Dissecting Selective Translation of HSP90 mRNA in Mammalian Cells

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Abstract

Mammalian Hsp90 is a ubiquitous molecular chaperone that undergoes selective translation under stress. However, the precise control mechanism of HSP90 translation is yet to be elucidated. Polysome profiling has revealed that HSP90α mRNA is selectively translated, although global translation is inhibited during heat shock. A genetic screen identified two ribosomal proteins, RPL36A and RPL42, as translation regulators of yeast HSP90. I found that knockdown of either RPL36 or RPL36A, mammalian homologs of the yeast ribosomal proteins, modulates HSP90α expression under basal and heat shock condition, suggesting that the selective translation mechanism is conserved between humans and yeast. Profile expression in rhabdomyosarcoma cell line revealed a correlation between HSP90 protein levels and RPL36/RPL36A expression, suggesting that they might be the drivers behind elevated HSP90 expression. Interestingly, a higher level of RPL36 and RPL36A rendered cells less sensitive to HSP90 inhibitor, suggesting that they may be predictors of HSP90 inhibitor resistance.

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I dedicate this thesis to my best friend and husband, Majid Afana, who expects nothing but the best from me and for me; to my mom, who taught me to always believe in myself, work hard and never let obstacles stand in the way of my goals; to my dad, who taught me that "the will to persevere is often the difference between failure and success". I am indebted to my family for their emotional support and for encouraging me to always reach for the stars and chase my dreams.

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LIST OF ABBREVIATIONS

17-AAG	17-(Allylamino)-17 demethoxygeldanamycin
Act D	Actinomycin D
AS	Amino acid sequence
aRMS	Alveolar RMS
BCA	Bicinchoninic acid assay
CDK4	Cyclin-dependent kinase 4
CDS	Coding sequence
CHX	Cyclohexamide
Ctrl	Control
cDNA	Complementary deoxyribonucleic acid
ct	Cycle threshold
DMEM	Dulbecco's modified Eagle medium
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
eRMS	Embryonal RMS
FBS	Fetal Bovine Serum
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
HS	Heat shock / 42˚C
HSMM	Primary human skeletal muscle myoblasts
IRES	Internal ribosome entry site

mRNA	Messenger ribonucleic acid
NHS	Non-heat shock / 37˚C
ORF	Open reading frame
PBS	Phosphate-Buffered Saline
PCR	Polymerase chain reaction
PMSF	Phenylmethylsulfonyl fluoride
RIPA	Radioimmunoprecipitaion Assay
RMS	Rhabdomyosarcoma
RNA	Ribonucleic acid
RNAi	RNA interference
RPL5	Ribosomal protein L5
RPL36	Ribosomal protein L36
RPL36A	Ribosomal protein L36A
RPL42	Ribosomal protein L42
RT	Reverse Transcription
qRT-PCR	Quantitative reverse transcription Polymerase chain reaction
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
S.E.M	Standard error of the mean
siRNA	small interfering RNA
siCtrl	Scrambled non-targeting small interfering RNA
siL36	small interfering RNA against RPL36
siL36A	small interfering RNA against RPL36A
uORF	Upstream open reading frame

UTR	Untranslated region
V/V%	Volume/Volume%
W/V%	Weight/Volume%

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Chapter 1: Introduction

1.1 Importance of translational regulation

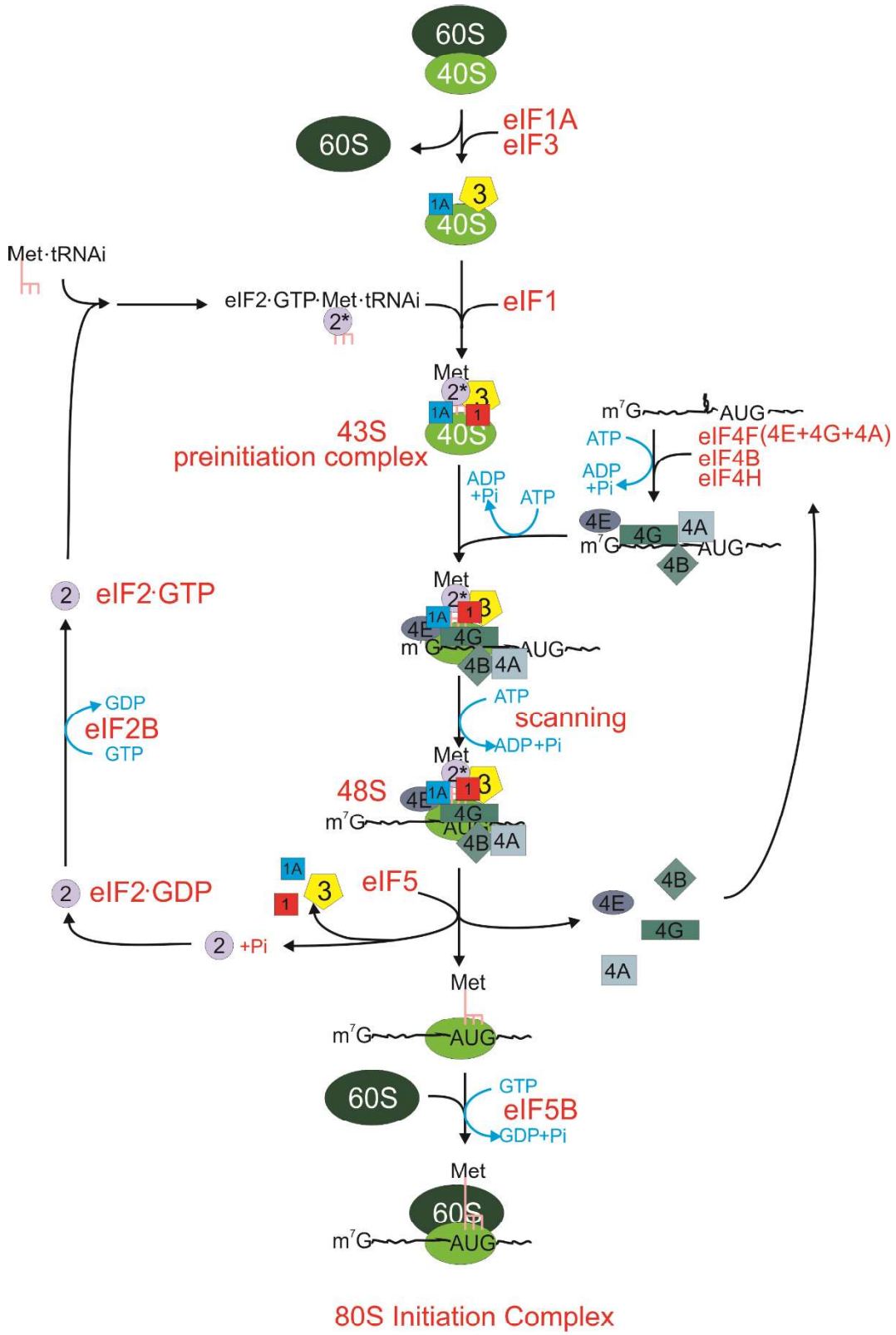
Gene expression is regulated at multiple steps, including transcription and translation. Transcriptional regulation includes modulating the production of a messenger RNA (mRNA) from DNA. mRNA processing, transport, and stability are part of post-transcriptional control that can affect the concentration and availability of mRNA. Translational regulation controls mRNA translation efficiency, allowing for a rapid control of protein expression. Although mRNA levels have been thought of as the rate-limiting step in global protein translation, studies have shown lack of correlation between mRNA and protein levels (Tebaldi et al., 2012; Schwanhausser et al., 2011; Nishizuka et al., 2003; Gygi et al., 1999;). Highly regulated protein synthesis consumes ~50% of cellular ATP (Li et al., 2014; Pace and Manahan, 2007; Princiotta et al., 2003). Therefore, it is more time and energy-efficient to regulate mRNA translation rather than deal with the aftermath of aberrant proteins. Moreover, certain cells like oocyte use translational control as their only method of protein regulation as they lack active transcription (Kronja and Orr-Weaver, 2011). Translational regulation plays a role key in cell cycle control. Hence, impaired regulation has been associated with numerous diseases (Schafer et al., 2015; Darnell, 2011; Kronja and Orr-Weaver, 2011).

There are two general modes of translation: global translation, which affects the translation of the bulk of cytoplasmic mRNAs, and mRNA specific translation, which affects a specific subset of mRNAs.

1.1.1. Global translation

Although translation is a continuous process, it can be divided into three distinct steps: initiation, elongation, and termination. Initiation is thought to be the rate limiting step of mRNA translation and is modulated by eukaryotic initiation factors (eIF) (Figure 1). The m^7G cap-structure at the 5' end and poly (A) tail regulatory sequence at the 3' end of the mRNA promote the initiation of translation, also known as cap-dependent translation (Figure 2).

Initiation involves the recruitment of the 40S ribosomal subunit in association with a ternary complex that contains an initiator tRNA (methionyl-$tRNA_i$), GTP and eIF2, in addition to eIF1, eIF1A, eIF3, and eIF5 (Figure 1). This complex is known as the 43S-initiation complex. Each eIF has a unique and distinct role in regulating translation initiation. For instance, eIF2 mediates the binding of the initiator tRNA to the 40S ribosomal subunit, whereas eIF1 and eIF1A assist in the scanning of the 5'UTR for an initiation codon. The 43S-initiation complex recognizes bound mRNA through the interaction of two scaffold proteins known as eIF3 and eIF4G. The recruitment of the 43S-initiation complex to the mRNA is mediated by eIF4E. It is an essential step in the activation of the mRNA as eIF4E's function is to recognize and physically bind to the cap structure. The scanning of the mRNA is facilitated by eIF4A, an RNA helicase, which is believed to unwind any secondary structures in the 5'UTR and is promoted by eIF4B and eIF4H. The interactions between eIF4A, eIF4E and eIF3 is mediated by the scaffold protein, eIF4G. Moreover, the simultaneous interaction of eIF4G and PABP (poly-a biding protein) allows the mRNA to be circularized, further enhancing translation. The 48S-initiation complex, (43S-initiation complex bound to mRNA) scans the 5' UTR until it identifies an initiation codon (AUG) in the appropriate context, followed by the



80S Initiation Complex

Figure 1: The mechanism of the eukaryotic translation initiation.

Cap-dependent translation is the mechanism used by vast majority of mRNAs. Initiation involves the recruitment of the 40S ribosomal subunit in association with several initiation factors (together known as 43S-initiation complex) followed by the binding and scanning of the 5' UTR until an initiation codon is identified. Following, the 60S ribosomal subunit joins the 43S-initiation complex turning into a catalytically active 80S ribosome. For more details, refer to the text. (Figure courtesy of Martin Holcik).

eIF5-catalyzed release of eIF2, eIF1, eIF1A, and eIF3 (Hershey et al., 2012; Holcik and Sonenberg, 2005). This reaction allows the 60S ribosomal subunit to join the 48S-initiation complex and become a catalytically active 80S ribosome.

Global mRNA translation can be modulated through altering the initiation factors' activity. There are two main regulatory pathways – altered phosphorylation status (reversible) and proteolytic degradation (irreversible). The first one usually includes eIF2α, eIF3, eIF4E and eIF4E binding proteins (4E-BP). The second one is usually centered around eIF4G, eIF2a, and PABP. For instance, eIF2α phosphorylation inhibits the recycling of Met-tRNA$_i$ and charging of the ternary complex. However, the phosphorylation of eIF4E decreases its binding to the cap structure increasing translation initiation, whereas its dephosphorylation leads to attenuation in global translation (Holcik, 2015; Liwak, and Holcik 2012; Pavitt and Ron 2012, Holcik and Sonenberg, 2005). The availability of eIF4E to assemble in the eIF4F complex is reduced by the competitive binding of dephosphorylated 4E-BP to eIF4E, inhibiting eIF4E interaction with the scaffold protein eIF4G. Moreover, the phosphorylation of the f subunit in eIF3 has been noted during apoptosis and is known to negatively regulate global translation. In addition to phosphorylation-mediated inhibition, the proteolytic cleavage of either eIF2α, eIF4G, or PABP by an apoptotic protein caspase-3 inhibits protein synthesis (Holcik, 2015; Liwak and Holcik, 2012; Gebauer and Hentze, 2004; Marissen et al., 2000; Bushell,et al., 1999). Although altered phosphorylation or cleavage of initiation factors leads to global inhibition of translation during stress, it may also promote the upregulation of transcript-specific translation.

1.1.2. Selective translation

A number of stresses such as DNA damage, heat shock, and hypoxia can lead to the attenuation of global translation, yet a specific subset of mRNAs whose protein products are necessary to cope with stress are effectively translated. Selective translation is regulated by unique elements that are found in the 5' and 3' UTR regions of the mRNA. This includes internal ribosomal entry site (IRES), upstream open reading frames (uORFs), and hairpins (Figure 2). The IRES element can recruit the 40S ribosome directly and bypass the binding requirement of the canonical initiation factors with the m^7G cap-structure, allowing translation to occur in a cap-independent manner (Hershey et al., 2012; Holcik and Sonenberg, 2005) (Figure 1). At least 3% of cellular mRNAs, many of which contain an IRES element, undergo cap-independent translation (Johannes at al., 1999). They frequently display inefficient translation during normal growth conditions. However, IRES-mediated translation dominates during conditions that result in global inhibition of cap-dependent translation (Hershey et al., 2012; Holcik and Sonenberg, 2005). Modification of eukaryotic initiation factors during stress may promote IRES-mediated translation. For example, during picarnovirus infection, viral proteases cleave eIF4G into two polypeptides fragments (c-terminal polypeptide and N-terminal polypeptide) and inhibit cap-dependent translation. However, cap-independent (IRES-mediated) viral mRNA translation was enhanced in this process, as the c-terminal polypeptide fragment maintained its binding activity towards eIF3 and eIF4A. Therefore, the picarnovirus hijacked the host's protein synthesis machinery by cleaving the canonical eIF4G, inhibiting the global translation of the host's mRNA, and then used the c-terminal fragment of the cleaved eIF4G to preferentially direct its IRES-mediated translation (Alvarez et al., 2003).

