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Hsp90 REGULATION OF MITOCHONDRIAL PROTEIN FOLDING: FROM ORGANELLE INTEGRITY TO CELLULAR HOMEOSTASIS

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Abstract

Although essential for energy production and cell fate decisions, the mechanisms that govern protein homeostasis, or proteostasis, in mitochondria are only recently beginning to emerge. Fresh experimental evidence has uncovered a role of molecular chaperones of the Heat Shock Protein-90 (Hsp90) family in overseeing the protein folding environment in mitochondria. Initially implicated in protection against cell death, there is now evidence that Hsp90-directed protein quality control in mitochondria connects to hosts of cellular homeostatic networks that become prominently exploited in human cancer.

Keywords

Apoptosis; autophagy; Hsp90; mitochondrial permeability transition; tumor progression

INTRODUCTION

Mitochondria produce cellular energy through oxidative phosphorylation in the electron transport chain [1], and orchestrate cell death/life decisions by controlling the initiation of apoptosis [2]. Both processes require multi-molecular and dynamic protein interaction complexes. Their functions depend on a highly regulated and adaptive protein quality control environment, ensuring proper folding of key effector proteins and efficient elimination of potentially toxic protein aggregates [3].

The unique anatomical features of mitochondria, with its double membrane-enclosed topology, its dependence on import of nuclear-encoded proteins from the cytosol, and the production of protein-modifying reactive oxygen species (ROS), add considerable challenges to the regulation of organelle protein homeostasis, or proteostasis. In addition, and differently from the ubiquitin-dependent degradative pathway in the cytosol, removal of aggregated or misfolded proteins in mitochondria depends on the function of selected AAA-type proteases [4]. Therefore, changes in mitochondrial integrity, defects in the protein import machinery, exposure to environmental stress, or aberrant ROS production, all have the potential to compromise the mitochondrial protein folding environment, with potentially catastrophic consequences.

Working in concert with AAA proteases [4], mitochondria utilize a network of chaperones, including Heat Shock Protein-70 (Hsp70) and the orthologs of the GroEL and GroEs

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CONFLICT OF INTEREST

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chaperones, Hsp60 and Hsp10, to continuously buffer proteotoxic stress within the organelle, and maintain flexible proteostasis to changing environments [3]. What is less clear is whether these mechanisms reach beyond the preservation of organelle integrity, and impact more general cellular functions [5]. For instance, there is clear evidence that the Hsp90-like molecule, Glucose-Regulated Protein 94 (Grp94) [6] is a critical regulator of ER proteostasis [6]. However, Grp94 has also been implicated in broader cellular processes of Ca^{2+} homeostasis, stress response, embryonic development, stem cell maintenance, host defense, and cell adhesion [7–10]. Recent studies have also expanded the list of key players overseeing mitochondrial protein folding quality control. Specifically, members of the Hsp90 family [11], including Hsp90 and its ATPase-directed homolog, Tumor Necrosis Factor Receptor-Associated Protein-1 (TRAP-1) [12] have now been localized to mitochondria, and implicated in organelle proteostasis. The structure-function properties of Hsp90s and their requirements for chaperone activity have been reviewed in detail elsewhere [13]. The nearly universal exploitation of this pathway in cancer is also known, along with the potential therapeutic opportunities offered by targeting chaperone-directed cellular adaptation [14].

In this contribution, I will review emerging data that connect Hsp90-directed protein folding in mitochondria [11] to broader signaling mechanisms of cellular homeostasis, including autophagy, inter-organelle stress response, apoptosis and compensatory induction of gene expression. The potential contribution of these processes to cancer is discussed.

Structure-function of mitochondrial Hsp90s

TRAP-1 is an Hsp90-like chaperone originally identified while screening for proteins associated with the cytoplasmic domain of the type 1 Tumor Necrosis Factor Receptor (TNFR1) [15]. In an independent screen, an Hsp90-like protein of ~75 kD that bound the Retinoblastoma (Rb) protein, and designated Hsp75, was also identified [16]. Sequence analysis later determined that TRAP-1 and Hsp75 were identical molecules.

