). HSP70s display weak ATPase activity and cyclically bind and release hydrophobic segments of unfolded and partially folded proteins in an ATP/ADP-dependent reaction cycle (for review, see Refs. 8, 34, 44). The complex consisting of ADP, HSP70, and nonnative polypeptides is relatively stable, thus preventing incorrect interaction between protein domains. Exchange of ADP against ATP results in a low-affinity complex that releases the substrate polypeptide rapidly and thus allows the folding process to advance (8, 34, 44). The binding and release of substrate polypeptides to HSP70s is modulated by cofactors (HSP40, Hip, BAG) that may also regulate ADP/ATP exchange or ATP hydrolysis (81). Structural features common to the various HSP70s are an ATPase domain localized in the NH_2 terminal, a peptide-binding region following the ATPase domain, and the COOH-terminal fragment, which may differ substantially between the various HSP70 subfamilies and may partake of the interaction with cochaperones and self-association of HSP70 monomers (43).

HSP73

HSP73, also termed HSC70 (70-kDa heat shock cognate protein), is the major constitutively expressed member of the HSP70 family. By using Western blot analysis, similar levels of HSP73 have been observed in all zones of the normal rat kidney (96). This distribution pattern is unaffected by chronic diuresis or continuous administration of the antidiuretic hormone analog dDAVP after chronic diuresis (95).

With the use of immunohistochemistry, HSP73 can be detected in the cortex in all tubular epithelial cells, in glomerular podocytes, and, although not consistently, in Bowman's capsule (66, 67, 96). The immunoreactivity for HSP73 is similar in the nucleus and cytoplasm in most of these cells except for podocytes, Bowman's epithelium, and proximal tubule cells, where nuclear expression is more pronounced (66, 67). In the outer and inner medulla, staining for HSP73 is evident in proximal straight tubules, thick ascending limbs of the loop of Henle, collecting ducts, the papillary epithelium, and papillary interstitial cells (96). This ubiquitous presence of HSP73 can be ascribed to the need, also of nonstressed cells, for assistance in protein folding, trafficking, and controlled degradation.

In puromycin aminonucleoside nephrosis, immunoreactivity to HSP73 is increased in the cytoplasm at the basolateral pole of the cells of the thick ascending limb of the loop of Henle, of distal tubule cells, and of collecting duct cells. In this case, intense staining is also noted in cells of mesangial localization. Because these alterations are associated with a greatly increased urine protein excretion, the authors of that study speculated that increased cytoplasmic levels of HSP73 may be related to increased tubular protein reabsorption or/and to cytoprotection against the adverse effects of proteinuria (66). In experimental acute renal failure, HSP73 is induced preferentially in the straight portion of the proximal tubule in both the degenerative and in the regenerative phase (88). This part of the nephron has been shown to be the major site of ischemia-induced damage to tubule cells (139). In gentamicin-induced acute tubular injury, HSP73 moves from the nucleus into the cytoplasm in proximal tubule cells and accumulates in fine granules, with a distribution pattern closely resembling that of lysosomes. Western blot analysis reveals that, at the same time, HSP73 is shifted from a detergent-soluble into a detergent-insoluble protein fraction (67). These observations suggest that in gentamicin-induced acute tubular injury HSP73 may participate in the lysosomal degradation of abnormal proteins (67).

HSP72

In the normal rat kidney, HSP72 mRNA abundance and the tissue content of HSP72, the most readily inducible member of the HSP70 family, increase steeply along the corticopapillary axis (23, 24, 83, 95, 96, 118, 143). Similar to HSP25/27 and αB-crystallin, HSP72 thus displays a distribution pattern closely paralleling the tissue solute concentration characteristic of the concentrating kidney. Consistent with this distribution pattern, in one study immunoreactivity to HSP72 was detected in the cortex in individual collecting duct cells only. In the outer medulla, all tubules were stained weakly, whereas in the papilla intense staining of collecting ducts and the epithelium lining the papilla was noted (96). In another study, however, in which a different antibody was used, staining was apparent in all cortical and medullary tubules after intravenous injection of vasopressin but not in the saline-injected control (145). With few exceptions (83, 118), changes in medullary solute concentrations are associated with corresponding changes in inner medullary HSP72 expression (23, 24, 95, 121). Because, in murine inner medullary collecting duct ($mIMCD_3$) cells, maximal HSP72 mRNA expression is already reached at ~500 mosmol/kgH_2O (143) and because the various stressors (high NaCl and urea concentrations, low pH) may act in concert to induce HSP72 (101), the variability in these results may be explained by differ-