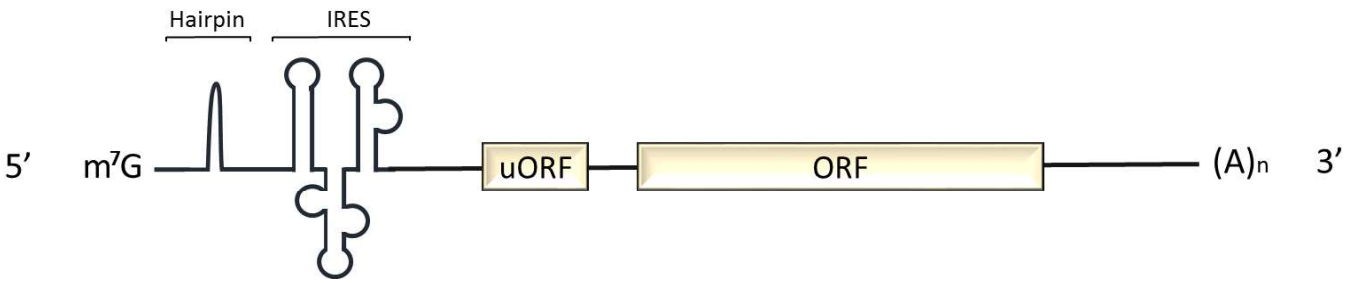

Hairpin
IRES
5’
m7G
uORF
ORF
(A)n
3’

Figure 2: Regulatory elements and sequences that can affect mRNA translation. Majority of the eukaryotic mRNAs contain a cap structure (m^7G) found at the 5' end and a poly(A) tail found at the 3' end, which promote translation initiation. Secondary structures such as hairpins, upstream open reading frame (uORFs), and internal ribosomal entry sites (IRES) affect translation efficiency. Hairpins decrease translation efficiency, whereas IRES elements increase translation efficiency. The main protein coding sequence is known as the open reading frame (ORF). An uORF is a secondary protein coding sequence found upstream of the main protein coding sequence. uORF negatively affect the translation efficiency of the main ORF.

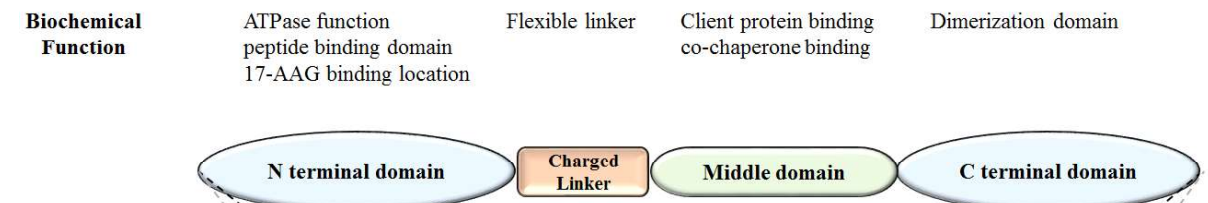
In addition to eIFs, many IRES elements require auxiliary proteins function properly. These factors, termed IRES trans-acting factors (ITAFs) are mostly RNA-binding proteins that play a pivotal role in regulating IRES-mediated translation. They function in remolding the IRES structure to either facilitate or block the binding of the ribosome to the mRNA.

1.2 Heat shock protein 90 (HSP90)

Heat shock protein 90 (HSp90) is a ubiquitously expressed molecular chaperone that accounts for 1-2% of the total cellular protein under physiological conditions, yet 4-6% under stress conditions (Finka and Goloubinoff, 2013; Tankiewicz et al., 2012; Picard, 1990). HSP90 has several important biological functions, including folding and maturation of proteins, assembling steroid hormone receptors, and kinases. It plays a key role in maintaining cellular homeostasis by modulating cell cycle factors, ubiquitin ligases, and helping in transportation and translocation of proteins (McCarthy et al., 2008; Whitesell and Lindquist, 2005; Picard, 2002; Yano et al., 1999; Zhang et al., 1999; Pratt, 1998; Bose et al, 1996). HSP90 is highly conserved and found among all kingdoms, except for archaea (Johnson, 2012; Chen et al., 2005). For example, human HSP90 and yeast HSP90 share 60% similarity in the identity of their amino acids (Johnson, 2012; Chen et al., 2005). Human HSP90 family is divided into three groups based on their cellular compartments: cytosolic HSP90, mitochondrial paralogue TRAP1, and an ER paralogue known as Grp94 (Chen et al., 2005).

As a result of a gene duplication 500 million years ago, cytosolic HSP90 has two isoforms: HSP90α (inducible under stress) and HSP90β (constitutively expressed) (Figure 3) (Chen et al, 2005; Krone et al., 1994). Despite their similarities, the two isoforms likely fulfill distinct

A


Biochemical Function
ATPase function
peptide binding domain
17-AAG binding location
Flexible linker
Client protein binding
co-chaperone binding
Dimerization domain
N terminal domain
Charged Linker
Middle domain
C terminal domain

B

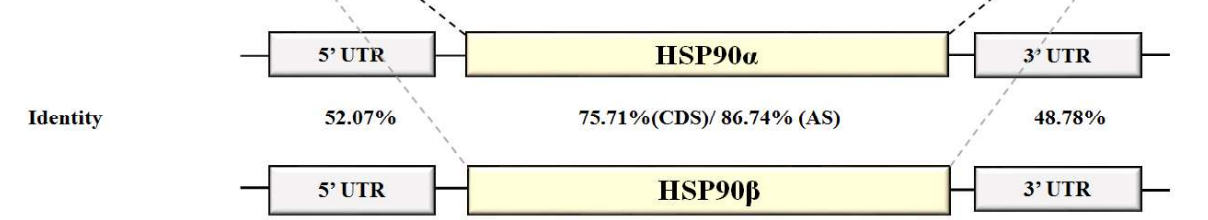

5' UTR
HSP90α
3' UTR
Identity
52.07%
75.71%(CDS)/ 86.74% (AS)
48.78%
5' UTR
HSP90β
3' UTR

Figure 3: HSP90α and HSP90β display functional similarity, yet differ in regulatory sequences of their mRNAs. (A) HSP90 protein is made of three main domains connected by a charged linker. The function of each domain is described in the figure. (B) HSP90α and HSP90β show 75.71% identity at the protein coding sequence (CDS) level and 86.74% at the amino acid sequence (AS) level. They display 52.07% identity in the 5' UTR and 48.78% identity in the 3'UTR.Clustal omega alignment program was used to calculate identity percentage.

cellular functions. HSP90α knockout mice are viable but sterile, whereas HSP90β knockout mice are embryonically lethal (Grad et al., 2010; Voss et al., 2000).

HSP90 protein is comprised of three structural domains with distinct functions: N-terminal domain (NTD), Middle domain (MD), and C-terminal domain (CTD) (Figure 3A). The function of HSP90 protein and its homologues is dependent on the NTD structure that contains an ATPase. The MD site, connected to the NTD by a charged link, is the client protein binding site for HSP90s clients or co-chaperones. The CTD site is responsible for the dimerization of HSP90 which is important for its function. Interestingly, the 5' and 3' UTRs of HSP90α share 52% and 48.7% identity, respectively, with the 5' and 3' UTR of HSP90β (Figure 3B). However, HSP90α and HSP90β share 75.7% identity at the protein coding sequence level and 86.7% at the amino acid sequence level, suggesting that these two proteins may be functionally similar, yet they are regulated under different mechanisms.

Several studies investigated the post-transcriptional regulation of HSP90 expression during stress (Silva et al, 2013; Duncan, 2008; Ahmed and Duncan, 2004). In yeast and *Drosophila*, the analysis of mRNAs associated with polysomes of stressed cells revealed an increase in translation efficiency of the inducible HSP90 under stress, concomitantly with a decrease in global translation of mRNAs (Silva et al, 2013; Duncan, 2008; Ahmed and Duncan, 2004). Although cap-dependent translation was inhibited, HSP90 mRNA was translated through a mRNA-selective mechanism which led to an increase in HSP90 protein levels with no correlating mRNA levels. It was postulated that this mechanism is mediated through an IRES element found in HSP90 5'UTR. Moreover, in cells lacking eIF4E and eIF4G, which are deemed necessary for cap-dependent translation, HSP90 mRNA was shown to be continually translated (Joshi-Barve et al., 1992). These experiments suggest that HSP90 mRNA has the

ability to recruit the ribosome to its mRNA through an IRES-mediated mechanism that may not necessarily be dependent on the availability eukaryotic initiation factors (Joshi-Barve et al., 1992). Although selective translation has been implicated to be part of HSP90's induction, the precise mechanism is yet to be fully elucidated.

Interestingly, an elevated expression of HSP90 has been noted in a number of aggressive tumours (Yano et al., 1999; Beliakoff and Whitesell, 2004; Pick et al., 2007; Wang et al., 2013; Lesko et al., 2007; Workman and Powers, 2007). Since HSP90 is a chaperone to many known oncogenes, *e.g* human epidermal growth factor receptor (Her2) and tumour suppressor protein 53 (P53), and cell cycle regulators (*e.g* cyclin-dependent kinase 4/6 (CDK4/CDK6)), its deregulation can lead to detrimental effects on the fate of the cell (Sharma et al., 2012).

1.3 Roles of ribosomes in translation

The ribosome is a highly conserved translation machinery that is responsible for protein synthesis. The human ribosome (80S) is composed of a small ribosomal subunit 40S and a large ribosomal subunit 60S. In mammalian cells, ribosomes consist of 4 rRNAs and 80 ribosomal proteins. 5S, 5.8S, and 28S rRNA and 47 proteins are found in the 60S ribosomal subunit, whereas 18S rRNA and 33 proteins are found in the 40S ribosomal subunit (Anger et al., 2013; Klinge et al., 2012; Ben-Shem et al., 2011; Khatter et al., 2015). Although ribosomes have been proposed to possess a fixed stoichiometry, different tissue types and the physiological conditions may lead to heterogeneous ribosomal proteins composition in the ribosome (Slavov et al., 2015; Reschke et al., 2013; Xue and Barna, 2012). For instance, developing tissues such as the face, eyes and neural tube have shown an enrichment in RPL38 expression (Kondrashov et al., 2011). However, RPL38 is not generally expressed in all adult tissues, suggesting that the translating polysome found during development is made of

different ribosomal composition in comparison to the translating polysome in mature tissues. (Kondrashov et al., 2011; Sahin et al., 2005). A quantitative gene-expression profile screen of 72 ribosomal proteins across various tissues and cell lines presented an evidence of substantial heterogeneity in these ribosomal proteins across different tissues/cells (Kondrashov et al., 2011; Xue and Barna, 2012; Bortoluzzi et al., 2001). This strongly suggests that there is tissue/cell-specific expression of ribosomal proteins and it is distinctively regulated. Furthermore, the amount of the ribosomes present on a given mRNA allows us to infer the rate of translation; higher ratios of ribosomes suggest a higher translation rate (Slavov et al., 2015). Thus, in an induced growth condition, more ribosomal proteins are needed for the assembly of the additional ribosomes to support the higher rate of translation.

More research is starting to focus on the additional function of ribosomal proteins within the ribosome. The ribosome filter hypothesis postulated that a ribosome can preferentially alter the translation of a subset of mRNAs (Mauro and Edelman, 2002). In fact, some ribosomal proteins have already been proven to play a key role in the regulation of selective mRNA translation, as a part of the ribosome. For example, the mitochondrial ribosomal protein L18 (MRPL18) plays a role in selectively translating the heat shock protein 70 (HSP70) mRNA under heat shock. The presence of the cytosolic form of MRPL18 alters the 80S ribosome affinity towards the 5'UTR of HSP70 mRNA, allowing its selective translation under heat shock (Zhang et al. 2014). In a different example, the vesicular stomatis virus (VSV) mRNA contains a long (~750-nt) highly structured 5'UTR that undergoes cap-dependent translation. Lowered levels of RPL40, but not other ribosomal proteins, lead to the inhibition of VSV infection by ~90% (Lee et al., 2013). The transcription of VSV mRNA was not affected; however, the polysome association with VSV mRNA and protein levels were largely reduced,

suggesting that the loss of VSV translation was due to the absence of RPL40 in the 80S ribosome. It's a unique example of a ribosomal protein allowing selective translation of an mRNA through cap-dependent translation.

Ribosomal proteins that have functional roles outside the ribosome are referred to as extra-ribosomal proteins and they can play an important in regulating translation as free ribosomes. RPL13a harbours an extra-ribosomal function that allows it to modulate ceruluplasmin mRNA expression. Exposure of cells to interferon γ for 24 hours mediates RPL13a phosphorylation, leading to its release from the 60S ribosomal subunit. The recruitment of free phosphorylated RPL13a as part of the interferon γ-activated inhibitor of translation (GAIT) complex to the 3'UTR of ceruluplasmin mRNA results in the silencing of ceruluplasmin translation. The recruitment of the 43S ribosome complex to the ceruloplasmin mRNA step was blocked by the phosphorylated extra-ribosomal RPL13A (Mazumder, 2003).

1.3.1. Ribosomes and diseases

Emerging research is now linking ribosomal abnormalities to developmental diseases and cancer (Xue and Barna, 2012; Song et al., 2011; Kim et al., 2004). A genetic screen in 2011 identified Rpl38 as a deleted gene in mice who have deformations in their axial skeleton formation. Further studies confirmed that Rpl38 controls the expression of Hox mRNAs that are necessary for proper development (Song et al., 2011; Kondrashov et al., 2011). Mutations in the ribosomal genes, that code for the 40S subunit (RPS7, RPS17, RPS10, RPS24, and RPS24) and 60S subunit (RPL5, RPL11, and RPL35A), lead to a bone marrow disorder known as diamond-blackfan aneaemia (DBA) (Boria et al., 2010). People who suffer from DBA have physical abnormalities and are more susceptible to other diseases. Studies have also shown that cancerous tissues and cells display an increased expression of specific ribosomal proteins

(Kondrashov et al., 2011; Song et al., 2011; Sahin et al., 2005; Kim et al., 2004; Bortoluzzi et al., 2001). For example, RPL39 is overexpressed in breast cancer cells, RPS11 and RPL7 are overexpressed in colorectal cancer, whereas RPL19 is over expressed in prostate cancer (Dave et al., 2014; Bee et al., 2006; Kasai et al., 2003). A tissue microarray identified high expression of RPL36 and RP36A in hepatocellular tumours, but did not detect them in healthy non-tumour tissues (Song et al., 2011; Kim et al., 2004). A vast number of these ribosomal proteins are currently being used as prognostic markers for aggressive and malignant cancers.