As far as tissue distribution, a ~2.4 kb TRAP-1 mRNA was originally detected in normal tissues, but more abundant in tumor cell lines [15]. By sequence alignment, TRAP-1 is 34% identical and 60% homologous to other Hsp90 family members [15], suggesting an origin from a common ancestor [17]. A TRAP-1 mRNA of 2225 nucleotides is transcribed from a single locus on chromosome 16p13 in humans, producing at least 14 independent mRNAs with extensive nucleotide polymorphism. At least 6 TRAP-1 protein variants (TRAP-1 1–6) are predicted, characterized by different splicing patterns, amino acid additions, or deletions. Translation of a main TRAP-1 mRNA generates a precursor protein of 704 amino acids, containing a mitochondrial import sequence of 59 amino acids, which is removed upon organelle import. A mature TRAP-1 protein of 645 amino acids contains a Bergerat ATP-binding fold found in Hsp90, gyrases and MutL [18], as well as several potential sites for post-translational modifications, including acetylation and phosphorylation. Differently from Hsp90, TRAP-1 does not contain a –COOH terminus MEEVD sequence, does not bind co-chaperones p23 or Hop (p60), and cannot substitute for Hsp90 in progesterone receptor maturation, i.e. acquisition of hormone-binding state [12]. There is no available structural information for TRAP-1. However, equilibrium binding studies suggest that this molecule is arranged in a tight homodimeric conformation [19], more stable than Grp94, and exhibits an heat shock-inducible ATPase activity that binds nucleotide approximately 10 times tighter than human or yeast Hsp90 [19]. Subcellular localization studies have confirmed that TRAP-1 is indeed a mitochondrial-compartmentalized chaperone [12; 20], predominantly localized to the matrix [20], with a smaller fraction present in the intermembrane space [21].

In addition to TRAP-1, recent studies identified a pool of Hsp90 to mitochondria [11]. One distinctive feature of this localization is that both TRAP-1 and Hsp90 selectively accumulate

in mitochondria of tumor cell lines and primary and metastatic cancer, *in vivo*, whereas their expression is low to undetectable in mitochondria of normal cells and tissues [11]. The mechanism of Hsp90 localization to tumor mitochondria has not been elucidated, as sequence analysis does not reveal the presence of a cleavable mitochondrial import sequence in the molecule. There have been suggestions that mitochondrial localization of Hsp90s is a hallmark of the malignant phenotype, observed for instance in Ras-transformed but not primary fibroblasts [11], even though the molecular requirements of this process have not been elucidated. Submitochondrial localization studies have suggested that Hsp90 is also predominantly localized to the organelle matrix, and, to a lesser extent, the intermembrane space [11].

Cytoprotective role of mitochondrial chaperones

The first function assigned to mitochondrial Hsp90s, and more extensively characterized for TRAP-1, was protection against apoptosis (Figure 1). Mammalian cells utilize two main pathways to activate apoptosis, or programmed cell death: an extrinsic pathway that involves the recognition of death receptor molecules at the cell surface [22], and an intrinsic pathway characterized by acute mitochondrial dysfunction [2], or permeability transition, which culminates with rupture of the organelle outer membrane [23]. TRAP-1 was first recognized to oppose mitochondrial dysfunction induced by chemotherapeutic agents, [24], or immune effector cytokines, for instance, granzyme M [25]. This response may be regulated by post-translational modifications, as TRAP-1 phosphorylation by the PTEN-Induced Putative Kinase, or PINK1 [26] results in enhanced cytoprotection against oxidative stress-induced apoptosis [21] (Figure 1). Conversely, inactivating mutations in PINK1, such as those found in patients with certain variants of Parkinson's disease (PD), triggered aberrant apoptosis and neuronal loss, suggesting that loss of TRAP-1 phosphorylation and cytoprotection may contribute to disease pathogenesis [27]. Changes in TRAP-1 stability also may be important for this survival pathway, as binding of TRAP-1 to a mitochondrial-localized, low molecular weight isoform of the Ca^{2+} binding protein, Sorcin [28] increased the stability of the chaperone against proteolytic degradation (Figure 1), and enhanced protection against apoptosis [28].