ences in the duration and/or extent of changes in medullary solute concentrations in the various studies. Experiments in cultured renal epithelial cells demonstrate that HSP72 is significantly induced only when medium osmolality is increased by solutes for which the cell membrane is poorly permeable (e.g., NaCl, mannitol) but not by readily permeating solutes (urea, glycerol) (21, 24, 99, 117, 118). This suggests that, in the renal inner medulla, the high extracellular NaCl concentration is a major determinant of the high HSP72 content. On the other hand, increases in renal papillary urea concentrations at stable extracellular NaCl concentrations are associated with increased HSP72 expression, suggesting that, in the kidney in vivo, high urea concentrations, possibly in combination with additional stress factors, may also induce HSP72 (101, 107). In addition, induction of HSP72 may also occur in a situation of exacerbated hypotonic stress. Hence, when the volume regulatory efflux of "organic osmolytes" from the cells of the inner medulla is impaired during rapidly falling extracellular solute concentrations, HSP72 expression is significantly enhanced (106), probably reflecting the severity of cell stress.

The intrarenal distribution of HSP72 and the effect of altered extracellular tonicity on HSP72 have led to the hypothesis that this HSP assists in the adaptation of medullary cells to high extracellular solute concentrations. A characteristic feature of this adaptation process is the accumulation of low-molecular-weight organic substances (organic osmolytes) by the cells of the renal medulla when extracellular tonicity is rising (for a review, see Ref. 6). The increase in the intracellular concentration of organic osmolytes, a process requiring hours and days, is preceded by HSP72 induction (123). Hence, it has been suggested that during the initial phase of adaptation to high extracellular tonicities, when intracellular ionic strength is high due to cell shrinkage (127), HSP72 stabilizes intracellular proteins and thus attenuates the denaturing effect of high intracellular concentrations of inorganic salts (21). In addition, osmotically obliged loss of water from cells enhances macromolecular crowding, thus augmenting the hazard of inappropriate interaction of proteins. It is reasonable to assume that in this situation an increased "chaperoning capacity" is beneficial.

In MDCK cells, induction of HSP72 by either heat shock or hypertonic stress is mitigated if uptake of betaine, a trimethylamine compound, by these cells is facilitated (123). This interaction between HSP72 expression and intracellular betaine accumulation was explained by the property of trimethylamines to stabilize proteins and hinder thermally induced protein unfolding (76, 123). However, hypertonicity-induced expression of HSP72 is not normalized in renal cells even after completion of osmolyte accumulation, in either the papilla in vivo (95) or cultured renal epithelial cells (99, 115). This observation suggests that HSP72 fulfills additional functions apart from providing cytoprotection against high intracellular concentrations of inorganic salts during the initial phase of adaptation to hypertonic stress. Evidence for such additional roles of HSP72 comes from experiments in MDCK cells exposed to elevated extracellular NaCl concentrations to increase intracellular HSP72 contents. These conditioned cells tolerate addition of urea (600 mM) to the hypertonic incubation medium far better than do nonconditioned cells, most of which die of apoptosis (99, 102). The observation that conditioned cells still display a high degree of urea tolerance even if they are depleted of methylamines before exposure to high urea concentrations, indicates that this protective effect is not mediated by methylamines (99). The cytoprotective function of HSP72 against high urea concentrations was underlined in studies demonstrating that inhibition of hypertonicity-induced HSP72 expression by either the p38 MAP kinase inhibitor SB-203580 or stable antisense transfection renders these MDCK cells susceptible to high urea concentrations (Fig. 1) (100). It is thus conceivable that the high intracellular HSP72 contents observed in the inner medulla of the concentrating kidney are vital for protection of these cells against the adverse effects of high urea concentration. The mechanism(s) underlying HSP72-mediated protection against high urea concentrations is at present unclear. It is conceivable that protein unfolding by urea is counteracted by the chaperoning activity of HSP72. Considering that high urea concentrations activate Jun NH_2-terminal kinases and cause apoptotic cell death (10, 102, 117), urea-induced apoptosis

