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MINI-REVIEW

Heat shock protein expression and change of cytochrome c oxidase activity: presence of two phylogenic old systems to protect tissues in ischemia and reperfusion

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Abstract Induction of heat shock proteins (hsp) has been shown to protect cells from ischemia by providing transient tolerance against myocardial injury and improving postischemic functional recovery. Attenuation of ATP depletion and earlier restoration of ATP content on reperfusion are thought to play a role in this scenario. Hsp induction is accompanied by altered enzyme activity of the respiratory chain, the major generator of ATP under physiological conditions. This report addresses the question whether processing and final assembly of the active holoenzyme cytochrome c oxidase (CcO, complex IV), member of the respiratory chain, is compromised under hypoxic conditions unless protected by stress proteins. Special focus is laid on function of the enzyme's subunits and importance of cellular energy availability and maintenance.

Keywords Heat shock proteins · Cytochrome c oxidase · Myocardial ischemia · Mitochondria · ATP- dependent enzyme inhibition

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Introduction

Approximately 250 Ma ago the atmospheric gas composition changed. In fact, the increase of oxygen in the late paleozoic atmosphere promoted the evolutionary development of tetrapod locomotion and secured energy delivery in these organisms (Dudley 1998).

Energy demand varies considerably and results from factors focussed on preservation of individual life answering to often rapid changes in environmental requirements. Each feature has to be covered by cellular respiration, the elementary biochemical reaction of which is situated in the mitochondrial organelles, containing at their inner membranes a complex consisting of five enzymes. These enzymes perform a sequential directed electron transfer within the mitochondrial inner membrane to complex IV (cytochrome c -oxidase) and finally to dioxygen for water production. The Electron Transfer Chain (ETC) maintains hydrogen ion pumping from the matrix into the inner membrane space by complex I (NADH- oxidase), complex III (cytochrome c -reductase), and complex IV. The resulting difference of charge sustains a mitochondrial membrane potential, which is driving the mitochondrial ATP synthase (complex V) for energy production. Because of the varying energy requirements the *regulation of respiration is essential for life*. In case of an immediate and huge exertion a rapid delivery of ATP has to be ensured. On the other hand, a permanent and accelerated transfer of electrons to dioxygen may be harmful due to the formation of active oxygen species.

Since Ashburner discovered the appearance of heat shock protein (hsp)—expression in puff structures of chromosomes of Drosophila melanogaster upon heat treatment, heat shock or stress-proteins have been intensively studied (Ashburner and Bonner 1979). Stress

proteins are expressed by cells in response to different stress conditions and are part of the complex of responses to ensure the functional integrity of the organism. For instance, expression of stress proteins is found in chronic heart failure (Niizeki et al. 2008), inflammation (Calderwood et al. 2007), at birth (Louapre et al. 2005), metabolic syndrome (Hooper and Hooper 2009) or even in case of exercise (Melling et al. 2004 and 2007). Not in each case their precise role in cell physiology is known, but evidence has accumulated linking stress protein expression to protection from cellular injury. For example, heat pretreatment can protect cells or organisms from a consecutive, otherwise even lethal damage. Currie studied the positive effect of hyperthermic pretreatment on recovery of contractile function of isolated rat hearts after ischemia (Currie et al. 1988). Concurrent with these data a protective role of short hyperthermic pretreatment in the arrested ischemic rabbit heart with subsequent improved hemodynamic function associated with an increased expression of hsp70 was shown by Vogt et al. (2000). We showed in addition to the improvement of contractility, coronary blood flow, oxygen consumption reduced release of lactate from myocardium. On electron microscopic examination improved preservation of postischemic mitochondrial ultrastructure was found (Currie et al. 1988; Sammut et al. 2001) accompanied by improved mitochondrial respiratory and complex activity, which was associated with an upregulation of hsp32, 60 and 72 in rats (Sammut et al. 2001). Transgenic animal models have then been able to show that overexpression of stressproteins itself mimics the protective effect of heat pretreatment (Williamson et al. 2008; Jayakumar et al. 2000; Lin 2001; Sammut and Harrison 2003) thereby arguing for a direct effect of stressproteins in this scenario. The stressprotein family comprises a number of proteins, labelled according to their apparent molecular weight, which—apart from their role in cytoprotection—are renowned for their chaperone activities enabling correct protein folding, translocation of proteins through membranes, and/or targeting of proteins for correct assembly, each in its own compartment. The cytoprotective effect of stressproteins during ischemia/reperfusion seems to rely–at least in part–on increased allocation of members of the respiratory chain enzymes (hsp10, hsp60, hsp72) and protection against oxidative stress damage (hsp32) (Sammut and Harrison 2003).

Mitochondrial complex IV, also called Cytochrome c oxidase (CcO), is composed of 13 subunits and being the terminal and rate limiting enzyme of the respiratory chain is of special interest to us. The corresponding genes of the subunits are either mitochondrial- (3 subunits: I, II and III) or nuclear-coded (10 subunits: IV, Va and b, VI a,b and c, VII a,b and c and VIII) according to the *Kadenbach*-classification. Tissue specific isoforms of nuclear-coded subunits have been shown to exist probably taking into account differences in energy demand in tissues such as skeletal/heart muscle and non-muscle tissue (Grossmann and Lomax 1997; Hüttemann et al. 2001; Lee et al. 2005). CcO activity is regulated by the ATP: ADP ratio, where ATP acts as an allosteric inhibitor (Arnold and Kadenbach 1997), also known as the second mechanism of respiratory control as opposed to the first mechanism of respiratory control, which is based on the inhibition of respiration by high $\Delta\Psi_m$, the electrical part of the proton motive force Δp across the inner mitochondrial membrane (Arnold and Kadenbach 1999). The allosteric ATP-inhibition of CcO, suggested to be switched on and off by reversible phosphorylation of subunits via signaling pathways, is as yet not fully understood (Vogt et al. 2007a). In vitro experiments first postulated regulation of CcO-activity via phosphorylation of subunits, which could later be confirmed by Hüttemann and coworkers by experiments in bovine mitochondria where cAMP dependent phosphorylation of tyrosine 304 of CcO subunit I led to strong CcO inhibition (Lee et al. 2005). Allosteric ATP-inhibition of CcO can be influenced by binding of 3,5-Diiodothyronine to subunit Va of CcO (Arnold et al. 1998), oxidation of cardiolipin in the inner mitochondrial membrane and by endogenously produced NO or lipid peroxides (Brunori et al. 2004; Paradies et al. 1997; Ludwig et al. 2001), but in general is rapidly abolished under stress conditions.