As far as potential second messenger(s) of this anti-apoptotic pathway, production of reactive oxygen species (ROS) [25] has been consistently associated with upregulation of TRAP-1 levels in various cell types [29] (Figure 1). The underlying mechanism for this response is presently unknown, but TRAP-1-positive cells become functionally resistant to oxidative stress (H_2O_2)-induced apoptosis, and exhibit decreased expression of oxidative markers [30]. It is also possible that other cellular stress conditions may affect TRAP-1-dependent cytoprotection. Consistent with this possibility, recent proteomics data identified TRAP-1 as a *bona fide* hypoxia-regulated gene, consistently upregulated in response to low oxygen tension [31] (Figure 1). Taken together, these data suggest that TRAP-1 levels in mitochondria may be dynamically regulated, potentially in response to diverse forms of cellular stress, and functionally positioned to adaptively enhance the organelle protein folding environment, and promote cell survival (Figure 1). However, whether the regulation of TRAP-1 by cellular stress is transcriptional or post-transcriptional, or whether this mechanism participates in the differential chaperone recruitment to tumor *versus* normal mitochondria [11], has not been elucidated.

Consistent with a differential expression of TRAP-1 in tumor *versus* normal mitochondria, microarray analyses identified TRAP-1 as one of the target genes upregulated by the Myc oncogene [32]. This finding mirrors retrospective immunohistochemical studies of patient series, in which TRAP-1 levels have been found consistently elevated in primary human tumors, while expressed at very low levels, and sometimes undetectable, in matched normal tissues, *in vivo* [11]. In prostate cancer, the differential expression of TRAP-1 provided an

attractive disease marker, abundantly represented in prostatic intraepithelial neoplasia (PIN), all Gleason grade primary tumors, and metastatic disease to bone and lymph nodes, while largely undetectable in normal prostatic epithelium or benign prostatic hyperplasia [33]. The tumor-selective expression of TRAP-1 may be important for disease outcome. Gene signatures of tumor progression typically contain TRAP-1 [34], and its cell survival function has been associated with the acquisition of multi-drug resistance [35], a hallmark of advanced disease in different tumor types.

Hsp90 regulation of the mitochondrial permeability transition pore

Recent studies suggested potential mechanistic requirements for how mitochondrial Hsp90s may control organelle cell death [11]. Accordingly, both Hsp90 and TRAP-1 were found to form a physical complex with cyclophilin D (CypD) (Figure 1), a matrix peptidyl prolyl isomerase (PPI) [11], which functions as a pivotal component of the organelle permeability transition pore (PTP) [2]. There is a general consensus that opening of the PTP is a molecular prerequisite for organelle apoptosis, heralding loss of inner membrane potential, increased permeability to solutes, remodeling of the cristae, and ultimately rupture of the outer membrane with release into the cytosol of apoptogenic intermembrane space proteins, most notably, cytochrome c [2].