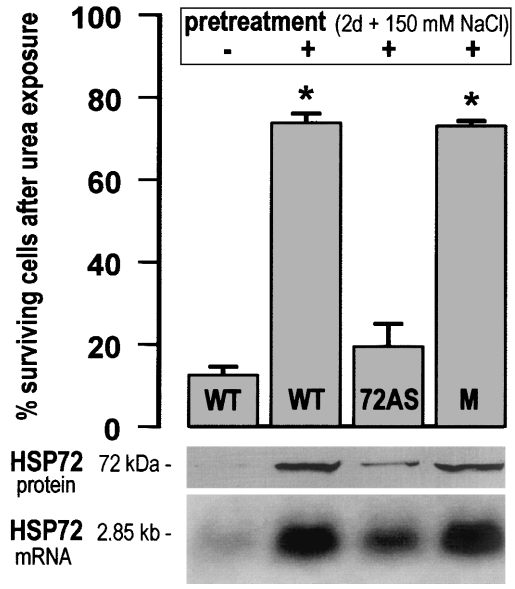

Fig. 1. Survival of Madin-Darby canine kidney (MDCK) cells after urea exposure parallels heat shock protein 72 (HSP72) expression. MDCK cells either kept in isotonic medium or preconditioned for 2 days in hypertonic medium (addition of 150 mM NaCl to induce HSP72 expression) were exposed to 600 mM urea in the respective media. Cells with low HSP72 levels [wild-type (WT) cells kept in isotonic medium and HSP72-antisense transformants (72AS)] display low survival rates in contrast to cells with high HSP72 contents [WT and mock-transfected (M) cells preconditioned with hypertonic medium]. Differences in HSP72 expression are demonstrated by representative Western and Northern blots. Part of the data is from Ref. 100. $*P < 0.05$ vs. WT and 72AS cells.

may also be prevented by interference of HSP72 with activation of Jun NH_2-terminal kinases or steps further downstream in the death-signaling pathway (16, 38, 57, 90).

As shown in numerous investigations, HSP70 may provide cytoprotection against stress factors other than high urea concentrations. Enhancement of HSP72 expression in cultured renal epithelial cells is associated with significantly improved cytoprotection against subsequent heat stress or ATP depletion (12, 105, 136, 140). Similarly, the greater tolerance of the immature compared with the mature nephron to anoxic, heat, and oxygen stress is assumed to be linked to the superior capacity of immature tubules to express HSP72 (41). Also relevant in this context is the recent finding that inducible nitric oxide synthase-knockout mice are more resistant to renal ischemia than are wild-type control animals, a phenomenon ascribed to increased HSP72 expression in the cortex of the inducible nitric oxide synthase-knockout mice (79). Although demonstrating a positive correlation between the intracellular HSP72 content and cytoprotection, these studies, of course, do not establish a causal relationship between these parameters. Evidence against HSP70 being a "universal" cytoprotectant has been provided by Zager et al. (146), who demonstrated that enhanced HSP70 production in the renal cortex was poorly correlated with cytoprotection against subsequent in vitro hypoxic injury to proximal tubule segments isolated from the respective cortices. In addition, transfection experiments in a porcine tubular-like cell line (LLC-PK_1 cells) have shown that overexpression of HSP72 does not protect against injury caused by hypoxia in combination with glucose deprivation (136).