Under hypoxic conditions a coordinated down-regulation of mitochondria-coded CcO subunit I and II and nuclear-coded CcO subunit IV and Vb mRNA was found associated with a decrease in mitochondrial transcription factor A. In addition, changes in the composition and activity of the enzyme complex have been observed accompanied by alterations in cellular ATP concentrations (Vijayasarathy et al. 2003). Avadhani and coworkers found that subunits I, IV and V are selectively phosphorylated after ischemia/reperfusion, leading to impaired CcO function (Fang et al. 2007; Prabu et al. 2006). These findings were confirmed by Johnson and coworkers who showed a marked decrease in subunits I and V after ischemia/reperfusion which was only partially inhibited by preconditioning (Yu et al. 2008). The myocardial genetic program is dependent on the functional status. The response of failing and healthy myocardium seems to be independent of species differences. Even in the early 1975 Sin et al. found an increase in mitochondrial protein synthesis after heat treatment and discussed what kind of proteins are produced for cytoprotection. In a ^{31}phosphorus- NMR- study, addressing this question higher myocardial HEP- content and lower pH reduction during cardiac ischemic arrest was detected when hsp were initially induced (Vogt et al. 2007b) so that adaptive changes in protein synthesis of respiratory complexes, subunit composition and enzyme activity have to be assumed. In a gene chip study of a rat heart, it was shown that a brief ischemic

episode activates a protective gene machinery including the strong up regulation of mRNA transcripts for heat shock proteins (Simkhovich et al. 2003). Especially, heat shock proteins such as hsp 70 participate in the response on short durations of reversible ischemia and reperfusion for myocardial preconditioning (Das and Maulik 2006). The involvement of stress protein -expression in case of hypoxic distress in a clinical feature e.g. severe instable angina was proven by the special increased appearance of IgG- antibodies directed against hsp 70 in the sera of patients suffering from coronary heart disease and underlines the therapeutic relevance of the chaperone induction for mitochondrial physiology. The helper function of the hsp expression in this concern could result in an increased internalization of apoproteins of CcO resulting in restoration of enzyme content and reduced enzymatic oxygen turn over for better energetic recovery in reperfusion and moderate electron flux while keeping HEP- production constant and avoiding additional ROS generation from respiratory chain complexes I, II and III.

With the focus on myocardial energy status, the influence of heat shock protein induction as chaperones upon the synthesis of the respiratory chain complexes especially the cytochrome c oxidase (CcO, complex IV) considered as a terminal effector for the electron transfer on dioxygen and mitochondrial membrane potential production is hereby especially of interest (Fig. 1). Additional hyperthermic induced chaperone activity proves itself to be tissue protective because CcO from hypoxia exposed cells exhibited altered subunit content.

CcO subunit composition and enzyme activity

The assembly of CcO is a highly differentiated process. The synthesis of active enzyme requires a coordinated production of mitochondrial and nuclear encoded subunits and additionally the insertion of five types of cofactors, including two hemes, three copper ions and one of each Zinc, Magnesium, and Sodium ions (Carr and Winge 2003).

Moreover, the transcriptional regulation of nuclear CcO structural genes is determined by oxygen concentration and glucose availability (Fontanesi et al. 2006). Additionally, the understanding of the beneficial effect of heat shock protein expression will be complicated by the variations of the CcO subunit content in different tissues and different myocardial compartments which is adaptively changed during hypoxia and regulated under the influence of thyroid hormone (Sheehan et al. 2004; Vijayasarathy et al. 1998). For instance, immunoblot analysis showed that the levels of ubiquitously expressed subunits IV and Vb are about 8–12-fold lower in liver mitochondria as compared to the heart, kidney and brain. The heart enzyme with higher abundance of CcO IV and Vb showed lower turnover number while the liver enzyme with lower abundance of these subunits exhibited a higher turnover number (Sheehan et al. 2004; Vijayasarathy et al. 1998). In case of hypoxia, a coordinated down regulation of mitochondrial-encoded COX I and II and nuclear-encoded COX IV and Vb mRNAs during hypoxia is suggested. Hypoxia also causes a severe decrease in mitochondrial transcription rates and an associated decrease in mitochondrial transcription factor A leads to a decrease in total cellular heme and ATP pools and decline in mitochondrial function.

Decreased mitochondrial heme aa3 content was associated with decreased levels of CcO subunit I, IV and Vb, though the catalytic efficiency of the enzyme (turn over rates for cytochrome c oxidase) remained nearly the same. Increased glycolytic flux and alterations in the kinetic characteristics of the CcO were suggested to be based on two mechanisms by which hypoxic cells maintain adequate ATP levels to sustain life processes. Reoxygenation almost completely reversed hypoxia-mediated changes in COX mRNA contents, rate of mitochondrial transcription, and the catalytic activity of CcO enzyme (Vijayasarathy et al. 2003). Fang et al. mapped the sites of ischemia/reperfusion-induced phosphorylation of cytochrome c oxidase (CcO) subunits in rabbit hearts by using a combination of Blue Native/Tricine gel electrophoresis and nano-LC-MS/MS approaches. Precursor ion scanning combined with neutral loss scanning found mature CcO subunit I phosphorylated at tandem Ser115/Ser116 positions, subunit IV at Thr52 and subunit Vb at Ser40, respectively. These sites are highly conserved in mammalian species. Molecular modeling suggests that phosphorylation sites of subunit I face the inter membrane space while those of subunits IV and Vb face the matrix side (Fang et al. 2007). Hypoxia and myocardial ischemia/reperfusion have an effect on the structure and function of CcO. Hypoxia and cAMP-mediated inhibition of CcO activity were accompanied by the phosphorylation of subunits I, IV, and Vb and markedly increased reactive oxygen species production by the enzyme complex. Both subunit phosphorylation and enzyme activity were effectively reversed by 50 nM H89 or 50 nM myristoylated peptide inhibitor (MPI), specific inhibitors of protein kinase A, but not by inhibitors of protein kinase C. In rabbit hearts subjected to global and focal ischemia, CcO activity was inhibited in a time-dependent manner and was accompanied by phosphorylation as in hypoxia. Additionally, CcO activity and subunit phosphorylation in the ischemic heart were nearly completely reversed by H89 or MPI, added to the perfusion medium. Hyperphosphorylation of subunits I, IV, and Vb was accompanied by reduced subunit contents of the immunoprecipitated CcO complex. Most interestingly, both inhibitors added to the perfusion medium dramatically reduced the ischemia/reperfusion injury to the myocardial