What is still being debated, however, is the detailed molecular arrangement of a PTP [36], as proteins long thought to be physical components of the pore, including the voltage-dependent anion channel (VDAC) [37], and the adenine nucleotide translocator (ANT) [38], turned out to be dispensable for apoptosis in gene knockout studies. There is also uncertainty as to whether a rigid PTP model best captures current evidence in cell death regulation [2]. Alternatively, a dynamic PTP model has been proposed, in which aggregated and/or misfolded proteins formed at the outer mitochondrial membrane in response to cellular stress may acquire the ability to conduct solutes, thus promoting permeability transition [39]. Notwithstanding these controversies, gene knockout experiments confirmed that CypD is involved in PTP opening, and required for at least certain forms of apoptosis, particularly those arising from oxidative stress stimuli [40; 41]. A pivotal role of CypD in orchestrating apoptosis has been also validated in animal studies, where deletion of CypD resulted in protection against cell death in various disease models [42], including neurodegeneration [43], *in vivo*. A physical interaction between Hsp90s and CypD [11] would seem ideally positioned to regulate PTP opening via a protein folding-dependent mechanism, potentially opposing oxidative stress and ROS-mediated cellular damage [21; 24; 25]. Clearly, this possibility seems to be best accommodated by a model of a “dynamic” PTP [39], where key effectors of permeability transition are misfolded and aggregated proteins during cellular oxidative stress(es). How a chaperone-CypD complex could ultimately gate mitochondrial permeability transition is not currently known, and no structural information is presently available for TRAP-1/Hsp90- CypD interaction. It is therefore premature to suggest that these complexes conform to a chaperone-client protein interaction [13]. On the other hand, acute silencing of TRAP-1 in tumor cells has been consistently associated with CypD-dependent mitochondrial apoptosis, and this response was reversed by the CypD inhibitor, Cyclosporine A (CsA) [11], reaffirming the concept that chaperone-directed protein folding may critically regulate at least certain forms of apoptosis. However, to specifically test this model, inhibitors of Hsp90 ATPase activity that could *selectively* target the mitochondria were needed.

Despite the wealth of structurally diverse small molecule Hsp90 antagonists currently in (pre)clinical development [44], recent studies demonstrated that none of these compounds had the ability to penetrate and/or accumulate in mitochondria [45]. The reason(s) behind this lack of subcellular accumulation is not known, but it may have to do with the complex, double-membrane topology of mitochondria, and its differential membrane potential. To

overcome this limitation, a new class of small molecule Hsp90 inhibitors selectively engineered to accumulate in mitochondria was recently synthesized. These compounds, designated Gamitrinibs (GA mitochondrial matrix inhibitors) have a combinatorial design, in which the ATPase inhibitory component of 17-allylamino demethoxygeldanamycin (17-AAG), a well characterized Hsp90 antagonist [44], is fused through a linker to a “mitochondriotropic” moiety, provided by either 1 to 4 tandem repeats of guanidinium or, alternatively, tryphenylphosphonium [45].

Consistent with this mitochondrial-targeting structure, Gamitrinibs readily accumulated in mitochondria of normal or tumor cells, and inhibited the ATPase activity of TRAP-1 and Hsp90 within the organelle [45]. Functionally, this was associated with sudden collapse of mitochondrial integrity, with loss of membrane potential, release of cytochrome c, and massive tumor cell death by both caspase-dependent and – independent mechanisms [45]. Biochemically, CypD became insoluble and aggregated within mitochondria of Gamitrinib-treated cells, reinforcing a role of Hsp90-directed CypD (re)folding [39] in PTP opening [2]. These results also brought back the prospect of targeting Hsp90s for cancer therapy [44]. This possibility has been aggressively pursued over the past decade [44], and despite the development of a large series of structurally diverse ATPase antagonists, these agents showed only modest activity in the clinic, further hampered by significant toxicity and limited efficacy in combination [46]. Whether the poor clinical activity reflected the inability of these agents to accumulate in mitochondria [45] is currently unknown. On the other hand, preclinical data uncovered important differences in the anticancer activity of Hsp90 inhibitors that do or do not target the mitochondrial pools of the chaperone. Specifically, and differently from non-mitochondrially targeted 17-AAG, Gamitrinib efficiently killed naïve and drug-resistant tumor cells, exhibited broad anticancer activity against genetically disparate tumor types [47], and suppressed localized and metastatic tumor growth in xenograft or genetic mouse models of cancer [47; 48]. Another attractive feature of Gamitrinib when considered as a potential anticancer agent was its selectivity of action. By targeting Hsp90 activity solely in mitochondria, Gamitrinib had no effect on chaperone-directed folding in the cytosol, leaving unscathed the stability of important client proteins, for instance Akt or Chk1, or the Heat Shock Factor (Hsf)-regulated chaperone, Hsp70 [45; 47]. Finally, as Hsp90s accumulate preferentially, if not exclusively, in mitochondria of tumor cells, Gamitrinibs exhibited a favorable safety profile, with no overt organ or systemic toxicity, *in vivo* [45; 47].