The idea that, under specific circumstances, HSP70 may act as a cytoprotectant has prompted a series of investigations examining the role and the putative protective effects of HSP72 in acute renal failure (5, 23, 31, 58, 110, 120, 121, 137, 138). These studies have shown that both ischemic and nephrotoxic renal failure induce HSP72 in the cortex (23, 31, 121) and outer medulla (91, 121). Within the cortex HSP72 is barely detectable in control kidneys (see above). However, by using immunocytochemistry in an animal model of postischemic acute renal failure, HSP72 localizes primarily to the apical domain of proximal tubule cells during the early reflow period (15 min), a time at which morphological alterations of the cytoskeletal terminal web and brush border are most conspicuous and basolateral membrane-resident proteins (Na-K-ATPase) appear in the apical domain (86, 137). Subsequently (2, 6, and 24 h reflow), HSP72 immunoreactivity disperses to a vesicular pattern, partly in colocalization with lysosomes, throughout the cytoplasm. Weak and diffuse cytosolic staining for HSP72 is seen at all reflow intervals; nuclear or nucleolar staining is not observed at any time (137). In contrast to nonischemic controls, HSP72 is found during the early reflow period not only in the soluble but also in the microsomal fraction of total kidney homogenates (137). This agrees with the observation that, at the end of the ischemic period and during the first hour of reperfusion, the amount of detergent-soluble HSP72 in total kidney homogenate and medullary tissue specimens decreases rather than increases, suggesting that during this period part of the cytosolic HSP72 moves into the cytoskeletal or/and microsomal fraction(s) (121, 137). On the other hand, during the early reflow period, Na-K-ATPase, normally present only in the detergent-insoluble protein fraction, appears in the soluble, cytosolic protein fraction, probably reflecting the ischemia-induced loss of anchorage of Na-K-ATPase to cytoskeletal structures (5). The subsequent disappearance of Na-K-ATPase from the soluble fraction is accompanied by an increase in Na-K-ATPase in the membrane-cytoskeletal complex and parallels the accumulation of HSP72 in both the soluble and insoluble fractions. ATP enhances the release of HSP72 from the membrane-cytoskeletal complex and preserves Na-K-ATPase association with this complex (5). Taken together, these findings are consistent with the idea that, during acute renal failure, HSP72 participates in the degradation of irreparably damaged proteins, assists in the refolding and correct assembly of partially denatured or/and disassembled proteins, and contributes to the restructuring of the cytoskeleton and restitution of epithelial cell polarity.

Although in both nephrotoxic and postischemic acute renal failure HSP72 expression is induced in the cortex, inner medullary HSP72 expression has been shown to decrease (23, 121). Because in both cases this phenomenon is associated with reduced urine osmolalities, the attenuated expression of HSP72 in the inner medulla of the injured kidneys has been attributed to the diminished osmotic stress in this kidney zone (23, 121).

Results of studies in which the effects of hyperthermic preconditioning on the degree of ischemia-induced renal injury have been examined are variable. Perdrizet et al. (109, 110) have shown that total body hyperthermia, followed by recovery, causes enhanced HSP72 production and protects renal allografts from cold and warm ischemia. In contrast, but consistent with Zager's results (Ref. 146 and see also above), Joannidis et al. (58) have been unable to demonstrate any beneficial effects of hyperthermic preconditioning on structure and function of kidneys subjected to transient ischemia 48 h after hyperthermia, despite HSP72 induction. Interpretation of these divergent results is complicated by differences in the experimental protocol and in the animal species employed. The degree of kidney damage inflicted by the preconditioning stress, the magnitude of HSP72 induction and its time course, and the induction of additional, putative cytoprotectants are but a few of the multiple factors that may determine the overall cytoprotective effect of a specific preconditioning maneuver.

There is evidence indicating that both hypertonic and ischemic/hypoxic stress induce HSP72 by activating heat shock factor 1 (HSF1) (41, 138, 145). In response to heat or other stresses, inactive HSF monomers, constitutively present in the cytoplasm, are converted into DNA-binding homotrimers that trans-

locate to the nucleus. HSF in its trimeric state binds to a consensus target sequence, the HSE, present in the promoter regions of heat shock genes, but does not per se necessarily activate transcription (22, 128). Intracellular acidification may promote HSF trimerization and DNA binding (32, 149). Acquisition of full transcriptional activity appears to be correlated with hyperphosphorylation of HSF1 (144). The observation that inhibition of p38 kinase, a MAP kinase activated by hypertonic stress (10, 122, 143), reduces hypertonicity-induced HSP72 expression (100, 122) is consistent with the idea that p38 kinase-mediated hyperphosphorylation of HSF1 may be important for the induction of HSP72 in response to hypertonic stress. In nonepithelial cells, binding of HSF1 to the HSE is enhanced by both hypo- and hypertonic stress without activation of *hsp72* gene transcription (1, 18). The degree of HSF1 phosphorylation, however, was not assessed. Of note, evidence is emerging that several transcription factors may enhance or reduce HSF-mediated transcription of *hsp70* and *hsp90* genes (129).