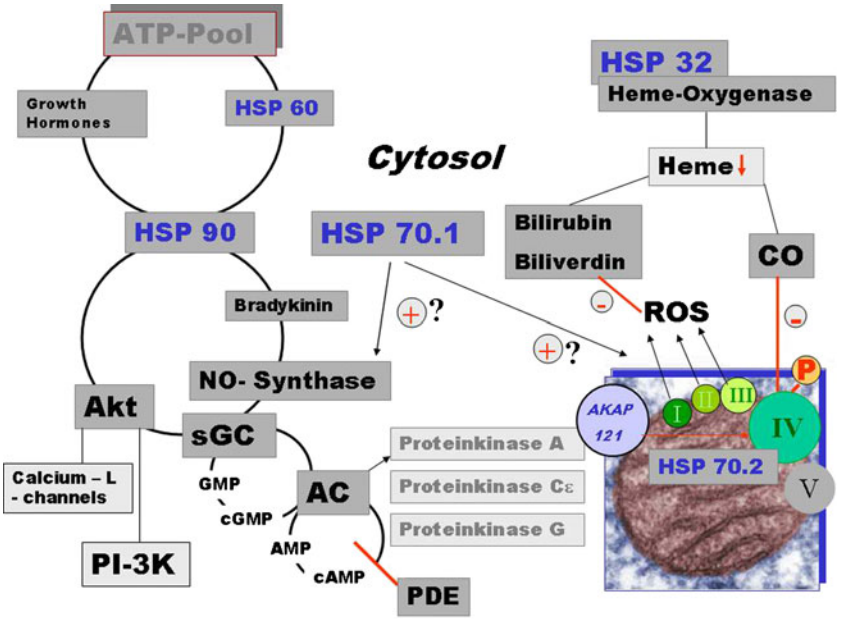

Fig. 1 Possible role of heat shock- protein interaction with respiratory complexes in case of severe trauma and cell damage: Ischemia and reperfusion result in an attenuation of protein synthesis and activation of mitochondrial enzyme complexes (Racay et al. 2009) for the increase of cellular ATP- level. *hsp 90* in combination with *Bradykinin* (released via its receptor), several hormones and *Akt* influences the activation of *NO-synthase*. Production of NO in turn itself induces relaxation of blood vessels for the increased local blood flow, and also participates in cytochrome c oxidase partial inhibition by excessive *ROS* release by mitochondrial complexes I, II and III (because of the reduced electron transmission). Hereby, NO moderates oxygen exchange between myoglobin and cytochrome c oxidase (Giulivi et al. 2006; Cooper and Giulivi 2007). Interaction of *hsp 90* and *hsp 60* results in proper conformation of newly synthesized proteins and activates *Akt* (Protein-kinase B) for the regulation of *Calcium- L- channels*, essential for ionotropic myocardial improvement, but is also involved in the phosphoinosityl-3-kinase (*PI-3 K*) signaling. The *hsp 90/NO- synthase* complex activates a soluble guanylate cyclase (*sGC*) for further increase of adenylate cyclase (AC) activity essential for *protein kinase* activation. At this stage Phosphodiesterase inhibitors' (*PDE*) action seems to be beneficial for cardiac insufficiency treatment because their action for the reduction of *cAMP* degradation and in turn higher *Protein kinase A* activation may possibly induces phosphorylation of subunit 1 of the CcO (P) by the protein kinase A anchoring protein (*AKAP 121*) which is suggested to be important for the further possible regulation of CcO activity. Increased oxygen supply to the tissue is associated with higher heme synthesis and regulated by the heme oxygenase (*hsp 32*) that breaks down the heme disk for *CO* and *Bilirubin/Biliverdin* release. Beneficial effects of this pathway could result in another partial CcO inhibition (in turn lower ROS production) and the action of Bilirubin and Biliverdin as antioxidants. *Cytosolic hsp 70.1* and *mitochondrial hsp 70.2* sustain transport of nuclear apoproteins into mitochondria for final protein translation

tissue. These results pointed out the possibility of using CcO activity modulators for controlling myocardial injury associated with ischemia and oxidative stress conditions (Prabu et al. 2006).

The composition and activity of CcO was studied in mitochondria from rat liver, brain, kidney and heart and also in different compartments of the bovine heart to see whether any correlation exists between known oxidative capacity and COX activity. In yeast, CcO subunit composition is regulated by COX5a and COX5b gene transcription in response to high and low oxygen concentrations, respectively. In mammalian cells, the expression of the COX4-1 and COX4-2 isoforms is regulated in an oxygen dependant way. When oxygen availability is reduced, hypoxia-inducible factor 1 (HIF-1) reciprocally regulates COX4 subunit expression by activating transcription of the genes encoding COX4-2 and LON, a mitochondrial protease that is required for COX4-1 degradation. The effects of manipulating COX4 subunit expression on CcO activity, ATP production, oxygen consumption, and reactive oxygen species generation indicate that the COX4 subunit switch is a homeostatic response that optimizes the efficiency of respiration at different oxygen concentrations. Thus, mammalian cells response to hypoxia by altering CcO subunit composition (Fukuda et al. 2007). The catalytic activity of mammalian CcO is regulated by binding of ATP to the N-terminus of subunit IV. This causes an allosteric inhibition of the enzyme at a high energy level and thus plays an important role in adjusting energy production to cellular energy requirements. Differences in the kinetic behaviour of CcO found in neurons and astrocytes can be addressed to a differential, but cell type-specific, expression of the CcO subunit IV-2 isoform. Besides CcO isoform IV-1, which is ubiquitously transcribed in all mammalian tissues, low levels of CcO isoform IV-2 were detected in cerebellar neurons, but not

in cortical astrocytes. Under conditions of oxygen deprivation, transcription of CcO IV-2 is induced in astrocytes and further up-regulated in cerebellar granule cells. Elevated transcription levels of the CcO IV-2 isoform are accompanied by an abolition of the allosteric inhibition of CcO by ATP. This suggests a pivotal role of CcO as an oxygen sensor on example of brain function (Horvat et al. 2006).

Heat shock protein involvement in CcO assembly

Heat shock proteins are known as molecular chaperones and regulators of the cellular homeostasis. They differ in their expression levels under normal, unstressed conditions as well as in their spatial and sub cellular distribution. Although only little is known about the extent, cooperation and time dependency of their expression, these proteins are up-regulated in response to various stressors.

Hsp 32 or heme- oxygenase is an essential component in the catabolism of heme. Induction of the stress inducible HO-1 occurs in response to heat shock, oxidative stress, thiol reacting reagents, heavy metals, inflammatory mediators and certain growth factors. The majority of the protein is localized to the endoplasmatic reticulum, but is also found at the plasma membrane and mitochondria. Products of the heme oxygenase have important physiological effects. Carbon monoxide is a potent vasodilator while biliverdin and its product bilirubin act as antioxidants. The modulation of HO-expression is useful for protection against oxidative stress and ischemia. Endogenous carbon monoxide participates in the control of respiration. It might play a role in the ventilatory adaptation to hypoxia, as low oxygen is a potent inducer of HO-1. In neural structures that express HO-1, generation of NO was often found although the regulatory interaction remains still unclear (Prabhakar 1998). Heme is involved in the transcriptional regulation of nuclear COX structural genes by oxygen concentration and by carbon source availability. In the presence of oxygen concentrations equal to or higher than 0.5 μM, COX5a is induced in a heme-dependent way by a transcriptional activator. Heme also induces the expression of the ROX1 gene though the transcriptional factor Hap1p. The product of ROX1 is a translational repressor able to bind at the promoter sequence of COX5b and represses its expression. COX6 is a nuclear aerobic gene induced in the presence of oxygen concentrations equal or higher than 1 μM in a heme-dependent way (Fontanesi et al. 2006 and 2008). Inhibition of cell respiration by endogenous CO through its interaction with CcO contributes to cell activation under hypoxic conditions (D'Amico et al. 2006). Enhancement of mitochondrial transport carriers and CcO activity was found in kidney after up-regulation of HO-1. Interestingly, increased phosphorylation of AKT and levels of BcL-XL proteins were also found so that the cytoprotective mechanisms of HO-1 against oxidative stress is based on multiple steps including increase of anti-apoptotic proteins (Di Noia et al. 2006). Hsp 32 (HO-1) induction is mediated by a redox-sensitive mechanism so that an improved antioxidant defense in cardiomyocytes when N-acetyl-L- cysteine was administered results in a lower expression of hsp 32 mRNA (Borger and Essig 1998).