1.4 *Rationale and hypothesis*

Using polysome profiling in yeast, our collaborators have identified hsp/hsc82 (yeast HSP90 homologues) as two yeast genes that are up-regulated at the level of protein synthesis in response to stress while global translation was impaired, suggesting a selective translation mechanism (Silva et al., 2013). A follow-up genetic screen performed by Dr. Golshani's laboratory at Carleton University has identified several yeast genes potentially involved in the translational control of hsp/hsc82 in response to stress. Two of these candidate genes, namely RPL36A and RPL42, have homologs in mammalian cells, also known as RPL36 and RPL36A. When RPL36A and RPL42 are deleted in the yeast model, HSP90's ability to be induced is blunted. These findings suggest that yeast HSP90 is selectively translated under stress, and that the selective translation is controlled by several yeast genes including RPL36A and RPL42. I therefore ***hypothesize that ''RPL36 and RPL36A regulate the selective translation of mammalian HSP90 in response to cellular stress***." My goal was to validate that mammalian HSP90 is regulated at the level of translation and that altering levels of RPL36 and RPL36A will affect HSP90's ability to be induced by heat shock stress. Furthermore, physiological relevance of HSP90 is explored in rhabdomyosarcoma model.

Chapter 2: Materials and Methods

2.1 Cell Culture and Transfection

HeLa cells were maintained at 37˚C in 5% CO_2 in complete DMEM (DMEM supplemented with 10% Fetal Bovine Serum, 1% glutamine, 100,000 U/L penicillin and 100 g/L streptomycin; HyClone). Human Rhabdomyosarcoma (RMS) cell lines (RH18, RH30, RH36, RD, and RH41) were a gift from Dr. P. Houghton (Department of Hematology-Oncology, St. Jude Children's Research Hospital, Memphis, TN) and were cultured in RMPI 1640 media. The human RMS cell line Kym-1 was purchased from the JCRB, Japan, and were cultured in DMEM-F12 media. Primary human skeletal muscle myoblasts (HSMM) were a gift from Dr. Kyle Cowan (CHEO, Ottawa).

For transient siRNA transfections, Lipofectamine RNAiMax reagent (Invitrogen) was used. Double stranded siRNA targeting RPL36 (Sense: 5’ AAGCUGCUGCCAAGAAAGAtt 3’, Antisense: 5’ UCUUUCUUGGCAGCAGCUUtt 3’; (Santa-Cruz, sc-97541) and RPL36A (Sense: 5’ CAGUUCUAAGUGUCAUCUUtt 3’. Antisense: AAGAUGACACUUAGAA CUGtt ; Santa-Cruz, sc-91135) were purchased from Santa Cruz and non-targeting control siRNA (Sense: 5’UUC UCC GAA CGU GUC ACG U 3’. Anti-sense: 5’ACG UGA CAC GUU CGG AGA 3’; Qiagen, cat #102720) was used as a negative control. HeLa cells were transfected for 48 or 72 hours in 6-well plates with either 5 nM of siRPL36A or 15 nM of RPL36 siRNA, respectively. RD cells were transfected for 48 hours with either 15 nM of siRPL36A or 20 nM of RPL36 siRNA. Kym-2 cells were transfected for 48 hours with either 40 nM of siRPL36A or 40 nM of RPL36 siRNA.

For heat shock experiments in HeLa cells, 48 to 72 hours after siRNA transfection, the media was removed from the wells. The wells were then replenished with complete DMEM media

without antibiotics which was heated to either 37˚C or 42˚C and then put in an incubator at 37˚C (non-heat shocked cells, Control) or 42˚C (heat shocked cells, Treated). After 45 minutes, the media was aspirated from the wells and each well was rinsed with phosphate-buffered saline (PBS). The cells were harvested using RIPA Buffer.

2.2 RNA Extraction and PCR

Total RNA extraction and purification was performed using RNAzol® RT (Sigma Aldrich) as follows: Media was aspirated from the wells and then 1ml of RNAzol® RT was used to cover one well in a 6-well plate. Following, 0.4 ml of RNAse-free water was added per 1 ml RNAzol® RT used for homogenization. The samples were then shaken vigorously for 15 second and then allowed to stand at RT for 15 minutes. After the incubation, the samples were centrifuged at 12,000 x g for 15 minutes at 4˚C and then the supernatant was transferred to a new tube. The mix was allowed to incubate at RT for 10 minutes after the addition of equal amount of 100% isopropanol to the supernatant to precipitate the RNA. After the incubation, the mix was centrifuged at 12,000 x g for 10 minutes at RT. Supernatant was discarded and the pellet was washed with 0.6 ml of 75% ethanol and then centrifuged at 6,000 x g for 3 minutes. The previous step was repeated twice. Ethanol was then aspirated and the RNA pellet was solubilized, without drying, in RNAse-free water. Solubilized RNA was vortexed for 5 minutes at RT and then RNA readings were taken using the Nano-drop1000. Reverse transcription (RT) was performed using qScript™ cDNA SuperMix (Quanta Bioscience) and the following reaction mix was prepared for a final volume of 20µL: 4 µL of qScript™ cDNA SuperMix (5x), 1µg of RNA template and then add H_2O up to a final volume of 20µL. cDNA reaction mix was then incubated in the PCR machine using following

conditions: 5 minutes at 25˚C, 30 minutes at 42˚C, and 5 minute at 85˚C. 1µl of cDNA product was used to perform quantitative real time (qRT-) PCR using 10 µL of SYBR green Master Mix (Qiagen), 2µL of primers, 7µl of H_2O, and 1µL of cDNA. Quantitect primers RPL36, RPL36A, HSPA90AA, HSP90AB, Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and β-Actin were used for endogenous mRNA quantification. qRT-PCR was performed using the Eppendorf qPCR machine and its associated software. Cycling conditions: 95˚C for 15 min, ˚C for min, 40 cycles. Melting curve was also obtained.

mRNA Target	Catalogue Number	Company
HSP90α	QT01848273	Qiagen (QuantiTect primers)
HSP90β	QT01002624	Qiagen (QuantiTect primers)
RPL36	QT00219779	Qiagen (QuantiTect primers)
RPL36A	QT01668030	Qiagen (QuantiTect primers)
GAPDH	QT00079247	Qiagen (QuantiTect primers)
β-Actin	123172H11 123172H12	Invitrogen

Table 1: List of qRT-PCR primers used in this study

2.3 Western Blotting

Cells were harvested in radioimmunoprecipitation assay RIPA buffer (50mM Tris-CL pH 7.4, 1mM EDTA, 150 mM NaCl, 1%np40, 0.5% Deoxycholic acid, 0.05% SDS) with the addition of protease inhibitors (1µM PMSF, 1µM Leupeptin, 1µM Aprotonin, 1µM Pepstatin) and then kept on ice for 30 minute, followed by centrifugation at 14,000 rpm in a 4˚C bench top centrifuge. The supernatant was then collected, and bichoninic acid assay (BCA, Thermofisher) was used to determine protein concentration.

Equal protein amounts (10-20 µg) were diluted in Laemlli Buffer supplemented with 5% (v/v) Dithiothreitol (DTT) (Bio-Rad) and resolved on 10% SDS-PAGE, transferred to PVDF membrane using wet transfer (1 hr at 100V). The levels of the following proteins were investigated: HSP90 (Anti-mouse, 1:2500 in 1% milk PBST, Calbiochem-386040), RPL36 (Anti-rabbit, 1:500 in 1% milk TBST, Abcam- ab138032), , RPL36A (Anti-mouse, 1:700 in 1% milk PBST, Santa Cruz- sc-100831), RPL5 (Anti-rabbit, 1:1000 in 1% milk PBST, Abcam-ab74744), CDK4 (Anti-mouse, 1:5000 in 1% milk PBST, Cell Signalling- #2906), β-Actin (Anti-mouse, 1:10,000 in 1%milk PBST, Abcam- ab6276), α-Tubulin (Anti-mouse, 1:10,000 in 1% milk PBST, Abcam- ab7291). Secondary antibodies used were α-mouse (Cell Signalling) and α-rabbit (Bio-rad) and were developed using ECL (GE Healthcare), or West PICO substrate (Pierce) to visualize protein on film (GE Healthcare). Alternatively, Alexa 680-, or Alexa 780-conjugated (LI-COR Biosciences) secondary antibodies were used followed by detection using LI-COR Odyssey infrared scanner (LI-COR Biosciences). Densometry analyses were performed using the LI-COR Odyssey software.

2.4 Polsyome Profiling

HeLa cells from three to four 10cm plates per condition were plated for 24 hours prior to harvesting. Following day, cells were incubated with 0.1 mg/ml CHX for 3 minutes, washed with cold CHX-PBS and then collected in tubes to be pelleted by centrifugation at 300 xg. The supernatant was discarded and the pellet was lysed in polysome lysis buffer (100 mM KCL, 5 mM $MgCl_2$, 10 mM HEPES pH 7.4, 2% Triton X-100, 140U/ml, 0.1 mg/ml Cyclohexamide (CHX) for 15 min. Supernatant was spun at 12,000rpm to remove cellular debris. Equal OD254 units were loaded on top of a 15-45% sucrose gradient column. The columns were centrifuged for 90 minutes at 38,000 rpm at 4˚C in SW41Ti rotor in Optima L-100 Xp Ultracentrifuge (Beckman Coulter). Gradients were fractioned into 1 ml/fractions using an ISCO gradient fractionation system (Teledyne ISCO Inc) and RNA/protein was monitored at 254 nm. Fractions were flash frozen in liquid nitrogen and stored at -80˚C. Total RNA was isolated from individual fractions by TRIzol LS® (Roche) according to the following protocol: A ratio of 1:3 was maintained between the volume of sucrose fraction used and the TRIzol LS®reagent added. Samples were homogenized by pipetting and down several time after the addition of TRIzol LS® and then incubated at RT for 5 minutes. O.2 mL of Chloroform was added per 0.75 mL of TRIzol LS®reagent used for homogenization. Mixture was then shaken vigorously for 15 seconds, incubated for 15 minutes at RT, and then centrifuged at 12,000 x g for 15 minutes at 4˚C. Aqueous phase of sample was placed into a new tube along with equal volume of 100% isopropanol. After 10 minute RT incubation, the mixture was centrifuged at 12,000 x g for 10 minutes at 4˚C. Supernatant was removed and the remaining RNA pellet was washed with 1 mL of 75% ethanol and vortexed. Sample was then centrifuged at 7,500 x g for 5 minutes at 4˚C and the supernatant was discarded. The RNA pellet was allowed to partially air dry, then was re-suspended in RNase-free water and incubated in a heat block at 55˚C for

15 minutes. Equal volumes of RNA were used in cDNA synthesis. PCR and RT-PCR were performed as described in RNA Extraction and PCR.

2.5 Cell cytotoxicity and YOYO-1 activity assay

For cyctotoxicity assays, $1x10^4$ RD cells and $8x\ 10^3$ Kym-1 cells were seeded a day before the treatment. The day of the treatment, cells were treated with increasing concentrations of 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG) (Sigma Aldrich) or 0.1% DMSO and were incubated with 100nM of YOYO-1 dye (Molecular probes). Uptake of the YOYO-1 fluorescent dye was monitored over 48 hour using the INCUCYTETM ZOOM Live-Cell Imaging System (Essen Bioscience). Cell death activity, normalized yoyo-1 positive cells, was calculated as the number of green fluorescent cells divided by the confluence. Fold cytotoxicity was calculated as Log 2 of normalized yoyo-1 positive cells.

2.6 Statistical Analysis

Data are expressed as a mean +/- standard error of the mean (s.e.m). Unless otherwise stated, all results were obtained through a minimum of three independent experimental replications. *t*-tests and one way-ANOVA were used to determine data significance using GraphPad Prism version 5.00 for Windows.

Chapter 3: Results

3.1 HSP90 mRNA is selectively translated under heat shock condition.

The heat shock protein 90, HSP90, has been extensively studied as it regulates numerous cellular processes (Mayer and Le Breton, 2015; Silva et al., 2013; Kozeko, 2010; Csermely et al., 1998; Bose et al., 1996). The HSP90 mRNA has a unique secondary structure in its 5' UTR allowing it to undergo selective translation under stress (Silva et al., 2013; Ahmed and Duncan, 2004). The precise molecular mechanism responsible for HSP90 upregulation during heat shock is not fully understood.