BiP/GRP78

The endoplasmic reticulum (ER)-resident member of the 70-kDa family of HSPs is termed immunoglobulin heavy chain binding protein (BiP) or glucose-regulated protein (GRP78). BiP/GRP78 assists in the co- and posttranslational translocation of proteins into the ER and participates in the correct folding and assembly (and possibly in the orderly degradation) of proteins in the ER lumen (for review, see Refs. 13, 69). In $mIMCD_3$ cells, BiP/GRP78 expression is stimulated by exposure to cadmium or tunicamycin but not by oxidant stress or hypertonic stress exerted by elevated NaCl or mannitol concentrations (118). Because glucose starvation stimulates BiP/GRP78 accumulation, the observation that exposure of $mIMCD_3$ cells to increased concentrations of the relatively impermeant glucose reduced BiP/GRP78 expression is not unexpected. In MDCK cells or mouse kidney cells (primary culture), chemical ATP depletion or low pH induces BiP/GRP78 (62, 68). The observation that, after transient interruption of renal blood flow, BiP/GRP78 mRNA is clearly increased in the injured kidney is consistent with these in vitro results (68). Enhanced expression of ER stress proteins in MDCK cells induced by proteasome inhibitors or tunicamycin confers increased resistance to heat stress and antimycin A-induced ATP depletion (15). Conversely, suppression of BiP/GRP78 expression in LLC-PK_1 cells by antisense transfection heightens the susceptibility to alkylating toxicants (80). Hence, after renal injury, an increased abundance of ER-resident molecular chaperones may accelerate the folding and assembly of disordered and newly synthesized, nonnative proteins in the ER lumen and the degradation of severely damaged proteins and thus enhance cell recovery.

A major trigger for enhanced expression of BiP/GRP78 appears to be the accumulation of abnormally folded and misassembled proteins in the ER lumen (68). A coherent picture of the "sensor(s)" monitoring the accumulation of unfolded/misassembled ER-resident proteins and the signaling pathway relaying this information to the nuclear transcription machinery is only now beginning to emerge (25, 124, 135, 141).

THE HSP90 FAMILY

HSP90 (reviewed in Refs. 8, 17, 34, 113) is a major soluble cytosolic protein that is expressed under both normal and stressful conditions. Two mammalian isoforms (HSP90α, HSP90β), encoded by separate genes and usually forming homodimers, are known. HSP90 displays only weak ATPase and chaperoning activity, i.e., prevention of aggregation of unfolded polypeptides and assistance in their refolding. However, in cooperation with several cochaperones, one of which is HSP70 (HSC70), HSP90 makes a vital contribution to the maturation of signal-transducing proteins such as steroid hormone receptors or protein kinases. Highly orchestrated interactions between the immature, low-affinity steroid receptor and the HSC70/HSP90 system are required to shape the mature receptor characterized by high hormone affinity (37, 53). In the absence of steroid hormones, cytosolic steroid receptors are associated with HSP90 and accessory chaperone-related peptides and thus cannot bind to steroid-responsive elements. After the binding of the hormone to the high-affinity receptor, the HSP90-containing inhibitory complex dissociates. This entails activation of the receptor by the exposition of its DNA-binding site and subsequent translocation of the activated receptor, presumably along cytoskeletal tracts (40), into the nucleus and binding to steroid-responsive elements. Although HSEs are important for the transcriptional control of both the *hsp90α* and *hsp90β* genes, the transcriptional control of the two genes appears to differ and the expression of the two isoforms may be regulated in a tissue-specific manner (129, 147).

In the normal kidney, HSP90 immunoreactivity is most pronounced in the distal convoluted tubule and in the cortical and medullary collecting duct. Weak staining is found in the thick ascending limb of the loop of Henle, in podocytes, and in the parietal epithelium of Bowman's capsule. Other segments of the nephron as well as blood vessels and interstitial cells display little or no immunoreactivity at all (82). This intrarenal distribution of HSP90 is reminiscent of that of mineralo- and glucocorticoid receptors (33), thus mirroring the functional significance of HSP90/steroid hormone receptor interaction. After ischemia, HSP90 is induced rapidly and transiently in the pars recta of the proximal tubule and in the thick ascending limb of the loop of Henle. A renewed surge of HSP90 expression in these nephron segments is observed in the regenerative phase of these tubular epithelia (88). In gentamicin-induced acute renal failure, HSP90 expression is stimulated in injured proximal tubule cells and accumulates in lysosomes and nucleoli (108). In cisplatin-induced renal failure, a rapid and sustained increase in the expression of HSP90 is observed in the epithelial

cells of the loop of Henle. In the cells of the pars recta of the proximal tubule, the major site of cisplatin-mediated injury, HSP90 immunoreactivty appears in the nucleus and, particularly, in the cytoplasm, later in the regenerative phase (119). From these observations it was concluded that HSP90 may assist in the disposition of damaged proteins and synthesis of new proteins (88, 108, 119). In addition, HSP90 may be a component of the signal cascade, ultimately leading to regeneration and differentiation of the injured tubular epithelium. This idea is consistent with the observation that in the kidney HSP90 decreases dramatically during postnatal development (27).