Cronje et al. point out that the biochemical paradigm for CO is driven by the WARBURG- hypothesis, where CO alters oxygen- dependent functions by binding heme proteins in a competitive inverse relation to oxygen partial pressure. High oxygen partial pressure accelerates CO elimination and toxicity resolution, but with more oxygen, CO-exposed tissues exposed less oxidative stress (Cronje et al. 2004). Although it is interesting that CcO activity was found to be initially elevated after heat shock (unpublished data) but the induction of mitochondrial HO-1 was found to be associated with a decrease of mitochondrial heme content with a selective reduction in protein expression of CcO subunit I which is coded by the mitochondrial genome and synthesized in the mitochondria depending on heme availability and ultimately resulting in the decreased enzyme activity (Converso et al. 2006). On one hand, these results may confirm recent observations of a loss of subunit I in case of severe ischemia and PKC- ε inhibition in a open-chest coronary ligation model of rats (Yu et al. 2008). While on the other hand, in case of stress, ROS production may result in a selective oxidation of subunit I on Tryptophan 334. Electron transfer through the aromatic networks moves the free radicals generated at the binuclear center to the surface-exposed tryptophans where they produce hydroxytryptophan (Lemma-Gray et al. 2007). Modifications of the conformation can prevent a loss of subunit 1 as a result of reduced affinity by the used monoclonal antibodies. Moreover, conformational alterations could affect the enzymatic activity and malfunction is suspected to sustain ROS production. Repair of the enzymatic structure is performed by hsp induction.

In combination with hsp 10, the **hsp 60** binds newly synthesized polypeptides and facilitates their folding to the native state via one or more rounds of ATP hydrolysis. Moreover, these “chaporinins” have been shown to coordinate the re-folding of partially denatured proteins. Therefore, it makes coincidence with the hsp 70.2 mRNA induction located mostly in the mitochondria reasonable (Endo 1991; Pfanner et al. 1991). Additionally, there seems to be a feasible link between hsp 60- and hsp 90 mRNA. The hsp 60 and IGF- 1 receptor signaling protect cardiac muscle against injury. The abundance of cardiac IGF- 1 receptor can be upregulated by hsp 60 (Chen et al. 2005). In case of developing diabetic cardiomyopathy, hsp 60 was

found to be down regulated and leads to subsequent reduction of IGF-1 receptor signaling. To clarify the role of hsp 90 in this context, the effects of hsp 90 inhibition were measured on activation of Akt. Because of Akt, phosphorylation induced by IGF-1 or insulin was found to be decreased by facilitating phosphatase- mediated dephosphorylation of Akt, a general buffer effect of hsp 90 on the magnitude and duration of activation of proliferative and survival- promoting signaling responses is suggested (Meares et al. 2004). On example of hsp90 alpha -mRNA level, it was shown in earlier studies its up-regulation by growth factors via tyrosin kinase receptors (Jérôme et al. 1991). Although not completely studied until now, the interaction of hsp 60 and 90 could correlate because of these findings as found in our study. Most mitochondrial proteins are nuclear-encoded and synthesized as preproteins on polysomes in the cytosol. They must be translocated into mitochondria. For the transportation, the translocase of the outer membrane (TOM) and the translocation complexes of the inner membrane (TIM) mediate the preprotein import. Studies on the mechanism of mitochondrial protein import revealed the imperative function of the membrane potential across the inner membrane. Energy utilization for the transport process is performed by ATP hydrolysis. The hsp70 family is herein identified as ATPases involved in the protein import (Truscott et al. 2001). Both the inner mitochondrial membrane potential and the matrix heat shock protein 70 are essential to release the preproteins from the TOM complex, but on the other hand mitochondria employ different mechanisms for the translocation of multispanning proteins across the aqueous intermembrane space (Frazier et al. 2003).

Hsp 70 family contains multiple homologues ranging in size from 66 kDa to 78 kDa. The major stress inducible hsp 70 are the highly homologous genes hsp 1A1 and hsp A1B, also referred to as hsp 70.1 and hsp 70.2, respectively. Activation of hsp 70 is coordinated by binding of ATP at the N-terminus, causing a conformational change that opens a cap of a variable an α -helical domain, allowing interacting of the hydrophobic peptide binding domain with a number of proteins in their unfolded, misfolded or denatured state. In association with co-chaperones, hsp 70 is involved in the folding of newly synthesized, but also refolding of misfolded or denatured proteins. Moreover, the coordination of protein trafficking is performed and hereby these proteins are enabled to inhibit apoptosis. Post-exercise induction of hsp 70 mRNA involves the activation of heat shock transcription factor (HSF-1), which includes itself phosphorylation events in myocardial cells through protein kinase A (PKA) and protein kinase C (PKC) (Melling et al. 2004), events important for phosphorylation states of CcO (Hüttemann et al. 2008; Vogt et al. 2007a, b). Increased expression of the hsp 70 family is found in different mitochondrial pathophysiological scenarios like unstable angina (Valen et al. 2000), chronic heart failure (Genth-Zotz et al. 2004) or hypoxia and reperfusion (Williamson et al. 2008), where in the latter case a protection of mitochondria against damage was due to a decrease in reactive oxygen species (ROS) leading to preservation of mitochondrial complex function and ATP formation. This effect is probably related to translocation of hsp 70 to plasma membranes as induced by insulin (Li et al. 2006), inhibiting Fas- mediated apoptosis (Zhao et al. 2007) and when accumulating in the nucleus by the known classic reduction of the activity of the poly(ADP- ribose) synthetase (PARS) down to 50%, which consumes high-energy phosphates (HEP) excessively in the reoxygenated state (Kawana et al. 2000).

Clinical relevance of these data was evaluated in heart surgery suggesting that blood cardioplegia can induce an increment in the expression of hsp 70–1 confirming its protective role in ischemia/reperfusion injury (Vittorini et al. 2007). The action of the protective benefit seems to be time dependent and optimal when induced at least 2 h before surgical intervention (Schmitt et al. 2002). These interesting data correlate with preliminary findings (unpublished data) concerning an inhibition of myocardial CcO activity at this time and give an interesting clinical feature for the confirmation of the theory of the second mechanism of respiratory control (Kadenbach et al. 2009). Supporting this idea, Hampton et al. found that hsp70.1 and hsp70.3 are required for late-phase protection against infarction following ischemic preconditioning in mice (Hampton et al. 2003).

The molecular chaperones of the **hsp 90** family are major chaperones located in the cytosol and the endoplasmatic reticulum. Hsp 90 is a part of the defense network that regulates protein folding and assembly, requiring both ATP and co-chaperones (e.g. hsp 70), and participates in hormone signaling, cell growth and differentiation through binding of additional proteins like e.g. Akt. An increase in the association of hsp90 with endothelial nitric oxide synthase (eNOS) is well recognized for increasing NO production. The mechanisms by which hsp90 modulates eNOS is suggested that the tyrosine kinases, either directly or indirectly, and hsp90-dependent signaling pathways acts in concert to suppress uncoupled eNOS activity. Herein, involvement of Nostrin and Nosip factors are confirmed (Dedio et al. 2001; Ou et al. 2004; Zimmermann et al. 2002).