Inter-organelle signaling by mitochondrial Hsp90s

Altogether, the results obtained with Gamitrinibs [47; 48] argue that mitochondrial Hsp90s may oppose cell death by controlling CypD (re)folding in PTP (Figure 2), and, in turn, this may provide an adaptive and survival advantage for genetically heterogeneous tumors. However, is it possible that chaperone control of protein folding goes beyond the organelle’s confines to affect broader cellular responses? To begin probing this question, recent experiments used sub-optimal concentrations of Gamitrinib [45], and for shorter incubation times. The rationale behind this protocol was to perturb chaperone-directed organelle protein folding, but to a degree insufficient to trigger irreversible collapse of mitochondrial integrity and cell death. Under such conditions of non-lethal proteotoxic stress, inhibition of mitochondrial Hsp90s triggered the accumulation of insoluble and misfolded proteins, and expression of organelle stress markers [49], but without significantly decreasing cell viability.

One of the first responses induced by these conditions was a dramatic activation of autophagy in tumor cells [49] (Figure 2). Morphologically, cells treated with non-cytotoxic concentrations of Gamitrinib filled up with autophagosomes containing mitochondrial-reactive material, suggestive of mitophagy, and exhibited conversion of microtubule-

associated light chain 3 protein to a lipidated, membrane-bound form, a canonical autophagosome marker [49]. Autophagy is a process of self-digestion of subcellular organelles that aims at maintaining cellular energy levels during stress conditions [50]. Its role in cancer is complex and likely biphasic, with autophagy antagonizing the earlier phases of tumor growth, but providing an adaptive, survival advantage in more advanced stages of malignancy [51]. In the case of mitochondrial proteotoxic stress induced by targeting Hsp90s, autophagy functioned as a potent survival signal likely activated as a compensatory response. Accordingly, genetic ablation of the essential autophagy gene, *atg5* or pharmacologic inhibitors of autophagosome formation, converted non-cytotoxic concentrations of Gamitrinib into effective tumor cell killing regimens [49].

There is extensive evidence linking autophagy to the control of mitochondrial function [52], and data have also suggested that changes in the organelle protein folding environment couples to autophagy as a way to clear damaged and potentially noxious mitochondria [53]. The precise signaling mediators that link defective mitochondrial protein folding after Hsp90 targeting to activation of autophagy have not been elucidated. One attractive model taken from the mechanism of neuronal quality control may involve the role of PINK1 (Figure 1). This is a mitochondrial kinase that phosphorylates TRAP-1 in mitochondria [21], and has been implicated in promoting the recruitment of the E3 ubiquitin ligase, Parkin [54] to mitochondria that have lost differential inner membrane potential (an invariable occurrence after inhibition of mitochondrial Hsp90s [11]). In turn, a PINK1-Parkin signaling axis has been proposed to transduce an “eat me” signal in damage mitochondria, which results in the collapse of the organelle network into perinuclear clusters positive for the autophagosome marker, LC3 [55]. Whether a similar PINK1-dependent mechanism clears mitochondria with defective TRAP-1 function has not yet been investigated. As an alternative model, the activation of autophagy after disabling mitochondrial Hsp90s may involve secondary activation of endoplasmic reticulum (ER) stress [56], a condition associated with defective mitochondrial protein folding [49] (see below).