Western blotting technique was used to analyse the expression of HSP90 protein in HeLa cells under basal temperature (37˚) (non-heat shock, NHS), and under heat shock (HS) temperature (42˚) for 45 minutes (Figure 4A). It was found that there was ~3.5 fold increase in HSP90 protein under HS. In attempt to dissect whether the upregulation of HSP90 protein expression is due to enhanced transcription, I investigated the levels of HSP90α mRNA, an inducible isoform of HSP90, and HSP90β, a constitutive isoform of HSP90, under basal and heat shock temperatures (Figure 4B). It was found that there were no significant changes in HSP90α and HSP90β mRNA levels at HS in comparison to NHS. To investigate the role of translation in HSP90 induction, I examined the association of HSP90 mRNA with the translating ribosomes in heat shocked cells by polysome profiling (Faye et al., 2014). Following the incubation of HeLa cells in either basal or heat shock conditions, polysomes were prepared by sucrose gradient centrifugation and analysed. Polysome profiles of heat shocked HeLa cells show an increase in the abundance of monosomes and a collapse in their polysome profile, indicative of inhibition of global protein synthesis (Figure 4C). To examine the association of HSP90 with translating ribosomes, polysome distribution of HSP90α, HSP90β, Actin, and GAPDH

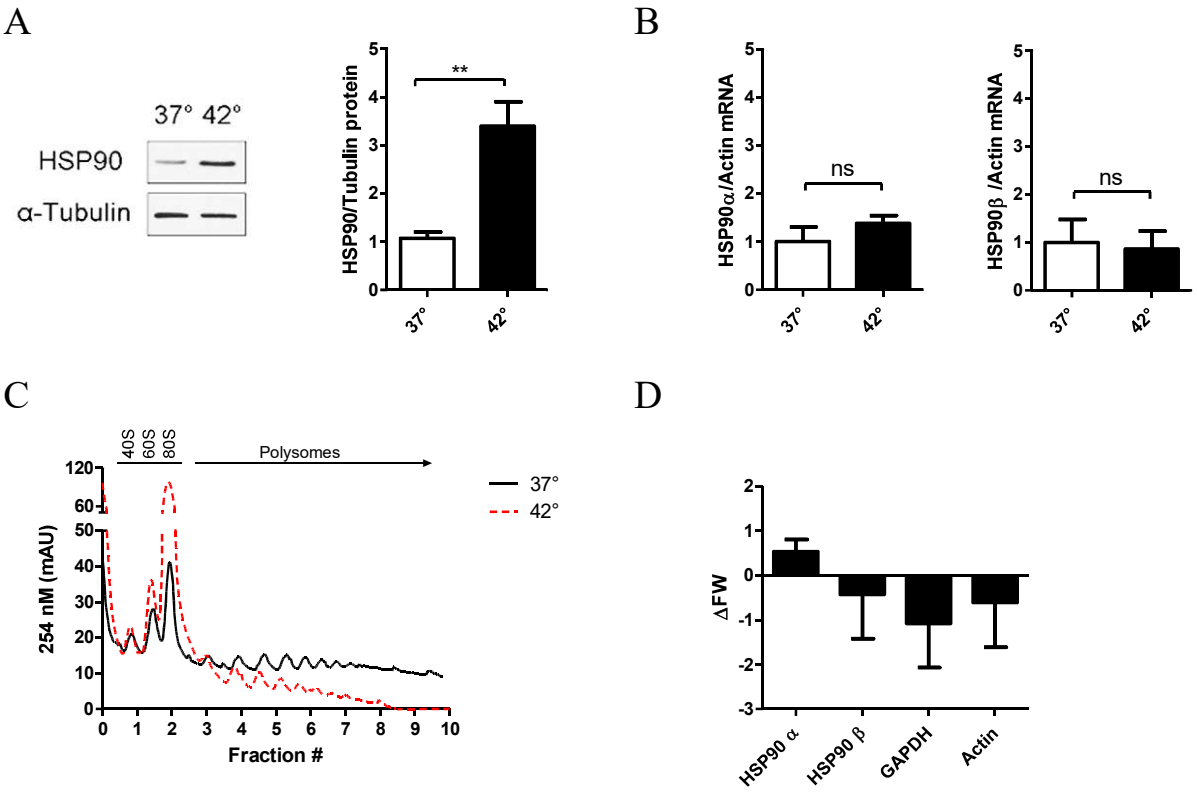

A
37° 42°
HSP90
α-Tubulin
HSP90/Tubulin protein
**
37°
42°
B
HSP90α/Actin mRNA
ns
HSP90β /Actin mRNA
ns
C
40S
60S
80S
Polysomes
254 nM (mAU)
Fraction #
37°
42°
D
ΔFW
HSP90 α
HSP90 β
GAPDH
Actin

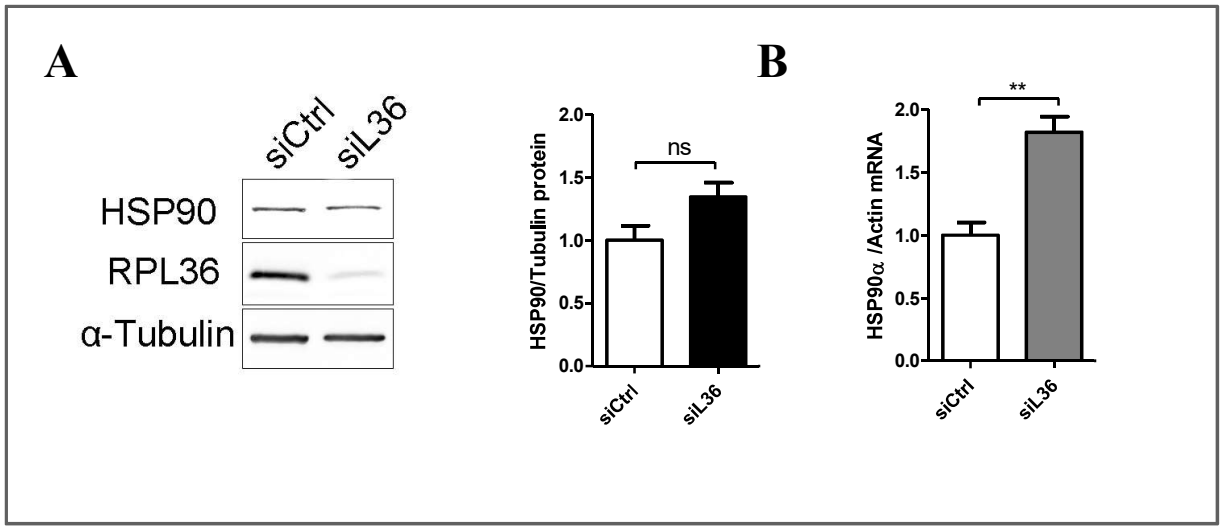
Figure 4: HSP90 undergoes selective translation during heat shock. (A) Left panel: western blot analysis indicating an increase in HSP90 expression when HeLa cells are heat shocked at 42˚C for 45 minutes. Right panel: quantification of HSP90 protein levels relative to tubulin (**, P<0.025). (B) Heat shock does not significantly affect HSP90α or HSP90β mRNA levels. Total RNA was isolated followed by cDNA synthesis by reverse transcription. Quantitative PCR (qPCR) was used to determine the levels of HSP90α and Actin mRNAs; values are expressed as HSP90α relative to Actin (ns, non-significant). (C) HeLa cells were either maintained at 37˚C as a control or were heat shocked at 42˚C for 45 minutes, and then cell lysates were harvested and subjected to polysome analysis. HeLa cells which were heat shocked at 42˚C exhibit increase in monosomes and collapsed polysomes profile when compared to control cells grown at 37˚C. (D) The distribution of HSP90α, HSP90β, Actin, and GAPDH mRNAs in each polysome fraction as shown in panel (C) was calculated as $\Delta F_{w\ method}$ (Chiluiza et al., 2011) (N=2).

mRNAs was determined in each fraction using qRT-PCR (Figure 1D). Importantly, HSP90α mRNA association with polysomes did not decrease during heat shock. In contrast, polysome association of HSP90 β, Actin, and GAPDH mRNAs was repressed during heat shock. This data suggests that HSP90α is selectively translated following heat shock, despite the inhibition of global translation.

3.2 RPL36 and RPL36A regulate HSP90 expression at different levels.

Silva et al. (2013) examined mRNA translation efficiency of yeast cells during acetic acid treatment, a condition mimicking amino acid starvation. They observed that treated cells exhibited inhibition of global translation, but a small number of mRNAs, including HSP90 remained efficiently translated supporting the notion of selective translation of HSP90 under stress. Based on these observations, a genetic regulator screen was performed by Dr. Golshani's lab in Carleton University to identify yeast genes that are potentially involved in the translational control of yeast HSP90. Two of the hits, ribosomal protein L36A (RPL36) and ribosomal protein L42 (RPL42), have well conserved mammalian homologs known as ribosomal protein L36 (RPL36) and ribosomal protein L36A (RPL36A), respectively. Both of these ribosomal proteins are a part of the 60S ribosome subunit (Khatter et al., 2015; Anger et al., 2013; Klinge et al., 2012; Wilson et al., 2012). To determine whether RPL36 and RPL36A are regulators of HSP90 expression in mammalian cells, HeLa cells were transfected with non-targeting (Ctrl) siRNA, RPL36 siRNA, and RPL36A siRNAs for 48 hours and 72 hours, respectively, and were then put under either NHS temperature or HS temperature for 45 minutes. The effect of each siRNA on the HSP90 protein and mRNA levels was then assessed using western blotting and qRT-PCR (Figure 5-6).

37°C - Basal temperature



42°C - Heat shock temperature

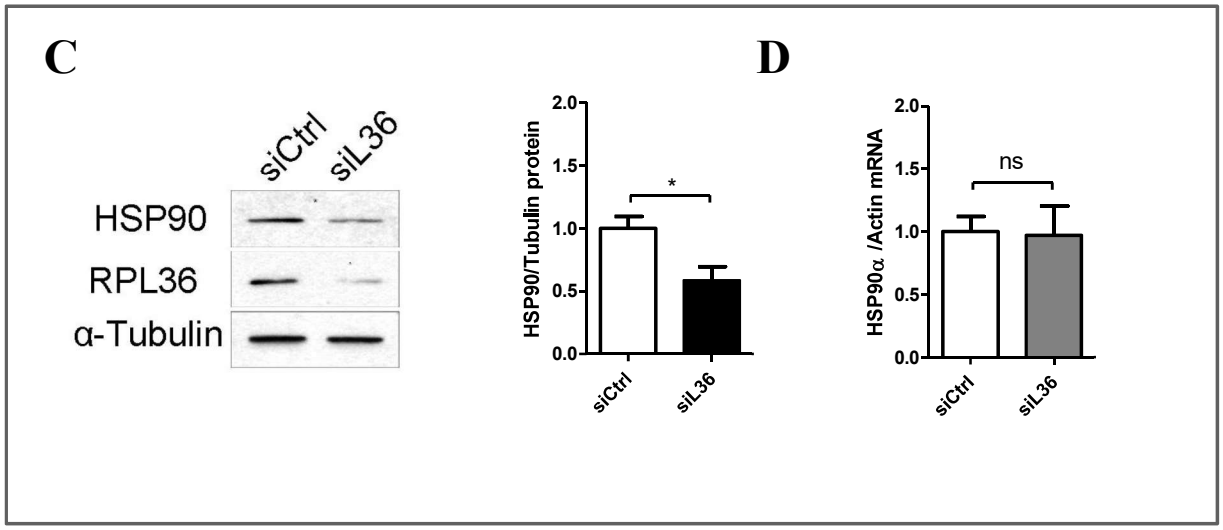
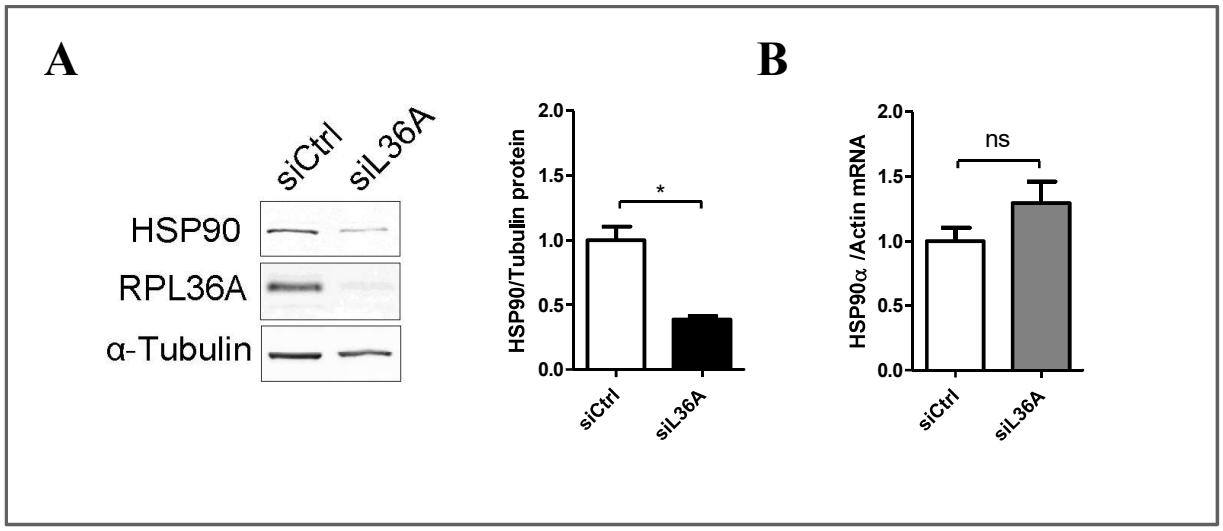


Figure 5: RPL36 regulates HSP90 expression under heat shock. (A, C) Left panel: western blot of HeLa cells treated with non-targeting siRNA (siCtrl), or RPL36 siRNA (siL36) treated cells under basal temperature at 37˚C (A) or under heat shock at 42˚C (C). Right panel: Quantification of western (*, $P<0.05$. ns, non-significant). (B, D) Steady-state mRNA levels were measured by qRT-PCR in control, non-targeting siRNA (siCtrl), or RPL36 siRNA (siL36) treated cells under basal temperature at 37˚C (B) or under heat shock at 42˚C (D) (**, $P<0.01$).