Energy maintenance and protein import

The cytochrome c oxidase is the terminal enzyme of the mitochondrial respiratory chain. It has the key role in

the regulation of high energy phosphate production (Kadenbach et al. 1998 and 2000).

Meanwhile it is known, that the biogenesis of the Eukaryotic proteins requires the coordinated action of two genes. More than 20 additorial nuclear- encoded factors act at all levels of the biogenetic process (Fontanesi et al. 2006). Recent studies with yeast mutants indicate that most catalytic core unassembled subunits are posttranslationally degraded in the meaning of specific processing of apoproteins. Investigations of CcO in yeast *Saccharomyces cerevisiae* confirmed the accumulation of subunits during CcO biogenesis targeting subunit I. COX1p is a mitochondrially encoded catalytic subunit which acts as a seed around until the full complex is assembled (Fontanesi et al. 2008). Therefore, the role of subunit 1 located directly inside the inner mitochondrial membrane and hardly modified from both sides seems to be pivotal for the whole enzyme's action although it will alterate its activity dependent on its phosphorylation status (Lee et al. 2005). From previous work, we know that the assembly of the 13 subunits of CcO starts with the association of subunit I and IV (Nijtmans et al. 1998). New mechanisms of CcO assembly were recovered dependent on the presence of COX1p. The biogenesis and stability of the fully assembled enzyme is sustained by the available electron carrier cytochrome c and the mitochondrial ATPase (Fontanesi et al. 2008). Moreover, the coincidence of heat shock protein expression and increased mitochondrial complex activity was already shown (Sammut et al. 2001 and 2003). It has to be assumed that specific hsp induction directly promotes gene induction for mitochondrial protein complexes and plays a role in certain steps of posttranscriptional enzymatic degradation for full function. A shift of respiration from State 4 to stage 3 but finally resulting in a relapse into preexisting ATP-dependent inhibition of CcO is myocardial protective against the ischemic impact (Kadenbach et al. 2009). The beneficial effect of hsp induction even for cardiac function has already been shown in direct animal models without (Vogt et al. 2000 and 2007b) or with using gene transfection (Jayakumar et al. 2000 and 2001). One example of hsp 70 induction is that hypoxia and reoxygenation procedures result in a redox dependent activation of STAT 1 and thus an improved defense mechanism against apoptosis. By addition of a STAT1-specific inhibitor, fludarabine, the fraction of apoptotic cells after hypoxia and reoxygenation were significantly increased. STAT1 was activated and sequential phosphorylation of Tyr701 and Ser727 was observed which could be inhibited by the antioxidant N-acetyl-L-cysteine. Tyrosine and serine phosphorylation of STAT1 was mediated by Janus kinase 2 and phosphoinositide 3-kinase/Akt kinase respectively in a redox-dependent manner following STAT1-induced hsp70 expression and the suppression of apoptosis occurred concomitantly. STAT1 activation, in a redox-dependent manner, following hypoxia and reoxygenation may play crucial roles in cell survival, at least partly via HSP70 induction (Terui et al. 2004).

Activation of transcription seems to be important for energy maintenance in tissue and mitochondrial production of high energy phosphates. Mitochondria contain their own translocation machineries of the inner and outer membrane (Tim and Tom complexes), which are involved in cardioprotection because of their role in the prevention of ischemia induced decrease of Tom 20 by ischemic preconditioning (Boengler et al. 2006; Wagner et al. 2009). Nuclear encoded precursor proteins are synthesized in the cytosol and eventually unfold with the help of cytosolic chaperones, such as Hsp70. Peptides with cleavable N-terminus presequence are recognized by the TOM complex and subsequently interact with Tom20/Tom22 (Tom70/Tom37 for non-cleavable peptides) and transfer these proteins to the protein-conducting channel of the TOM complex, Tom40. Once these precursors are translocated to the intramitochondrial space they associate with the Tim17/Tim23 complexes. Mitochondrial hsp70 and Tim44 facilitate the translocation of these precursors from the inner membrane into the matrix. It has to be pronounced that the transport across the inner membrane is dependent on the mitochondrial membrane potential and ATP hydrolysis. Inside the matrix the precursor peptides are further processed by the mitochondrial-processing peptidase which cleaves the mitochondrial targeting sequences and allows the protein to fold into its natural configuration with the help of chaperone proteins (Bowers and Ardehali 2006). Moreover, there are numerous proteins that might be generated without a presequence. In these cases, the energy needed for these proteins to pass across the outer membrane to travel through the intermembrane space and target the inner-membrane surface is suggested to be provided by conformational changes involving import components that seem to have natively unfolded structures. Hydrophobic import substrates become organized into partially assembled forms within the translocon, present targeting signals and induce conformational changes in translocase subunits (de Marcos-Lousa et al. 2006). Although the process of protein import for the mitochondrial respiratory complexes (e.g. cytochome c oxidase) are not fully understood, these mechanisms become important in case of ischemia and reperfusion because of appearing improperly folded proteins, needing refolding and degradation or a mediation of protein assembly of the import machinery. Cells have developed elaborate protein quality control systems that recognize these proteins and one such quality control system is the "unfolded protein response". Recent work indicates that in the heart, this kind of response is activated during acute stresses, including ischemia/reperfusion, as well as upon longer term stresses that lead to cardiac

hypertrophy and heart failure (Glembotski 2008). Herein, protein translocation pathways to the mitochondrial matrix and inner membrane underlie a redox regulation that determines itself protein folding. However, protein import pathways into the intermembrane space remain complicated in that different precursors use different approaches. Proteins that contain a bipartite N-terminal targeting sequence engage the Tim23 translocon and then transfer arrests. Translocation depends on the presence of a mitochondrial membrane potential ($\Delta\psi$). The presequence is proteolytically cleaved in two steps by processing peptidases in the mitochondrial matrix and intermembrane space. Representatives include cytochrome b_2, apoptosis inducing factor, and cytochrome c peroxidase. Other intermembrane space proteins that lack an N-terminal targeting sequence are imported and then fold around cofactors or acquire disulfide bonds. Import is independent of $\Delta\psi$, emphasizing that the inner membrane translocons are bypassed (Koehler and Tienson 2009). Coordination of translocator function and motor proteins in mitochondrial protein import seems to be performed by the Tim 23 and Tim 50 interaction. Tim23-Tim50 interactions also facilitate a late step of protein translocation across the inner membrane by promoting motor functions of mitochondrial hsp70 in the matrix (Tamura et al. 2009). These data leave no doubt about strong correlations between specific protein demand and subsequent protein import and the value of mitochondrial membrane potential. Beside the mentioned role of hsp 70, we have to pronounce the interaction of hsp's in cases of reparation, regeneration and adaptation. Western blot analysis already revealed constitutive expression of these Hsp90, Hsc70, Hsp60 and Hsp70 early in postnatal development as well as in the adult. Developmental profiles of these hsps, during this time of extensive cell differentiation, suggest that they are differentially regulated in postnatal development with tissue-specific differences. Interestingly, the developmental expression of subunit IV of cytochrome oxidase was similar to that of Hsp60, a protein localized predominantly to mitochondria (D'Souza and Brown 1998). Even findings in normal human skin after hyperthermia confirm, indicate and pronounce the complexity of hsp dynamics which results in tissue protection (Wilson et al. 2000).