Accordingly, targeting mitochondrial Hsp90s with Gamitrinib is associated with profound changes in the gene expression profile of tumor cells [49] (Figure 2). This involves the upregulation of stress response transcription factor, CCAAT Enhancer Binding Protein (C/EBP β), and its dimerization partner C/EBP homology protein, CHOP, and increased expression of several molecular chaperones, including Hsp70 and Grp78 [49]. In particular, Grp78 (also called BiP) is a ubiquitous chaperone that functions in adaptive protein folding quality control in the ER and the plasma membrane, with the latter more likely linked to receptor-type recognitions [57]. It also has an important pro-survival function, which has been mechanistically linked to differential assembly of Bcl-2 homodimers [58]. Together with compensatory autophagy, the induction of Grp78 may further elevate the apoptotic threshold in tumor cells undergoing mitochondriotoxic stress, affording better survival. Mechanistically, these are classical hallmarks that underscore an unfolded protein response (UPR), a gene expression program that aims at reducing the organelle protein load while also promoting the correct folding of aggregated protein(s), or, conversely, their elimination [3]. The fact that Hsp90s accumulate preferentially in mitochondria of tumor cells [11] is consistent with such an adaptive model, potentially well suited to enhance the protein buffering capacity of transformed cells, which are especially at risk of proteotoxic stress for their higher biosynthetic needs.

The best characterized UPR is the one originating in the ER due to defective protein post-translational modifications [59; 60] (Figure 2). The molecular requirements of this pathway, the elucidation of three separate but intertwined UPR signaling branches, and the coupling of this response to the control of apoptosis mediated by Bcl-2 family members have been revealed in considerable detail [59; 60]. There has also been increasing evidence that

changes in the protein folding environment in mitochondria may couple to the activation of a *bona fide* UPR [3]. Accordingly, mutations in mitochondrial matrix proteins, inhibition of mitochondrial DNA transcription, or interference with the organelle protease clearing machinery have all been linked to activation of a mitochondrial UPR [61; 62]. Recent findings have suggested that mitochondrial proteotoxic stress may activate two independent UPR pathways depending on the topographic location of aberrant protein folding, whether in the matrix, which leads to increased expression of CHOP and endonuclease G [63], or in the intermembrane space, a process that results in Estrogen Receptor (ER)-dependent transcription of genes of the mitochondrial respiratory chain [64]. The molecular requirements of these processes are still being elucidated, but initial evidence points to a compensatory role of the mitochondrial protease OMI (also known as HTRA2), and the cytosolic proteasome system in reducing the load of unfolded proteins within mitochondria, and before their intra-mitochondrial accumulation, respectively [65].