37°C - Basal temperature



42°C- Heat shock temperature

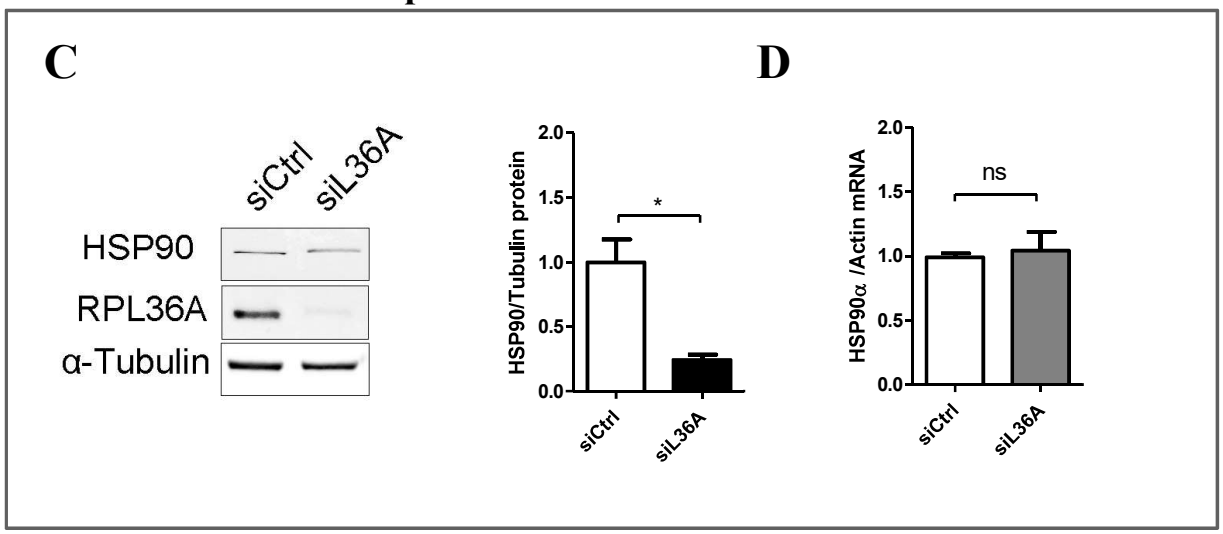


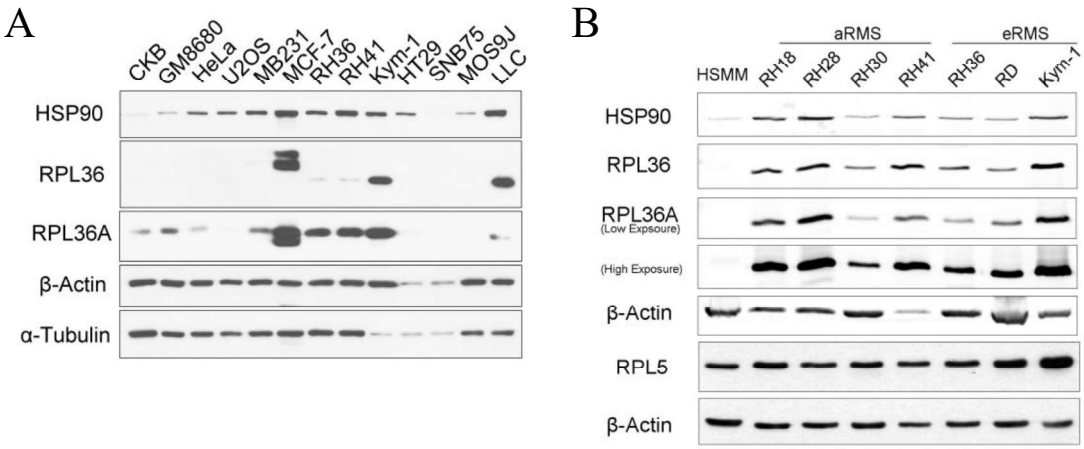
Figure 6: RPL36A regulates HSP90 expression under basal level and heat shock stress. (A, C) Left panel: western blot of HeLa cells treated with non-targeting siRNA (siCtrl), or RPL36A siRNA (siL36A) treated cells under basal level at 37˚C (A) or under heat shock at 42˚C (C). Right panel: Quantification of western (*, $P<0.05$. ns, non-significant). (B, D) Steady-state mRNA levels were measured by qRT-PCR in control, non-targeting siRNA (siCtrl), or RPL36A siRNA (siL36A) treated cells under basal level at 37˚C (B) or under heat shock at 42˚C (D).

When HeLa cells were transfected with RPL36 siRNA (siL36) under basal temperature (Figure 5A-B), there was no significant change in HSP90 protein levels. However, there was a significant increase in HSP90α mRNA level. When HeLa cells were transfected with siL36 under heat shock temperature, HSP90 protein level significantly dropped, whereas HSP90α mRNA level remained unchanged. In contrast, when HeLa cells were transfected with RPL36A siRNA (siL36A) under basal temperature (Figure 6A-B), there was a significant decrease in HSP90 protein level. However, there was no change in HSP90α mRNA level. When HeLa cells were transfected with siL36A under heat shock temperature, HSP90 protein level decreased, whereas HSP90α mRNA level did not change. This data shows that RPL36 and RPL36A regulate HSP90 expression under both heat shock and basal temperature.

3.3 High RPL36 and RPL36A expression correlates with high HSP90 expression in cancer cells.

Previous studies have noted that HSP90 expression is elevated in various cancer cell lines and primary tumours (Wang, 2013 ; Zagouri, 2012; Simpson, 2010; Annamalai, 2009; Okamoto, 2008; Milicevic, 2008; McCarthy, 2008; Workman, 2007; Pick et al, 2007; Whitesell, 2005; Beliakoff, 2004). I confirmed this by examining the western blot expression profile of HSP90 in various cancer cell lines (Cervical Cancer (HeLa), Osteosarcoma (U2OS), Breast Cancer (MB231, MCF-7), Rhabdomyosarcoma (RH36, RH41, Kym-1), Colon Cancer (HT29), Glioma (SNB75, MOS9J), and Lung Carcinoma (LLC)) compared to normal fibroblast (Normal fibroblast (CKB, GM8680)) (Figure 7A).

My previous data suggests that RPL36 and RPL36A drive HSP90 expression, so I was curious to investigate whether these ribosomal proteins were the reason behind elevated expression of



RD Cells

Kym-1 Cells

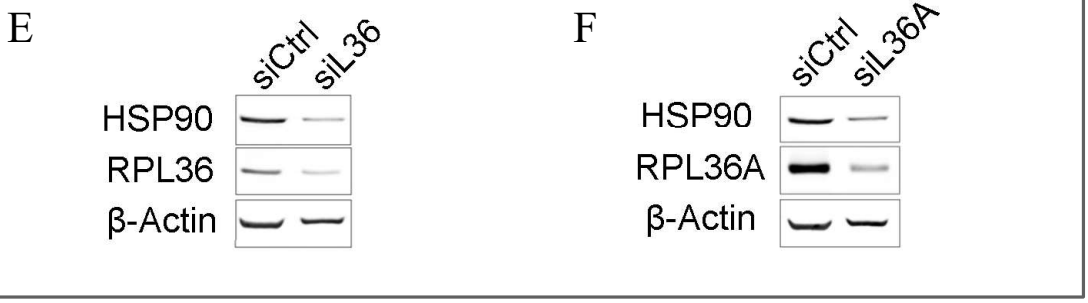


Figure 7: High RPL36 and RPL36A expression correlates with high HSP90 expression in cancer cells. (A) Western blot expression profile of HSP90, RPL36, and RPL36A in various cancer cell lines compared to normal fibroblast (Normal fibroblast (CKB, GM8680), Cervical Cancer (HeLa), Osteosarcoma (U2OS), Breast Cancer (MB231, MCF-7), Rhabdomyosarcoma (RH36, RH41, Kym-1), Colon Cancer (HT29), Glioma (SNB75, MOS9J), and Lung Carcinoma (LLC)). (B) Western blot expression profile of HSP90, RPL5, RPL36, and RPL36A in Rhabdomyosarcoma (RMS) cell lines in comparison to normal Human Skeletal Muscle Myoblast (HSMM). (aRMS (RH18, RH28, RH30, and RH41), eRMS (RH36, RD, and KYM-1)). (C-D) RD cells treated with (C) RPL36 or (D) RPL36A siRNA and control, non-targeting siRNA (siCtrl). Cell lysates were harvested and subjected to western blot analysis. (E-F) KYM-1 cells treated with (E) RPL36 or (F) RPL36A siRNA and control, non-targeting siRNA (siCtrl). Cell lysates were harvested and subjected to western blot analysis.

HSP90 in cancer. To investigate whether elevated levels of HSP90 correlate with elevated levels of RPL36 and/or RPL36A, the same samples were used to examine expression of RPL36 and RPL36A. It was interesting to note that various cancer cells with high HSP90 expression correlated with high RPL36 and RPL36A expression. In particular, Rhabdomyosarcoma (RMS) cell lines, (RH36, RH41, Kym-1) seemed to show high HSP90 level correlating with high RPL36 and RPL36A (Figure 7A). Therefore, RMS cell lines were chosen to be the focus of my further experiments. A western blot expression profile of HSP90, RPL5, RPL36, and RPL36A in a panel of RMS cell lines in comparison to normal Human Skeletal Muscle Myoblast (HSMM) was examined (Figure 7B). RMS, a malignant skeletal muscle tumour, is divided into two subtypes: alveolar RMS (aRMS), which includes RH18, RH28, RH30, And RH41, and embryonal RMS (eRMS), which includes RH36, RD, and Kym-1 (Egas-Bejar and Huh, 2014; Hinson et al., 2013). All RMS examined showed high HSP90 levels when compared to HSMM and this correlated with high levels of RPL36 and/or RPL36A. In contrast, levels of RPL5 (ribosomal protein L5) remained unchanged.

To examine whether RPL36 and/or RPL36A are the drivers of HSP90 expression in RMS, two cell lines, RD and Kym-1, were chosen to study the effect of siL36 and siL36A on HSP90 protein levels. Although RD and Kym-1 are both eRMS, Kym-1 cells display higher HSP90 protein level than RD cell. Reducing the levels of RPL36 and RPL36A using siRNA in RD (Figure 7C-D) and Kym-1 cell lines (Figure 7E-F) resulted in a marked decrease in HSP90 protein levels in both cell lines, suggesting that it may be a common mechanism in these cell lines.

3.4 Higher HSP90 levels in RMS correlate with resistance to HSP inhibitor 17-AAG.

HSP90 inhibitors have been used, alone and in combination with other drugs, to treat various cancers that have high HSP90 expression (Mori et al., 2015; Brennan et al., 2014; Hirshfield and Ganesan, 2014; Zagouri et al., 2013; Usmani et al., 2009; Shimamura and Shapiro, 2008). I was interested into examining the sensitivity of different cell lines, with variable HSP90 expression, towards a known HSP90 inhibitor, 17-demethoxygeldanamycin (17-AAG). I hypothesized that Kym-1 cells would be less sensitive to 17-AAG in comparison to RD cells, which has a relatively lower HSP90 protein level. A live-cell imaging approach was used to monitor cell death using a fluorescent probe, YOYO-1. 17-AAG was used to treat RD and Kym-1 cells with increasing doses over the course of 48 hours. Kym-1 cells were less sensitive to 17-AAG (Figure 8A) in comparison to RD cells (Figure 8B). Fold cytotoxicity of YOYO-1 at 48 hour revealed that RD cells showed a ~20 fold induction in cytotoxicity at 10μM, whereas Kym-1 showed ~8 fold induction at the same dose ($P<0.0001$).

To confirm HSP90 inhibitor activity of 17-AAG, cyclin-dependent kinase 4 (CDK4), a client of HSP90 chaperone, was used as a marker (Figure 8E-F). CDK4 was decreased in both cell lines confirming the activity of the inhibitor. This data indicates that different levels of HSP90 expression leads to variable sensitivity to HS90 inhibitor and that high RPL36 and RPL36A levels drive the elevated HSP90 expression in RMS cells. In conclusion, RPL36 and RPL36A can be potentially used as prognostic markers for HSP90 inhibitor drug resistance in RMS.

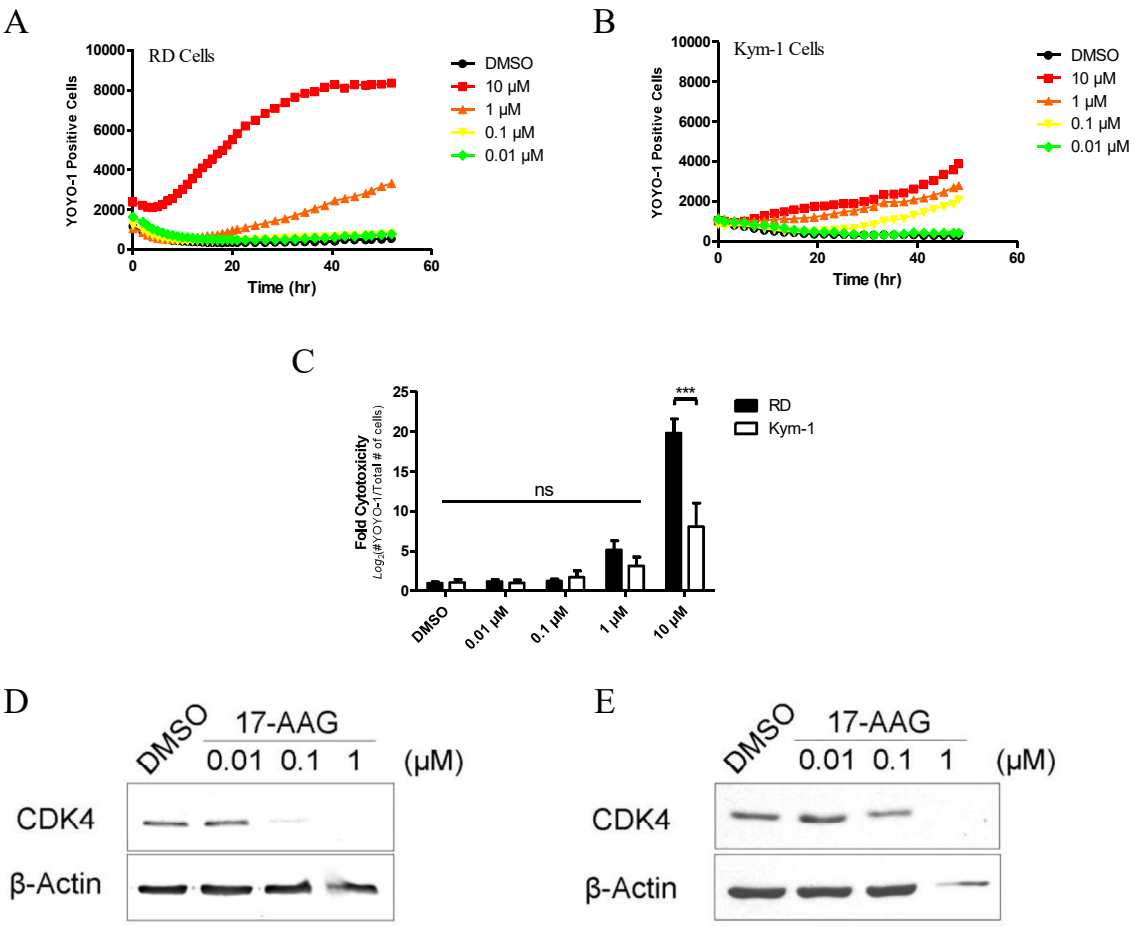

A
RD Cells
YOYO-1 Positive Cells
Time (hr)
DMSO
10 μM
1 μM
0.1 μM
0.01 μM
B
Kym-1 Cells
YOYO-1 Positive Cells
Time (hr)
DMSO
10 μM
1 μM
0.1 μM
0.01 μM
C
Fold Cytotoxicity
Log2(#YOYO-1/Total # of cells)
ns
***
RD
Kym-1
DMSO
0.01 μM
0.1 μM
1 μM
10 μM
D
DMSO
17-AAG
0.01 0.1 1
(μM)
CDK4
β-Actin
E
DMSO
17-AAG
0.01 0.1 1
(μM)
CDK4
β-Actin

Figure 8: HSP90 levels in RMS correlate with resistance to HSP inhibitor 17-AAG. (A-B) Measurement of cytotoxicity in real time using YOYO-1 fluorescence in (A) RD and (B) KYM-1 cells, which were treated with increasing doses of 17-(Allylamino)-17-demethoxygeldanamycin (17-AAG), a known HSP90 inhibitor. YOYO-1 Positive cells were normalized to confluency. (***, P< 0.0001 (C) Fold cytotoxicity of YOYO-1 at 48h from (A-B). RD cells are shown in black and Kym-1 cells are shown in white. (D-E) A client of HSP90 chaperone, Cyclin-dependent kinase 4 (CDK4), was used as a marker of HSP90 inhibitor activity.