Organisms respond to stress such as ischemia, heat and hypoxia with the synthesis of heat shock proteins. Mostly, in mammals it was shown that hearts pretreated with heat present enhanced resistence to ischemia. Because of the previous experimental works of Sammut et al. (Sammut et al. 2001), we know about the increased activities of all the respiratory chain enzyme complexes after induced heat shock. The correlations between the different species of heat shock proteins suggest an interactive network and helper function of involved chaperones in the assembly of mitochondrial proteins. Therefore, the pivotal defence mechanism of heat shock protein expression is suggested to result in higher energy phosphate delivery. Moreover, in concern of ΔG_{ATP}, the available energy that can be derived from ATP hydrolysis under given conditions suggesting a slight increase but finally reduced ΔG_{ATP} after heat shock results in alterations of hsp induction through ATP dependent action of HSF1 (Chang et al. 2001). In concern of bioenergetics, the study of both polarographic respiration of CcO and its production of HEP, e.g. ADP and ATP shows changes of the extramitochondrial [ATP]/[ADP] ratio from about 100 to 5 converting mitochondria from the resting state (state 4) to the fully active state (state 3). The importance of the adenine nucleotide translocator in this transition was already demonstrated by the influence of its specific inhibitor carboxyatractyloside (Kunz et al. 1981). After heat shock the V_{max} of ATP synthesis is accelerated (Vogt et al. 2007b) and is supposed to increase turn over rates of the metabolic enzymes. Increased $\Delta\psi$ is required for the higher activity of ATP synthase. In coupled stages, $\Delta\psi$ and the latter one is again determined by the CcO-activity so that higher rates are required (Kunz et al. 1981). To maintain ATP constant in the cell, mitochondria must sense cellular ATP utilization and transduce this demand to F0-F1-ATPase. Kushmerick et al. found that the apparent kinetic order of the transduction function of the signal cytosolic ADP concentrations is at least second order and not first order as has been assumed (Jeneson et al. 1996). The inhibition of CcO by intramitochondrial ATP is accompanied by a change of hyperbolic into sigmoidal kinetics. These results came from a study describing sigmoidal relationship between the ascorbate respiration of reconstituted cytochrome c oxidase and intraliposomal ADP concentrations. The possible role in the control of oxidative phosphorylation and cell respiration in this circumstance was noted (Arnold and Kadenbach 1999).

As far as the impact of heat shocked myocardium in the relaxing period is concerned, it seems to be feasible that increasing amounts of ATP are generated by heat stress induction through initially higher activity of mitochondrial enzyme complexes but successively followed by increased mitochondrial enzyme content after accelerated protein translation through a hsp bystander effect.

References

Arnold S, Kadenbach B (1997) Cell respiration is controlled by ATP, an allosteric inhibitor of cytochrome-c oxidase. Eur J Biochem 249:350–354

Arnold S, Goglia F, Kadenbach B (1998) 3,5-Diiodothyronine binds to subunit Va of cytochrome-c oxidase and abolishes the allosteric inhibition of respiration by ATP. Eur J Biochem 252:325–330

Arnold S, Kadenbach B (1999) The intramitochondrial ATP/ADP-ratio controls cytochrome c oxidase activity allosterically. FEBS Lett 443:105–108

Ashburner M, Bonner JJ (1979) The induction of gene activity in drosophilia by heat shock. Cell 17:241–254

Boengler K, Gres P, Cabestrero A, Ruiz-Meana M, Garcia-Dorado D, Heusch G, Schulz R (2006) Prevention of the ischemia-induced decrease in mitochondrial Tom20 content by ischemic preconditioning. J Mol Cell Cardiol 41:426–430

Borger DR, Essig DA (1998) Induction of HSP 32 gene in hypoxic cardiomyocytes is attenuated by treatment with N-acetyl-L-cysteine. Am J Physiol 274:H965–H973

Bowers M, Ardehali H (2006) TOM20 and the heartbreakers: evidence for the role of mitochondrial transport proteins in cardioprotection. J Mol Cell Cardiol 41:406–409

Brunori M, Giuffrè A, Forte E, Mastronicola D, Barone MC, Sarti P (2004) Control of cytochrome c oxidase activity by nitric oxide. Biochim Biophys Acta 1655:365–371

Calderwood SK, Mambula SS, Gray PJ Jr (2007) Extracellular heat shock proteins in cell signaling and immunity. Ann N YAcad Sci 1113:28–39

Carr HS, Winge DR (2003) Assembly of cytochrome c oxidase within the mitochondrion. Acc Chem Res 36:309–316

Chang J, Knowlton AA, Xu F, Wasser JS (2001) Activation of the heat shock response: relationship to energy metabolites. A (31)P NMR study in rat hearts. Am J Physiol Heart Circ Physiol 280: H426–H433

Chen HS, Shan YX, Yang TL, Lin HD, Chen JW, Lin SJ, Wang PH (2005) Insulin deficiency downregulated heat shock protein 60 and IGF-1 receptor signaling in diabetic myocardium. Diabetes 54:175–181

Converso DP, Taillé C, Carreras MC, Jaitovich A, Poderoso JJ, Boczkowski J (2006) HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. FASEB J 20:1236–1238

Cooper CE, Giulivi C (2007) Nitric oxide regulation of mitochondrial oxygen consumption II: molecular mechanism and tissue physiology. Am J Physiol Cell Physiol 292:C 1993–C 2003

Cronje FJ, Carraway MS, Freiberger JJ, Suliman HB, Piantadosi CA (2004) Carbon monoxide actuates O(2)-limited heme degradation in the rat brain. Free Radic Biol Med 37:1802–1812

Currie RW, Karmazyn M, Kloc M, Mailer K (1988) Heat-shock response is associated with enhanced postischemic ventricular recovery. Circ Res 63:543–549

D'Amico G, Lam F, Hagen T, Moncada S (2006) Inhibition of cellular respiration by endogenously produced carbon monoxide. J Cell Sci 119:2291–2298

Das DK, Maulik N (2006) Cardiac genomic response following preconditioning stimulus. Cardiovasc Res 70:254–263

de Marcos-Lousa C, Sideris DP, Tokatlidis K (2006) Translocation of mitochondrial inner-membrane proteins: conformation matters. Trends Biochem Sci 31:259–267

Dedio J, König P, Wohlfart P, Schroeder C, Kummer W, Müller-Esterl W (2001) NOSIP, a novel modulator of endothelial nitric oxide synthase activity. FASEB J 15:79–89

Di Noia MA, Van Driesche S, Palmieri F, Yang LM, Quan S, Goodman AI, Abraham NG (2006) Heme oxygenase-1 enhances renal mitochondrial transport carriers and cytochrome C oxidase activity in experimental diabetes. J Biol Chem 281:15687–15693

D'Souza SM, Brown IR (1998) Constitutive expression of heat shock proteins Hsp90, Hsc70, Hsp70 and Hsp60 in neural and non-neural tissues of the rat during postnatal development. Cell Stress Chaperones 3:188–199

Dudley R (1998) Atmospheric oxygen, giant Paleozoic insects and the evolution of aerial locomotor performance. J Exp Biol 201:1043–1050