In the case of mitochondrial Hsp90s, it is still unclear whether chaperone control of protein folding directly couples to nuclear gene expression, or, alternatively, whether it involves a mechanism of inter-organelle signaling with secondary activation of an ER UPR (Figure 2). There is only limited information as to how defects in the mitochondrial protein folding environment may directly signal nuclear activation of gene transcription. One model of such *direct* signaling has been worked out in *C.elegans*. Here, diffusible peptides generated in mitochondria through the proteolytic activity of the AAA protease ClpXP, promote nuclear translocation of the bZip transcription factor ZC376.7, thus activating gene expression [66]. Whether a similar mechanism occurs in mammalian cells, or in response to inhibition of mitochondrial Hsp90s, is not currently unknown (Figure 2). An alternative model of inter-organelle signaling is that the Hsp90-directed mitochondrial UPR sequentially activates a secondary ER stress response that in turn promotes gene expression [67]. The upregulation of stress markers [59], of compensatory chaperones like Grp78 [49], and the phosphorylation of the PERK substrate, eIF2 α (our unpublished observations) induced by targeting mitochondrial Hsp90s seems consistent with this model. Recent experiments also established a mechanistic crosstalk between mitochondrial and ER UPR signaling. In these studies, PKR activation in response to mitochondrial proteotoxic stress in a model of intestinal inflammation was associated with eIF2 α -dependent translational inhibition and concomitant activation of transcription of stress genes in a c-Jun-dependent mechanism [68]. Putative second messenger(s) of such a sequential pathway of mitochondria-to-ER inter-organelle signaling have not been identified. The extensive areas of physical juxtaposition between ER and mitochondria [67], populated by diverse classes of chaperones, appear ideally suited to transduce proteotoxic stress signals generated at the mitochondria-ER interface, and amplify compensatory gene expression programs [69]. Very recent data from our group demonstrated that non-lethal proteotoxic stress induced by inhibiting mitochondrial Hsp90s profoundly impaired cellular bioenergetics in disparate tumor cells [70] (Figure 2). Mechanistically, this pathway involved CypD-dependent detachment of the first enzyme of the glycolytic cascade, hexokinase II (HK-II), from the mitochondrial outer membrane, which in turn resulted in defective ATP production and acute cellular starvation characterized by phosphorylation of AMP-activated kinase (AMPK) and induction of a secondary ER UPR [70]. These data may be in line with other results regarding the ability of TRAP-1 to reverse cytotoxicity mediated by α -synuclein, a pre-synaptic protein whose aberrant over-expression has been associated with acute neurotoxicity [71]. In these experiments, TRAP-1 expression opposed α -synuclein-mediated oxidative stress, and restored ATP production via the activity of Complex I in the electron transport chain [72]. Taken together, a sudden decrease in ATP production under conditions of TRAP-1 inhibition [70] would provide a powerful stimulus to activate a secondary ER UPR, as inadequate energy to support protein post-translational modifications [73] has been previously shown to trigger a full ER UPR [74] (Figure 2).

One of the transcriptional networks shut down by Gamitrinib-induced mitochondrial UPR was Nuclear Factor κ B (NF κ B) gene expression (Figure 2) [49]. Suppression of NF κ B signaling with concomitant loss of several downstream target genes [49] was unexpected, and at variance with other models of organelle-coupled transcriptional responses. For instance, mitochondrial retrograde signaling in response to activation of mammalian target of rapamycin (mTOR) [75], or activation of ER stress [59], has been associated with *increased* NF κ B activation. Conversely, there is evidence that ER stress can suppress NF κ B activity in specific cellular contexts, for example inflammatory responses [76], and that this pathway involves C/EBP β and/or CHOP-dependent transcription [77]. This model is consistent with other data that silencing of CHOP by small interfering RNA rescued NF κ B transcriptional activity despite the presence of Gamitrinib, whereas forced expression of CHOP, alone, was sufficient to suppress NF κ B activity in these cells [49] (Figure 2). There has been intense interest in NF κ B as a survival pathway almost ubiquitously exploited in tumors, raising the possibility that targeting this cytoprotective function may enhance the efficacy of apoptosis-inducing anticancer therapies [78]. In support of this hypothesis, combining non-cytotoxic concentrations of Gamitrinib with a cell death inducer normally antagonized by NF κ B, i.e. TNF Receptor Apoptosis-Inducing Ligand (TRAIL) [79], resulted in complete killing of various glioblastoma cell types, and inhibition of intracranial glioblastoma growth in mice, with no detectable toxicity, *in vivo* [49]. These findings are reminiscent of molecular profiling studies that linked TRAP-1 expression to modulation of gene transcription [80], especially influencing proliferation-associated genes, or regulators of cell motility and metastatic spread [80].