Chapter 4: Discussion

Heat shock protein 90 (HSP90) is a highly conserved and ubiquitously expressed molecular chaperone which comprises up to 4-6% in stressed cells (Csermely et al., 1998; Borkovich et al., 1989; Finka and Goloubinoff, 2013). HSP90 plays a critical role in regulating key signalling factors such as cell cycle markers, kinases, and steroid receptors (Sharma et al., 2012; Wegele et al., 2004; Freeman and Yamamoto, 2002; Pratt, 1998; Picard et al., 1990). It is also involved in activation, maturation, translocation and transportation of its client proteins (Kim et al., 2006; Lindquist, 2009; Wayne et al., 2011; Lachowiec et al., 2015). There are two major isoforms of HSP90: HSP90α (inducible form) and HSP90β (constitutive form) (Chen et al., 2005; Zuehlke et al., 2015). HSP90α, the inducible form, is found at a higher level when cells are under stress (Zuehlke et al., 2015; Zhang et al., 1999; Krone et al., 1994). Previous studies suggested that the stress-inducible HSP90 is preferentially translated due to unique structure in its 5' UTR (Silva et al., 2013; Duncan, 2008; Ahmed and Duncan, 2004; Zhang et al. Joshi-Barve et al., 1992). Yet, the complete molecular understanding of HSP90 regulation during heat shock is missing.

Unpublished research performed by Dr. Golshani's lab in Carleton University identified two yeast proteins, RPL36A and RPL42A, as being involved in the translational control of yeast HSP90. This research focuses on testing the hypothesis that the mammalian homologues of RPL36A and RPL42A (RPL36 and RPL36A) regulate the translation of mammalian HSP90. This will be done by dissecting the translational regulation of HSP90 under stress and characterizing RPL36 and RPL36A as regulators of HSP90 in HeLa and rhabdomyosarcoma

cell lines. The physiological relevance of this finding was further examined in the context of drug resistance.

I have shown that HSP90α undergoes selective translation under heat shock. Although a marked 3-fold increase in the protein level was seen under heat shock, there was no significant increase in steady-state level of HSP90α mRNA under heat shock (Figure 4A-B). Consistent with published studies, my data suggests that the induction of HSP90 expression is largely due to post-transcriptional control. In fact, a previous study has also shown that there is a lack of correlation between HSP90 protein levels and mRNA expression in stressed cells (Tang et al., 2005). Moreover, cells treated with Actinomycin D (Act D), a transcriptional inhibitor, under heat shock affected the transcription of HSP90 mRNA. However, this treatment did not affect the translation of HSP90 protein under heat shock and has resulted in an increase in HSP90 protein levels (Ahmed and Duncan, 2004; Jacquier-Sarlin et al., 1995). My data is consistent with these studies in suggesting that post-transcriptional, more specifically translational, control plays a key role in HSP90 protein synthesis under heat shock.

To demonstrate that HSP90α is largely regulated by translational control under stress, I performed polysome profiling to examine the association of HSP90 mRNA isoforms with the translating ribosome under heat shock. I have demonstrated that heat shock impaired the global translation as reflected in the polysome profile (Figure 4C), showing a collapse in polysome fractions and an increase in the monosomal fractions. The relative distribution of HSP90α and HSP90β mRNA within the polysome profile suggested that HSP90α mRNA translation is enhanced under heat shock (Figure 4D). However, HSP90β mRNA translation is reduced along with other constitutively translated mRNA such as, actin and GAPDH. A global inhibition in translation is met with an increase in HSP90α mRNA association with translating

ribosomes and higher HSP90 protein levels, suggesting that HSP90α mRNA is selectively translated under heat shock. My data is consistent with previously published studies, and further confirms that regulation of HSP90 expression during heat shock occurs primarily at the levels of protein synthesis. Of note, this mechanism is likely universally conserved between different organisms, including yeast, drosophila, and humans (Joshi-Barve et al., 1992; Ahmed and Duncan, 2004; Duncan et al., 2008; Silva et al., 2013).

Silva et al. investigated altered translation of HSP90 mRNA under (acetic acid) stress. Using polysome profiling, they found that treating yeast cells with acetic acid led to a global inhibition of cap-dependent translation marked by a phosphorylation of eIF2α (Silva et al., 2013). They used a microarray analysis of polysome-associated mRNAs to investigate whether there were alternatively translated mRNAs under this condition. Interestingly, HSP90 mRNA was highly associated with polysomes under stress, although cap-dependent translation was inhibited. They concluded that HSP90 mRNA is controlled by selective translation under stress due to the increase of HSP90 mRNA association with polysomes and the lack of changes in mRNA levels

To identify the genetic determinants of yeast HSP90 translational control, Dr. Golashni's lab (Carleton University) has conducted a β-galactosidase reporter system screen (Samanfar and Golshani, unpublished). In this screen, the 5'UTR of yeast HSP90 was fused with β-galactosidase and then introduced it into yeast cells with non-essential gene deletions. Two of the several yeast hits, RPL36A and RPL42, have conserved mammalian homologues, RPL36 and RPL36A, found in the large ribosomal subunit 60S (Khatter et al., 2015; Klinge et al. l., 2012; Wilson and Doudna, 2012; Ben-Shem et al., 2011). I hypothesized that like in yeast,

RPL36 and RPL36A are regulators of HSP90 expression and altering levels of RPL36 and RPL36A will affect HSP90's ability to be translationally induced by stress.

Reducing the levels of RPL36 using siRNA treatment under basal temperature had no effect on HSP90 protein level; however, it led to a significant increase in steady-state level of HSP90α mRNA ($P<0.025$) (Figure 5A-B). The lack of correlation between the mRNA and protein level here suggests that it may be to be due to a decrease in translation efficiency of HSP90α mRNA. Under heat shock temperature, lower levels of RPL36 affect HSP90 protein levels considerably ($P<0.025$), whereas steady-state HSP90α mRNA level was not affected (Figure 5C-D). This data suggests that RPL36 is not necessary for the expression of HSP90 under basal temperature. However, lack of RPL36 under heat shock leads to a negative translational regulation of HSP90α mRNA, suggesting that RPL36 is essential for HSP90α mRNA translation and induced protein levels under heat shock.

On the other hand, reducing the levels of RPL36A using siRNA treatment under both basal temperature and heat shock temperature led to a robust decrease in HSP90 protein levels, yet no change in HSP90α mRNA level. These finding suggest that RPL36A is necessary, under basal temperature, for the HSP90 mRNA translation and that lowered levels of RPL36A lead to a decrease in the translation efficiency under both basal and heat shock temperature.

My data suggests that RPL36 and RPL36A are novel regulators of HSP90 mRNA translation; nothing has been published about their translational regulation of either HSP90 mRNA or other mRNAs. However, other ribosomal proteins have been previously shown to affect the translation of specific mRNAs, as a part of the ribosome and extra-ribosomal function; their function is not limited to catalyzing protein synthesis (Zhang et al., 2015; Volta et al., 2013; Warner and McIntosh, 2009; Mauro, 2002). For example, RPL27 is known to control the

induction and translation of p53 after DNA damage by selectively binding to the secondary structure found in its 5'UTR and increasing p53 mRNA translation efficiency (Takagi, 2005; Chen et al., 2012). Similarly, RPL38 is known to regulate Hox mRNA translation by facilitating the binding of the 80S subunit to the IRES of a specific subset of Hox mRNAs (Xue et al., 2015; Kondrashov et al., 2011). Reduced levels of RPL27 and RPL38 did not affect global translation of mRNAs; however, it affected the selective translation of specific mRNAs. Reintroducing their expression was shown to rescue the translation of their respective target mRNA and not a global set of mRNAs.

In the light of this data, the concept of RPL36 and RPL36A harbouring a specialized function affecting HSP90 mRNA translation under heat shock is very intriguing. There are several proposed mechanisms by which RPL36 and RPL36A, as a part of the ribosome, may exert control over HSP90 mRNA. The interaction of ribosomal proteins with regulatory elements found in the UTRs of the mRNA may pose as one of the mechanisms by which they can exert transcript-specific translational control. In addition, it has been suggested that ribosomes do not have a fixed stoichiometry as previously thought and that different growth conditions affect the stoichiometry of the ribosome; thus, possibly affecting translation efficiency of some mRNAs (Slavov et al., 2015; Ben-Shem et al., 2011; Gilbert, 2011). In fact, RPL36 and RPL36A play a role in stabilizing both the highly mobile L1 stalk protein and rRNA in the 80S ribosomal subunit, suggesting that they are needed for optimum translation efficiency (Khatter et al., 2015; Bulygin et al., 2013; Klinge et al., 2012). 18S and 28S rRNA are anchored to the L1 stalk, which is stabilized by RPL36 and RP36A, and bridge communication between the 60S ribosomal subunit and the 40S ribosomal subunit. Therefore, heat shock (a different growth condition) may affect the stoichiometry of the ribosome, allosterically altering the

ribosome's affinity through protein and rRNA interactions with RPL36 and RPL36A to selectively translate HSP90α mRNA.

Another possible explanation of how RPL36 and RPL36A exert transcript-specific translational control would be the activity of the ribosome separate from the polysome, also identified as extra-ribosomal function (Warner and McIntosh, 2009). Studies uncovered a selective translational control of ceruloplasmin mRNA mediated by an extra-ribosomal function of RPL13a. Once free phosphorylated RPL13a binds to the GAIT complex, they are recruited to the 3'UTR of ceruluplasmin mRNA resulting in the inhibition of ceruluplasmin translation. This may present a different mechanism by which RPL36 and RPL36A as extra-ribosomes specifically regulate HSP90α mRNA. Free RPL36 and RPL36A may potentially interact with the UTRs of HSP90α mRNA as a part of an extra-ribosomal function and block its translation under physiological temperature while promoting cap-independent translation under heat shock temperature. Lack of either RPL36 or RPL36A leads to the silencing of HSP90α mRNA translation.

Do RPL36 and RPL36A selectively affect HSP90 translation as a part of the ribosome or as a part of an extra-ribosomal function? What kind of a role does the 5'UTR of HSP90α play in this interaction? Future experiments may help further elucidate the exact mechanism behind RPL36 and RPL36A. Ribosome sucrose cushion experiment, which allows to discern free cytosolic ribosomes from complex-associated ribosomes, can be used to investigate whether these RPL36 and RPL36A act on HSP90 mRNA as a part of the 80S-mRNA complex or as a free extra-ribosome. To show the effect of RPL36 and RPL36A is dependent on the unique 5'UTR of HSP90α mRNA, firefly luciferase reporter, used in a construct system with various

deletions in the 5'UTR of HSP90α, can be transfected into cells under heat shock to confirm importance of the 5'UTR in these interaction.

Although I tried to examine the polysome profile of cells treated with siRNA against RPL36 and RPL36A under non-heat shock and heat shock temperature, the siRNA treatment affected the growth of cells, which prevented me from conducting polysome profiling. My data has excluded the transcriptional regulation of HSP90 mRNA, suggesting that the effect we are seeing is due to translational regulation or protein stability. Follow up experiments to further strengthen my data would include: (1) ruling out off-target effect, a second siRNA targeting a different seed should be used. Different siRNAs targeting the same gene should have comparable gene silencing efficacy and should induce similar changes in the gene expression. (2) Rescuing the endogenous levels of RPL36 and RPL36A by introducing a siRNA-resistant replacement plasmid. (3) Investigating the contribution of protein stability in the loss of HSP90 protein expression by looking at the rate of protein decay using pulse-chase technique. Cells would be cultured ("pulsed") with radioactive amino acids and then "chased" by a similar, non-radioactive amino acids.

Ribosomal proteins constituting the translating ribosome have been known to be altered in cancer cells compared to normal cells (de Las Heras-Rubio et al., 2014 Reschke et al., 2013; Warner and McInotosh, 2009). Further studies have also shown different ribosomal proteins may be necessary in a tissue-specific and developmental-state manner and that altered expression in these ribosomal proteins can lead to various diseases such DBA and cancer (Reschke et al, 2013; Gilbert, 2011; Song et al., 2011 Boria et al., 2010; Kim et al., 2004).