Endo T (1991) Co-operative binding of hsp60 may promote transfer from hsp70 and correct folding of imported proteins in mitochondria. FEBS Lett 293:1–3

Fang JK, Prabu SK, Sepuri NB, Raza H, Anandatheerthavarada HK, Galati D, Spear J, Avadhani NG (2007) Site specific phosphorylation of cytochrome c oxidase subunits I, IVi1 and Vb in rabbit hearts subjected to ischemia/reperfusion. FEBS Lett 581:1302–1310

Fontanesi F, Soto IC, Horn D, Barrientos A (2006) Assembly of mitochondrial cytochrome c-oxidase, a complicated and highly regulated cellular process. Am J Physiol Cell Physiol 29:C1129–C1147

Fontanesi F, Soto IC, Barrientos A (2008) Cytochrome c oxidase biogenesis: new levels of regulation. IUBMB Life 60:557–568

Frazier AE, Chacinska A, Truscott KN, Guiard B, Pfanner N, Rehling P (2003) Mitochondria use different mechanisms for transport of multispanning membrane proteins through the intermembrane space. Mol Cell Biol 23:7818–7828

Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, Semenza GL (2007) HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. Cell 129:111–122

Genth-Zotz S, Bolger AP, Kalra PR, von Haehling S, Doehner W, Coats AJ, Volk HD, Anker SD (2004) Heat shock protein 70 in patients with chronic heart failure: relation to disease severity and survival. Int J Cardiol 96:397–401

Giulivi C, Kato K, Cooper CE (2006) Nitric oxide regulation of mitochondrial oxygen consumption I: cellular physiology. Am J Physiol Cell Physiol 291:C1225–C1231

Glembotski CC (2008) The role of the unfolded protein response in the heart. J Mol Cell Cardiol 44:453–459

Grossman LI, Lomax MI (1997) Nuclear genes for cytochrome c oxidase. Biochim Biophys Acta 1352:174–192

Hampton CR, Shimamoto A, Rothnie CL, Griscavage-Ennis J, Chong A, Dix DJ, Verrier ED, Pohlman TH (2003) HSP70.1 and −70.3 are required for late-phase protection induced by ischemic preconditioning of mouse hearts. Am J Physiol Heart Circ Physiol 285:H866–H874

Hooper PL, Hooper PL (2009) Inflammation, heat shock proteins, and type 2 diabetes. Cell Stress Chaperones 14:113–115

Horvat S, Beyer C, Arnold S (2006) Effect of hypoxia on the transcription pattern of subunit isoforms and the kinetics of cytochrome c oxidase in cortical astrocytes and cerebellar neurons. J Neurochem 99:937–951

Hüttemann M, Kadenbach B, Grossman LI (2001) Mammalian subunit IV isoforms of cytochrome c oxidase. Gene 267:111–123

Hüttemann M, Lee I, Pecinova A, Pecina P, Przyklenk K, Doan JW (2008) Regulation of oxidative phosphorylation, the mitochondrial membrane potential, and their role in human disease. J Bioenerg Biomembr 40:445–456

Jayakumar J, Suzuki K, Khan M, Smolenski RT, Farrell A, Latif N, Raisky O, Abunasra H, Sammut IA, Murtuza B, Amrani M, Yacoub MH (2000) Gene therapy for myocardial protection: transfection of donor hearts with heat shock protein 70 gene protects cardiac function against ischemia-reperfusion injury. Circulation 102(Suppl 3):III302–III306

Jayakumar J, Suzuki K, Sammut IA, Smolenski RT, Khan M, Latif N, Abunasra H, Murtuza B, Amrani M, Yacoub MH (2001) Heat shock protein 70 gene transfection protects mitochondrial and ventricular function against ischemia-reperfusion injury. Circulation 104(Suppl 1):I303–I307

Jeneson JA, Wiseman RW, Westerhoff HV, Kushmerick MJ (1996) The signal transduction function for oxidative phosphorylation is at least second order in ADP. J Biol Chem 271:27995–27998

Jérôme V, Léger J, Devin J, Baulieu EE, Catelli MG (1991) Growth factors acting via tyrosine kinase receptors induce HSP90 alpha gene expression. Growth Factors 4:317–327

Kadenbach B, Napiwotzki J, Frank V, Arnold S, Exner S, Hüttemann M (1998) Regulation of energy transduction and electron transfer in cytochrome c oxidase by adenine nucleotides. J Bioenerg Biomembr 30:25–33

Kadenbach B, Hüttemann M, Arnold S, Lee I, Bender E (2000) Mitochondrial energy metabolism is regulated via nuclear-coded subunits of cytochrome c oxidase. Free Radic Biol Med 29:211–221

Kadenbach B, Ramzan R, Vogt S (2009) Degenerative diseases, oxidative stress and cytochrome c oxidase function. Trends Mol Med 15:139–147

Kawana K, Miyamoto Y, Tanonaka K, Han-no Y, Yoshida H, Takahashi M, Takeo S (2000) Cytoprotective mechanism of heat shock protein 70 against hypoxia/reoxygenation injury. J Mol Cell Cardiol 32:2229–2237

Koehler CM, Tienson HL (2009) Redox regulation of protein folding in the mitochondrial intermembrane space. Biochim Biophys Acta 1793:139–145

Kunz W, Bohnensack R, Böhme G, Küster U, Letko G, Schönfeld P (1981) Relations between extramitochondrial and intramitochondrial adenine nucleotide systems. Arch Biochem Biophys 209:219–229

Lee I, Salomon AR, Ficarro S, Mathes I, Lottspeich F, Grossman LI, Hüttemann M (2005) cAMP-dependent tyrosine phosphorylation of subunit I inhibits cytochrome c oxidase activity. J Biol Chem 280:6094–6100

Lemma-Gray P, Weintraub ST, Carroll CA, Musatov A, Robinson NC (2007) Tryptophan 334 oxidation in bovine cytochrome c oxidase subunit I involves free radical migration. FEBS Lett 581:437–442

Li G, Ali IS, Currie RW (2006) Insulin induces myocardial protection and Hsp70 localization to plasma membranes in rat hearts. Am J Physiol Heart Circ Physiol 291:H1709–H1721

Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH (2001) Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochodnrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation 103:1787–1792

Louapre P, Grongnet JF, Tanguay RM, David JC (2005) Effects of hypoxia on stress proteins in the piglet heart at birth. Cell Stress Chaperones 10(1):17–23

Ludwig B, Bender E, Arnold S, Hüttemann M, Lee I, Kadenbach B (2001) Cytochrome C oxidase and the regulation of oxidative phosphorylation. Chembiochem 2:392–403

Meares GP, Zmijewska AA, Jope RS (2004) Heat shock protein-90 dampens and directs signaling stimulated by insulin-like growth factor-1 and insulin. FEBS Lett 574:181–186

Melling CW, Thorp DB, Noble EG (2004) Regulation of myocardial heat shock protein 70 gene expression following exercise. J Mol Cell Cardiol 37:847–855

Melling CW, Thorp DB, Milne KJ, Krause MP, Noble EG (2007) Exercise-mediated regulation of Hsp70 expression following aerobic exercise training. Am J Physiol Heart Circ Physiol 293: H3692–H3698