GRP94, the ER-resident, constitutively expressed HSP90 homolog, is induced by glucose starvation, ATP depletion, and various maneuvers, causing misfolding and/or aggregation of secretory proteins (68, 80). GRP94 appears to assist in the correct folding of specific proteins in cooperation with other molecular chaperones such as BiP/GRP78, calnexin, and calreticulin and may be associated with misfolded ER proteins during ATP depletion (68, 69). Similar to BiP/GRP78, GRP94 expression is stimulated by transient ischemia in the kidney (68).

OTHER SELECTED MOLECULAR CHAPERONES

HSP47

HSP47 is a heat-inducible, ER-resident glycoprotein that interacts transiently with procollagen and has the ability to associate with collagens I-V and procollagens (97). This HSP participates in the processing and/or secretion of collagens and may prevent the secretion of procollagen with abnormal conformations. In a variety of renal diseases with glomerular sclerosis and/or tubulointerstitial fibrosis (experimental glomerulosclerosis in the remnant kidney, human diabetic and IgA nephropathies, gentamicin nephrotoxicity, unilateral ureteral obstruction, experimental glomerulonephritis), increased production of collagens is accompanied by enhanced HSP47 expression (19, 89, 116, 131, 132). This coincidence and the observation that inhibition of HSP47 expression by in vivo transfection with antisense oligonucleotides against HSP47 mRNA markedly reduces both collagen and HSP47 production in experimental glomerulonephritis, support the idea that HSP47 plays an important role in the pathogenesis of various fibrotic kidney diseases (132).

"Osmotic Stress Protein" 94

A cDNA encoding an 838-amino acid protein has been cloned from $mIMCD_3$ cells exposed to hypertonic stress (65). Osmotic stress protein 94 (Osp94) is a member of the HSP110 family, which is structurally related to the HSP70/BiP superfamily. Osp94 has a calculated molecular mass of 94 kDa and significant homology not only with HSP110 (65%) but also, albeit more weakly, with the HSP70 family (30%) (65). In the NH_2 half of the deduced protein, a putative ATP-binding site has been identified, and in the COOH-terminal half, a putative peptide-binding site (65), features that are common also to other members of the HSP family. Similar to HSP110, the expression of Osp94 mRNA in $mIMCD_3$ is significantly enhanced when the medium osmolality is elevated by using the relatively impermeant solutes NaCl, mannitol, and glucose. On the other hand, the rise in Osp94 mRNA expression is far less pronounced, or even absent, if the more permeant solutes urea or glycerol are employed instead (118). The increase in Osp94 mRNA induced by elevated NaCl concentrations is transient and reaches its maximum at ~8 h (118). Expression of Osp94 mRNA is enhanced not only by hypertonic stress but also by heat stress or cadmium. A decrease in Osp94 mRNA is seen after exposure to a low pH value or H_2O_2 (118). In the mouse kidney, the expression of Osp94 and HSP110 mRNAs parallels the corticomedullary osmotic gradient. This observation and the finding that water deprivation for 24 h causes Osp94 mRNA to increase in the inner medulla indicate that this stress protein participates in the adaptation of renal medullary cells to hypertonic stress (65).

In conclusion, in their function as molecular chaperones, HSPs play an important part in the "birth" of native, functionally competent proteins, in their intracellular trafficking and appropriate intracellular localization, and, finally, in their "death." In nonstressed cells, these vital, routine functions are served by a variety of HSPs, both constitutively expressed and highly inducible by stressors. In the kidney, a number of HSPs may fulfill specific, kidney-related functions. Participation in the maturation of steroid hormone receptors, in the contractility of mesangial cells, and in the adaptation of renal medullary cells to high, potentially damaging, extracellular NaCl and urea concentrations are examples of such functions. The observation that HSPs are induced in a variety of kidney diseases suggests that specific HSPs may have distinct functions in specific renal disease states. It can be expected that the application of modern molecular biological methods will contribute substantially to filling the manifold gaps in our knowledge and understanding of the role(s) played by this intriguing group of proteins in kidney physiology and pathophysiology.

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