While my data suggests that RPL36 and RPL36A regulate HSP90 expression in HeLa cells, I was curious to find out whether it was also true for other cancer cell lines. Various cancer cell

lines have been identified with elevated level of HSP90 expression (Slavov et al., 2009; Milicevic et al., 2008; Okamoto et al., 2008; Workman et al., 2007 ; Tang et al., 2005; Bagatell et al., 2004). To examine whether there is a correlation between elevated HSP90 levels and the expression of RPL36 and RPL36A, I examined the expression profile of HSP90, RPL36, and RPL36A proteins in various cancer cell lines (cervical cancer, osteosarcoma, breast cancer, rhabdomyosarcoma, colon cancer, glioma, and lung cancer) compared to normal fibroblast (Figure 7A). Consistent with the literature, I observed elevated HSP90 expression in the majority of the cancer cell lines tested. Interestingly, elevated HSP90 expression correlated with an elevated expression of RPL36 and RPL36A, particularly in Rhabdomyosarcoma (RMS) cells (RH36, RH41, and Kym-1). Therefore, I chose to examine this relationship in RMS cells in greater detail.

To further confirm whether there is a correlation between HSP90, RPL36 and RPL36A expression in RMS cell lines, I examined the expression profile of HSP90, RPL5, RPL36, and RPL36A proteins in RMS cells compared to normal human skeletal muscle myoblast (HSMM). Interestingly, majority (5 out of 7) of the RMS cell lines which exhibited elevated levels of HSP90 correlated with elevated levels of RPL36 and RPL36A. In addition, RPL5 did not seem to correlate with HSP90 expression and was found unchanged relative to β-actin protein. My data suggests that: (1) these two ribosomal proteins may be the drivers behind HSP90 expression in RMS cells, and potentially other cancerous cell lines. (2) There are common and unique differences in the ribosomal stoichiometric levels of different ribosomes between normal cells and cancerous cells as suggested previously in literature (Ben-Shem et al., 2011; Gilbert, 2011). That being said, it does not exclude a secondary and different function

for these ribosomal proteins as they could have "extra-ribosomal functions" separate from the ribosome itself (Gilbert et al., 2011; Warner and McIntosh, 2009).

To explore the notion that RPL36 and/or RPL36A play a role in the elevated HSP90 expression in RMS cells, I knocked down RPL36 or RPL36A and monitored HSP90 protein levels in RD and Kym-1 cells, two RMS cells expressing different levels of HSP90. Knocking down RPL36 and RPL36A using siRNA treatment in both cell lines resulted in a decrease in HSP90 expression. This data suggests that HSP90 regulation through RPL36 and RPL36A may be a common mechanism shared by different cancer cells.

Next, I was interested to examine the drug sensitivity of RMS cells with different levels of HSP90 expression. I postulated that cells with higher HSP90 expression, such as Kym-1, would be less sensitive to HSP90 inhibitor compared to cells with relatively lower HSP90 expression, such as RD cells. Using increasing doses of HSP90 inhibitor 17-AAG, Kym-1 cells indeed did show less sensitivity to HSP90 inhibitor exhibiting lower YOYO-1 positive cells in comparison to RD cells (Figure 8 A-B). Furthermore, I have shown that RD displayed a 20-fold increase in cytotoxicity, whereas Kym-1 displayed 8-fold increase in cytotoxicity (Figure 8C). To confirm HSP90 drug activity, Cyclin Dependent Kinase 4 (CDK4), a client target of HSP90, was investigated in the same cells which were treated with increasing doses of HSP90 inhibitor. A dose-dependent decrease in CDK4 was noted in RD cells starting 0.1 μM and in Kym-1 cells seemed to start at 1 μM. The results not only support my hypothesis in which cells shown to have higher level of HSP90 expression will be most likely be less sensitive to HSP90 inhibitor but also suggest that RPL36 and RPL36A may be used as predictors of 17-AAG resistance. In fact, elevated levels of RPL36 and RPL36A have been previously noted in hepatocellular carcinoma and were used as prognostic markers in

multidrug resistance (Song et al., 2011; Kim et al., 2004). Hepatocellular tumours displayed elevated expression of RPL36 and RPL36A, whereas these ribosomal proteins were not detected in healthy non-tumours. In the case of RMS, HSP90 inhibitor drug resistance may be rooted in the elevated levels of RPL36 and RPL36A, which further drive higher HSP90 expression. Thus, RPL36 and RPL36A can be potential prognostic markers and may present as future targets for cancer therapy.

Conclusion

Although heat shock has led to global inhibition of translation, I have shown that HSP90α is selectively translated under heat shock. I have characterized RPL36 and RPL36A as regulators of HSP90α mRNA translation under basal and heat shock conditions. These novel targets support the overlooked regulatory control of the ribosomal proteins on cap-independent translation of a selective subset of mRNAs. Furthermore, I explored the importance of these ribosomal proteins in driving the elevated expression of HSP90 in rhabdomyosarcoma cell lines. I found a correlation between the expression of RPL36 and RPL36A and the levels of HSP90 expression, suggesting that these ribosomal proteins can be driving HSP90-inhibitor resistance in rhabdomyosarcoma. Overall, my findings contribute to our current knowledge about the functions of ribosomal proteins in selectively regulating a specific mRNA, which plays an essential role in protecting cells from stress.

References

AHMED, R. & DUNCAN, R. F. 2004. Translational regulation of Hsp90 mRNA. AUG-proximal 5'-untranslated region elements essential for preferential heat shock translation. J Biol Chem, 279, 49919-30.

ANGER, A. M., ARMACHE, J. P., BERNINGHAUSEN, O., HABECK, M., SUBKLEWE, M., WILSON, D. N. & BECKMANN, R. 2013. Structures of the human and Drosophila 80S ribosome. Nature, 497, 80-5.

ANNAMALAI, B., LIU, X., GOPAL, U. & ISAACS, J. S. 2009. Hsp90 is an essential regulator of EphA2 receptor stability and signaling: implications for cancer cell migration and metastasis. Mol Cancer Res, 7, 1021-32.

BAGATELL, R., PAINE-MURRIETA, G. D., TAYLOR, C. W., PULCINI, E. J., AKINAGA, S., BENJAMIN, I. J. & WHITESELL, L. 2000. Induction of a heat shock factor 1-dependent stress response alters the cytotoxic activity of hsp90-binding agents. Clin Cancer Res, 6, 3312-8.

BAGATELL, R. & WHITESELL, L. 2004. Altered Hsp90 function in cancer: a unique therapeutic opportunity. Mol Cancer Ther, 3, 1021-30.

BANDHOLTZ, L., GUO, Y., PALMBERG, C., MATTSSON, K., OHLSSON, B., HIGH, A., SHABANOWITZ, J., HUNT, D. F., JORNVALL, H., WIGZELL, H., AGERBERTH, B. & GUDMUNDSSON, G. H. 2003. Hsp90 binds CpG oligonucleotides directly: implications for hsp90 as a missing link in CpG signaling and recognition. Cell Mol Life Sci, 60, 422-9.

BAUER, J. W., BRANDL, C., HAUBENREISSER, O., WIMMER, B., WEBER, M., KARL, T., KLAUSEGGER, A., BREITENBACH, M., HINTNER, H., VON DER HAAR, T., TUITE, M. F. & BREITENBACH-KOLLER, L. 2013. Specialized yeast ribosomes: a customized tool for selective mRNA translation. PLoS One, 8, e67609.

BEE, A., KE, Y., FOROOTAN, S., LIN, K., BEESLEY, C., FORREST, S. E. & FOSTER, C. S. 2006. Ribosomal protein l19 is a prognostic marker for human prostate cancer. Clin Cancer Res, 12, 2061-5.

BELIAKOFF, J. & WHITESELL, L. 2004. Hsp90: an emerging target for breast cancer therapy. Anticancer Drugs, 15, 651-62.

BEN-SHEM, A., GARREAU DE LOUBRESSE, N., MELNIKOV, S., JENNER, L., YUSUPOVA, G. & YUSUPOV, M. 2011. The structure of the eukaryotic ribosome at 3.0 A resolution. Science, 334, 1524-9.

BHAVSAR, R. B., MAKLEY, L. N. & TSONIS, P. A. 2010. The other lives of ribosomal proteins. Hum Genomics, 4, 327-44.

BORIA, I., GARELLI, E., GAZDA, H. T., ASPESI, A., QUARELLO, P., PAVESI, E., FERRANTE, D., MEERPOHL, J. J., KARTAL, M., DA COSTA, L., PROUST, A., LEBLANC, T., SIMANSOUR, M., DAHL, N., FROJMARK, A. S., POSPISILOVA, D., CMEJLA, R., BEGGS, A. H., SHEEN, M. R., LANDOWSKI, M., BUROS, C. M.,

CLINTON, C. M., DOBSON, L. J., VLACHOS, A., ATSIDAFTOS, E., LIPTON, J. M., ELLIS, S. R., RAMENGHI, U. & DIANZANI, I. 2010. The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update. Hum Mutat, 31, 1269-79.

BORKOVICH, K. A., FARRELLY, F. W., FINKELSTEIN, D. B., TAULIEN, J. & LINDQUIST, S. 1989. hsp82 is an essential protein that is required in higher concentrations for growth of cells at higher temperatures. Mol Cell Biol, 9, 3919-30.

BORTOLUZZI, S., D'ALESSI, F., ROMUALDI, C. & DANIELI, G. A. 2001. Differential expression of genes coding for ribosomal proteins in different human tissues. Bioinformatics, 17, 1152-7.

BOSE, S., WEIKL, T., BUGL, H. & BUCHNER, J. 1996. Chaperone function of Hsp90-associated proteins. Science, 274, 1715-7.

BRENNAN, G. T., RELIAS, V. & SAIF, M. W. 2014. Novel agents for the treatment of pancreatic cancer. JOP, 15, 110-3.

BULYGIN, K., MALYGIN, A., HOUNTONDJI, C., GRAIFER, D. & KARPOVA, G. 2013. Positioning of CCA-arms of the A- and the P-tRNAs towards the 28S rRNA in the human ribosome. Biochimie, 95, 195-203.

BUSHELL, M., MCKENDRICK, L., JANICKE, R. U., CLEMENS, M. J. & MORLEY, S. J. 1999. Caspase-3 is necessary and sufficient for cleavage of protein synthesis eukaryotic initiation factor 4G during apoptosis. FEBS Lett, 451, 332-6.

CHAN, Y. L., PAZ, V., OLVERA, J. & WOOL, I. G. 1993. The primary structure of rat ribosomal protein L36. Biochem Biophys Res Commun, 192, 849-53.

CHEN, B., PIEL, W. H., GUI, L., BRUFORD, E. & MONTEIRO, A. 2005. The HSP90 family of genes in the human genome: insights into their divergence and evolution. Genomics, 86, 627-37.

CHEN, J., GUO, K. & KASTAN, M. B. 2012. Interactions of nucleolin and ribosomal protein L26 (RPL26) in translational control of human p53 mRNA. J Biol Chem, 287, 16467-76.

CHENG, M. B., ZHANG, Y., ZHONG, X., SUTTER, B., CAO, C. Y., CHEN, X. S., CHENG, X. K., ZHANG, Y., XIAO, L. & SHEN, Y. F. 2010. Stat1 mediates an auto-regulation of hsp90beta gene in heat shock response. Cell Signal, 22, 1206-13.

CSERMELY, P., SCHNAIDER, T., SOTI, C., PROHASZKA, Z. & NARDAI, G. 1998. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther, 79, 129-68.

DALE, E. C., YANG, X., MOORE, S. K. & SHYAMALA, G. 1996. Cloning and characterization of the promoter for murine 84-kDa heat-shock protein. Gene, 172, 279-84.

DARNELL, J. C. 2011. Defects in translational regulation contributing to human cognitive and behavioral disease. Curr Opin Genet Dev, 21, 465-73.

DAVE, B., GRANADOS-PRINCIPAL, S., ZHU, R., BENZ, S., RABIZADEH, S., SOON-SHIONG, P., YU, K. D., SHAO, Z., LI, X., GILCREASE, M., LAI, Z., CHEN, Y., HUANG, T. H., SHEN, H., LIU, X., FERRARI, M., ZHAN, M., WONG, S. T., KUMARASWAMI, M.,

MITTAL, V., CHEN, X., GROSS, S. S. & CHANG, J. C. 2014. Targeting RPL39 and MLF2 reduces tumor initiation and metastasis in breast cancer by inhibiting nitric oxide synthase signaling. Proc Natl Acad Sci U S A, 111, 8838-43.

DE LAS HERAS-RUBIO, A., PERUCHO, L., PACIUCCI, R., VILARDELL, J. & ME, L. L. 2014. Ribosomal proteins as novel players in tumorigenesis. Cancer Metastasis Rev, 33, 115-41.

DE PAEPE, B., CREUS, K. K., MARTIN, J. J., WEIS, J. & DE BLEECKER, J. L. 2009. A dual role for HSP90 and HSP70 in the inflammatory myopathies: from muscle fiber protection to active invasion by macrophages. Ann N Y Acad Sci, 1173, 463-9.

DE SOUZA, H. S., WEST, G. A., REBERT, N., DE LA MOTTE, C., DRAZBA, J. & FIOCCHI, C. 2012. Increased levels of survivin, via association with heat shock protein 90, in mucosal T cells from patients with Crohn's disease. Gastroenterology, 143, 1017-26 e9.

DEFEE, M. R., QIN, Z., DAI, L., TOOLE, B. P., ISAACS, J. S. & PARSONS, C. H. 2011. Extracellular Hsp90 serves as a co-factor for NF-kappaB activation and cellular pathogenesis induced by an oncogenic herpesvirus. Am J Cancer Res, 1, 687-700.

DEHOUX, P., DAVIES, J. & CANNON, M. 1993. Natural cycloheximide resistance in yeast. The role of ribosomal protein L41. Eur J Biochem, 213, 841-8.