Niizeki T, Takeishi Y, Watanabe T, Nitobe J, Miyashita T, Miyamoto T, Kitahara T, Suzuki S, Sasaki T, Bilim O, Ishino M, Kubota I (2008) Relation of serum heat shock protein 60 level to severity and prognosis in chronic heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 102:606–610

Nijtmans LG, Taanman JW, Muijsers AO, Speijer D, Van den Bogert C (1998) Assembly of cytochrome-c oxidase in cultured human cells. Eur J Biochem 254:389–394

Ou J, Fontana JT, Ou Z, Jones DW, Ackerman AW, Oldham KT, Yu J, Sessa WC, Pritchard KA Jr (2004) Heat shock protein 90 and tyrosine kinase regulate eNOS NO* generation but not NO* bioactivity. Am J Physiol Heart Circ Physiol 286:H561–H569

Paradies G, Ruggiero FM, Petrosillo G, Quagliariello E (1997) Age-dependent decline in the cytochrome c oxidase activity in rat heart. mitochondria: role of cardiolipin. FEBS Lett 406:136–138

Pfanner N, Söllner T, Neupert W (1991) Mitochondrial import receptors for precursor proteins. Trends Biochem Sci 16:63–67

Prabhakar NR (1998) Endogenous carbon monoxide in control of respiration. Respir Physiol 114:57–64

Prabu SK, Anandatheerthavarada HK, Raza H, Srinivasan S, Spear JF, Avadhani NG (2006) Protein kinase A-mediated phosphorylation modulates cytochrome c oxidase function and augments hypoxia and myocardial ischemia-related injury. J Biol Chem 281:2061–2070

Racay P, Tatarkova Z, Drgova A, Kaplan P, Dobrota D (2009) Ischemia-reperfusion induces inhibition of mitochondrial protein synthesis and cytochrome c oxidase activity in rat hippocampus. Physiol Res 58:127–138

Sammut IA, Harrison JC (2003) Cardiac mitochondrial complex activity is enhanced by heat shock proteins. Clin Exp Pharmacol Physiol 30:110–115

Sammut IA, Jayakumar J, Latif N, Rothery S, Severs NJ, Smolenski RT, Bates TE, Yacoub MH (2001) Heat stress contributes to the enhancement of cardiac mitochondrial complex activity. Am J Pathol 158:1821–1831

Schmitt JP, Schunkert H, Birnbaum DE, Aebert H (2002) Kinetics of heat shock protein 70 synthesis in the human heart after cold cardioplegic arrest. Eur J Cardiothorac Surg 22:415–420

Sheehan TE, Kumar PA, Hood DA (2004) Tissue-specific regulation of cytochrome c oxidase subunit expression by thyroid hormone. Am J Physiol Endocrinol Metab 286:E968–E974

Simkhovich BZ, Marjoram P, Poizat C, Kedes L, Kloner RA (2003) Brief episode of ischemia activates protective genetic program in rat heart: a gene chip study. Cardiovasc Res 59:450–459

Sin YT (1975) Induction of puffs in drosophila salivary gland cells by mitochondrial factors. Nature 258:159–160

Tamura Y, Harada Y, Shiota T, Yamano K, Watanabe K, Yokota M, Yamamoto H, Sesaki H, Endo T (2009) Tim23-Tim50 pair coordinates functions of translocators and motor proteins in mitochondrial protein import. J Cell Biol 184:129–141

Terui K, Haga S, Enosawa S, Ohnuma N, Ozaki M (2004) Hypoxia/re-oxygenation-induced, redox-dependent activation of STAT1 (signal transducer and activator of transcription 1) confers resistance to apoptotic cell death via hsp70 induction. Biochem J 380 (Pt 1):203–209

Truscott KN, Pfanner N, Voos W (2001) Transport of proteins into mitochondria. Rev Physiol Biochem Pharmacol 143:81–136

Valen G, Hansson GK, Dumitrescu A, Vaage J (2000) Unstable angina activates myocardial heat shock protein 72, endothelial nitric oxide synthase, and transcription factors NFkappaB and AP-1. Cardiovasc Res 47:49–56

Vijayasarathy C, Biunno I, Lenka N, Yang M, Basu A, Hall IP, Avadhani NG (1998) Variations in the subunit content and catalytic activity of the cytochrome c oxidase complex from different tissues and different cardiac compartments. Biochim Biophys Acta 1371:71–82

Vijayasarathy C, Damle S, Prabu SK, Otto CM, Avadhani NG (2003) Adaptive changes in the expression of nuclear and mitochondrial encoded subunits of cytochrome c oxidase and the catalytic activity during hypoxia. Eur J Biochem 270:871–879

Vittorini S, Storti S, Andreani G, Giusti L, Murzi B, Furfori P, Baroni A, De Lucia V, Luisi VS, Clerico A (2007) Heat shock protein 70–1 gene expression in pediatric heart surgery using blood cardioplegia. Clin Chem Lab Med 45:244–248

Vogt S, Troitzsch D, Abdul-Khaliq H, Böttcher W, Lange PE, Moosdorf R (2000) Improved myocardial preservation with short hyperthermia prior to cold cardioplegic ischemia in immature rabbit hearts. Eur J Cardiothorac Surg 18:233–240

Vogt S, Rhiel A, Koch V, Kadenbach B (2007a) Regulation of oxidative phosphorylation by inhibition of its enzyme complexes via reversible phosphorylation. Curr Enzym Inhib 3:189–206

Vogt S, Troitzsch D, Abdul-Khaliq H, Moosdorf R (2007b) Heat stress attenuates ATP-depletion and pH-decrease during cardioplegic arrest. J Surg Res 139:176–181

Wagner K, Mick DU, Rehling P (2009) Protein transport machineries for precursor translocation across the inner mitochondrial membrane. Biochim Biophys Acta 1793:52–59

Williamson CL, Dabkowski ER, Dillmann WH, Hollander JM (2008) Mitochondria protection from hypoxia/reoxygenation injury with mitochondria heat shock protein 70 overexpression. Am J Physiol Heart Circ Physiol 294:H249–H256

Wilson N, McArdle A, Guerin D, Tasker H, Wareing P, Foster CS, Jackson MJ, Rhodes LE (2000) Hyperthermia to normal human skin in vivo upregulates heat shock proteins 27, 60, 72i and 90. J Cutan Pathol 27:176–182

Yu Q, Nguyen T, Ogbi M, Caldwell RW, Johnson JA (2008) Differential loss of cytochrome-c oxidase subunits in ischemia-reperfusion injury: exacerbation of COI subunit loss by PKC-epsilon inhibition. Am J Physiol Heart Circ Physiol 294:H2637–H2645

Zhao Y, Wang W, Qian L (2007) Hsp70 may protect cardiomyocytes from stress-induced injury by inhibiting Fas-mediated apoptosis. Cell Stress Chaperones 12:83–95

Zimmermann K, Opitz N, Dedio J, Renne C, Muller-Esterl W, Oess S (2002) NOSTRIN: a protein modulating nitric oxide release and subcellular distribution of endothelial nitric oxide synthase. Proc Natl Acad Sci U S A 99:17167–17172