Concluding remarks and future directions

In a relatively short span of only a few years, considerable progress has been made towards a better understanding of the function of molecular chaperones compartmentalized in subcellular organelles. Although much of the work has focused on the role of ER-localized chaperones in cellular homeostasis, recent evidence has brought into better focus the protein folding environment in mitochondria. Initially discounted as a consequence of non-physiologic stress conditions, such as deletion of mitochondrial DNA, there is now clear evidence that an intrinsic mitochondrial UPR affects broad aspects of cellular homeostasis. As briefly reviewed above, mitochondrial UPR signaling connects to general transcriptional responses of cellular adaptation, stimulation of compensatory autophagy, inter-organelle ER signaling, and bioenergetics. There is also evidence that these pathways are exploited in tumors, pointing to their importance in promoting the adaptation of malignant clones, while also presenting potential opportunities for novel molecular cancer therapeutics. Preclinical evidence collected with the mitochondrial-targeted Hsp90 inhibitors, Gamitrinibs, supports this hypothesis, and establishes the feasibility of a subcellularly-directed approach to cancer drug discovery [81], aimed at selectively disabling chaperone activity in specialized subcellular compartments.

Clearly, significant mechanistic and disease-relevant questions remain to be answered. For instance, much of the work has so far concentrated on mitochondrial-compartmentalized TRAP-1 and Hsp90 chaperones. However, could it be that other organelle chaperone(s) cooperate in similar mechanisms of mitochondrial adaptation, cell survival, autophagy or inter-organelle signaling? Preliminary evidence seems to support this possibility as the mammalian homolog of bacterial GroEL, Hsp60, has been found differentially expressed in tumor compared to normal mitochondria, and to physically associate with CypD, *in vivo* [82], in a reaction that antagonizes organelle permeability transition and apoptosis in tumor cells [11].

Another area of active investigation focuses on how mitochondrial proteotoxic stress may “communicate” with other organelles, especially ER, to potentially amplify a compensatory

and adaptive response. Preliminary data suggest that a critical second messenger in this putative axis may be decreased cellular ATP levels [70] due to defective mitochondrial bioenergetics [72]. But how is this pathway regulated? Do these data mean that mitochondrial Hsp90-directed protein folding control aerobic glycolysis, the inefficient but prevailing source of energy production in tumors [70], and this activity may also affect oxidative phosphorylation [1], perhaps in the form of Complex I function [72]? And if so, what are the molecular requirements of chaperone-dependent control of tumor cell metabolism?

And, finally, what are the broader implications of the pleiotropic signaling network apparently orchestrated by mitochondrial Hsp90s in tumors? Initial work has underscored the impact of this pathway on cell survival and adaptation. However, given the breadth of responses associated with mitochondrial Hsp90 signaling, it is plausible that other aspects of tumor cell behavior may also be affected, including those that are important for advanced disease, such as drug resistance and tumor cell invasiveness.

Given the fast pace of research in these exciting fields of investigation, the answers to some of these questions are undoubtedly forthcoming.

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Figure 1. Regulation of TRAP-1 cytoprotection and inhibition of mitochondrial apoptosis
 TRAP-1 forms a physical complex with the component of the permeability transition pore (PTP), cyclophilin D (CypD), and prevents pore opening via a protein (re)folding mechanisms. Proposed regulators of this pathway include production of reactive oxygen species (ROS) and hypoxia, which have been associated with increased expression of TRAP-1 in mitochondria, and binding of TRAP-1 to the Ca²⁺ binding-protein, Sorcin, which enhances TRAP-1 protein stability in mitochondria.

Figure 2. Impact of Hsp90 control of mitochondrial protein folding on inter-organelle signaling and general cellular homeostatic functions

Inhibition of mitochondrial Hsp90s activates apoptosis via opening of the permeability transition pore (PTP) and release of cytochrome c (Cyto c) in the cytosol. When titrated at non-lethal proteotoxic stress, this pathway results in induction of compensatory, pro-survival autophagy, stimulation of an endoplasmic reticulum (ER) unfolded protein response (UPR) and changes in gene expression with suppression of NF κ B-dependent transcription. Potential proposed regulators of these mechanisms are indicated. PQC, protein quality control.