DIDELOT, C., LANNEAU, D., BRUNET, M., BOUCHOT, A., CARTIER, J., JACQUEL, A., DUCOROY, P., CATHELIN, S., DECOLOGNE, N., CHIOSIS, G., DUBREZ-DALOZ, L., SOLARY, E. & GARRIDO, C. 2008. Interaction of heat-shock protein 90 beta isoform (HSP90 beta) with cellular inhibitor of apoptosis 1 (c-IAP1) is required for cell differentiation. Cell Death Differ, 15, 859-66.

DOUDNA, J. A. & RATH, V. L. 2002. Structure and function of the eukaryotic ribosome: the next frontier. Cell, 109, 153-6.

DU, S. J., LI, H., BIAN, Y. & ZHONG, Y. 2008. Heat-shock protein 90alpha1 is required for organized myofibril assembly in skeletal muscles of zebrafish embryos. Proc Natl Acad Sci U S A, 105, 554-9.

DUNCAN, R. F. 2008. Rapamycin conditionally inhibits Hsp90 but not Hsp70 mRNA translation in Drosophila: implications for the mechanisms of Hsp mRNA translation. Cell Stress Chaperones, 13, 143-55.

DUUS, J., BAHAR, H. I., VENKATARAMAN, G., OZPUYAN, F., IZBAN, K. F., AL-MASRI, H., MAUDUDI, T., TOOR, A. & ALKAN, S. 2006. Analysis of expression of heat shock protein-90 (HSP90) and the effects of HSP90 inhibitor (17-AAG) in multiple myeloma. Leuk Lymphoma, 47, 1369-78.

EGAS-BEJAR, D. & HUH, W. W. 2014. Rhabdomyosarcoma in adolescent and young adult patients: current perspectives. Adolesc Health Med Ther, 5, 115-25.

FAHL, S. P., HARRIS, B., COFFEY, F. & WIEST, D. L. 2015. Rpl22 Loss Impairs the Development of B Lymphocytes by Activating a p53-Dependent Checkpoint. J Immunol, 194, 200-9.

FALSONE, S. F., GESSLBAUER, B., TIRK, F., PICCININI, A. M. & KUNGL, A. J. 2005. A proteomic snapshot of the human heat shock protein 90 interactome. FEBS Lett, 579, 6350-4.

FAYE, M. D., GRABER, T. E. & HOLCIK, M. 2014. Assessment of selective mRNA translation in mammalian cells by polysome profiling. J Vis Exp, e52295.

FAYE, M. D. & HOLCIK, M. 2015. The role of IRES trans-acting factors in carcinogenesis. Biochim Biophys Acta, 1849, 887-97.

FINKA, A. & GOLOUBINOFF, P. 2013. Proteomic data from human cell cultures refine mechanisms of chaperone-mediated protein homeostasis. Cell Stress Chaperones, 18, 591-605.

FLYNN, J. M., MISHRA, P. & BOLON, D. N. 2015. Mechanistic Asymmetry in Hsp90 Dimers. J Mol Biol, 427, 2904-11.

FRASER, C. S. 2015. Quantitative studies of mRNA recruitment to the eukaryotic ribosome. Biochimie, 114, 58-71.

FREEMAN, B. C. & YAMAMOTO, K. R. 2002. Disassembly of transcriptional regulatory complexes by molecular chaperones. Science, 296, 2232-5.

FROLOVA, L. Y., MERKULOVA, T. I. & KISSELEV, L. L. 2000. Translation termination in eukaryotes: polypeptide release factor eRF1 is composed of functionally and structurally distinct domains. RNA, 6, 381-90.

GEBAUER, F. & HENTZE, M. W. 2004. Molecular mechanisms of translational control. Nat Rev Mol Cell Biol, 5, 827-35.

GILBERT, W. V. 2011. Functional specialization of ribosomes? Trends Biochem Sci, 36, 127-32.

GOETZ, M. P., TOFT, D. O., AMES, M. M. & ERLICHMAN, C. 2003. The Hsp90 chaperone complex as a novel target for cancer therapy. Ann Oncol, 14, 1169-76.

GRAD, I., CEDERROTH, C. R., WALICKI, J., GREY, C., BARLUENGA, S., WINSSINGER, N., DE MASSY, B., NEF, S. & PICARD, D. 2010. The molecular chaperone Hsp90alpha is required for meiotic progression of spermatocytes beyond pachytene in the mouse. PLoS One, 5, e15770.

GRAIFER, D., MALYGIN, A., ZHARKOV, D. O. & KARPOVA, G. 2014. Eukaryotic ribosomal protein S3: A constituent of translational machinery and an extraribosomal player in various cellular processes. Biochimie, 99, 8-18.

GRANDIN, N. & CHARBONNEAU, M. 2001. Hsp90 levels affect telomere length in yeast. Mol Genet Genomics, 265, 126-34.

GYGI, S. P., ROCHON, Y., FRANZA, B. R. & AEBERSOLD, R. 1999. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol, 19, 1720-30.

HERSHEY, J. W., SONENBERG, N. & MATHEWS, M. B. 2012. Principles of translational control: an overview. Cold Spring Harb Perspect Biol, 4.

HINSON, A. R., JONES, R., CROSE, L. E., BELYEA, B. C., BARR, F. G. & LINARDIC, C. M. 2013. Human rhabdomyosarcoma cell lines for rhabdomyosarcoma research: utility and pitfalls. Front Oncol, 3, 183.

HIRSHFIELD, K. M. & GANESAN, S. 2014. Triple-negative breast cancer: molecular subtypes and targeted therapy. Curr Opin Obstet Gynecol, 26, 34-40.

HOLCIK, M. & KORNELUK, R. G. 2000. Functional characterization of the X-linked inhibitor of apoptosis (XIAP) internal ribosome entry site element: role of La autoantigen in XIAP translation. Mol Cell Biol, 20, 4648-57.

HOLCIK, M. & SONENBERG, N. 2005. Translational control in stress and apoptosis. Nat Rev Mol Cell Biol, 6, 318-27.

HOUNTONDJI, C., BULYGIN, K., CRECHET, J. B., WOISARD, A., TUFFERY, P., NAKAYAMA, J., FROLOVA, L., NIERHAUS, K. H., KARPOVA, G. & BAOUZ, S. 2014. The CCA-end of P-tRNA Contacts Both the Human RPL36AL and the A-site Bound Translation Termination Factor eRF1 at the Peptidyl Transferase Center of the Human 80S Ribosome. Open Biochem J, 8, 52-67.

JACQUIER-SARLIN, M. R., JORNOT, L. & POLLA, B. S. 1995. Differential expression and regulation of hsp70 and hsp90 by phorbol esters and heat shock. J Biol Chem, 270, 14094-9.

JAYAPRAKASH, P., DONG, H., ZOU, M., BHATIA, A., O'BRIEN, K., CHEN, M., WOODLEY, D. T. & LI, W. 2015. Hsp90alpha and Hsp90beta together operate a hypoxia and nutrient paucity stress-response mechanism during wound healing. J Cell Sci, 128, 1475-80.

JOHANNES, G., CARTER, M. S., EISEN, M. B., BROWN, P. O. & SARNOW, P. 1999. Identification of eukaryotic mRNAs that are translated at reduced cap binding complex eIF4F concentrations using a cDNA microarray. Proc Natl Acad Sci U S A, 96, 13118-23.

JOHNSON, J. L. 2012. Evolution and function of diverse Hsp90 homologs and cochaperone proteins. Biochim Biophys Acta, 1823, 607-13.

JOSHI-BARVE, S., DE BENEDETTI, A. & RHOADS, R. E. 1992. Preferential translation of heat shock mRNAs in HeLa cells deficient in protein synthesis initiation factors eIF-4E and eIF-4 gamma. J Biol Chem, 267, 21038-43.

KAIGORODOVA, E. V., RYAZANTSEVA, N. V., NOVITSKII, V. V., BELKINA, M. V. & MAROSHKINA, A. N. 2011. The role of heat shock protein 90 in the regulation of tumor cell apoptosis. Bull Exp Biol Med, 150, 450-2.

KAMAL, A. & BURROWS, F. J. 2004. Hsp90 inhibitors as selective anticancer drugs. Discov Med, 4, 277-80.

KAPPELER, K. V., ZHANG, J., DINH, T. N., STROM, J. G. & CHEN, Q. M. 2012. Histone deacetylase 6 associates with ribosomes and regulates de novo protein translation during arsenite stress. Toxicol Sci, 127, 246-55.

KASAI, H., NADANO, D., HIDAKA, E., HIGUCHI, K., KAWAKUBO, M., SATO, T. A. & NAKAYAMA, J. 2003. Differential expression of ribosomal proteins in human normal and neoplastic colorectum. J Histochem Cytochem, 51, 567-74.

KHATTER, H., MYASNIKOV, A. G., MASTIO, L., BILLAS, I. M., BIRCK, C., STELLA, S. & KLAHOLZ, B. P. 2014. Purification, characterization and crystallization of the human 80S ribosome. Nucleic Acids Res, 42, e49.

KHATTER, H., MYASNIKOV, A. G., NATCHIAR, S. K. & KLAHOLZ, B. P. 2015. Structure of the human 80S ribosome. Nature, 520, 640-5.

KIM, J. H., YOU, K. R., KIM, I. H., CHO, B. H., KIM, C. Y. & KIM, D. G. 2004. Over-expression of the ribosomal protein L36a gene is associated with cellular proliferation in hepatocellular carcinoma. Hepatology, 39, 129-38.

KIM, T. S., JANG, C. Y., KIM, H. D., LEE, J. Y., AHN, B. Y. & KIM, J. 2006. Interaction of Hsp90 with ribosomal proteins protects from ubiquitination and proteasome-dependent degradation. Mol Biol Cell, 17, 824-33.

KLINGE, S., VOIGTS-HOFFMANN, F., LEIBUNDGUT, M. & BAN, N. 2012. Atomic structures of the eukaryotic ribosome. Trends Biochem Sci, 37, 189-98.

KOBAYASHI, N., TOYOOKA, S., SOH, J., YAMAMOTO, H., DOTE, H., KAWASAKI, K., OTANI, H., KUBO, T., JIDA, M., UENO, T., ANDO, M., OGINO, A., KIURA, K. & MIYOSHI, S. 2012. The anti-proliferative effect of heat shock protein 90 inhibitor, 17-DMAG, on non-small-cell lung cancers being resistant to EGFR tyrosine kinase inhibitor. Lung Cancer, 75, 161-6.

KONDRASHOV, N., PUSIC, A., STUMPF, C. R., SHIMIZU, K., HSIEH, A. C., XUE, S., ISHIJIMA, J., SHIROISHI, T. & BARNA, M. 2011. Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. Cell, 145, 383-97.

KOZEKO, L. E. 2010. [Heat shock proteins 90 kDa: diversity, structure, functions]. Tsitologiia, 52, 893-910.

KRONE, P. H. & SASS, J. B. 1994. HSP 90 alpha and HSP 90 beta genes are present in the zebrafish and are differentially regulated in developing embryos. Biochem Biophys Res Commun, 204, 746-52.

KRONJA, I. & ORR-WEAVER, T. L. 2011. Translational regulation of the cell cycle: when, where, how and why? Philos Trans R Soc Lond B Biol Sci, 366, 3638-52.

KULKARNI, A. P., MITTAL, S. P., DEVASAGAYAM, T. P. & PAL, J. K. 2010. Hsp90 mediates activation of the heme regulated eIF-2 alpha kinase during oxidative stress. Indian J Biochem Biophys, 47, 67-74.

LACHOWIEC, J., LEMUS, T., BORENSTEIN, E. & QUEITSCH, C. 2015. Hsp90 promotes kinase evolution. Mol Biol Evol, 32, 91-9.

LANGER, T., ROSMUS, S. & FASOLD, H. 2003. Intracellular localization of the 90 kDA heat shock protein (HSP90alpha) determined by expression of a EGFP-HSP90alpha-fusion protein in unstressed and heat stressed 3T3 cells. Cell Biol Int, 27, 47-52.

LEE, A. S., BURDEINICK-KERR, R. & WHELAN, S. P. 2013. A ribosome-specialized translation initiation pathway is required for cap-dependent translation of vesicular stomatitis virus mRNAs. Proc Natl Acad Sci U S A, 110, 324-9.

LEPRIVIER, G., ROTBLAT, B., KHAN, D., JAN, E. & SORENSEN, P. H. 2015. Stress-mediated translational control in cancer cells. Biochim Biophys Acta, 1849, 845-60.

LESKO, E., GOZDZIK, J., KIJOWSKI, J., JENNER, B., WIECHA, O. & MAJKA, M. 2007. HSP90 antagonist, geldanamycin, inhibits proliferation, induces apoptosis and blocks migration of rhabdomyosarcoma cells in vitro and seeding into bone marrow in vivo. Anticancer Drugs, 18, 1173-81.

LI, G. W., BURKHARDT, D., GROSS, C. & WEISSMAN, J. S. 2014. Quantifying absolute protein synthesis rates reveals principles underlying allocation of cellular resources. Cell, 157, 624-35.

LINDQUIST, S. 2009. Protein folding sculpting evolutionary change. Cold Spring Harb Symp Quant Biol, 74, 103-8.

LIU, B. & QIAN, S. B. 2014. Translational reprogramming in cellular stress response. Wiley Interdiscip Rev RNA, 5, 301-15.

LIWAK, U., FAYE, M. D. & HOLCIK, M. 2012. Translation control in apoptosis. Exp Oncol, 34, 218-30.

LO, C. W., CHANG, Y. S., CHAO, C. C., CHANG, M. D., CHANG, K. C. & LAI, Y. K. 2009. Control mechanisms of differential translation of Hsp90 isoforms in 9L rat gliosarcoma cells. J Cell Biochem, 107, 418-27.

LU, H., ZHU, Y. F., XIONG, J., WANG, R. & JIA, Z. 2015. Potential extra-ribosomal functions of ribosomal proteins in Saccharomyces cerevisiae. Microbiol Res, 177, 28-33.

LUKASIEWICZ, E., MIEKUS, K., KIJOWSKI, J., GOZDZIK, J., WILUSZ, M., BOBIS-WOZOWICZ, S., WIECHA, O. & MAJKA, M. 2009. High anti tumor activity against rhabdomyosarcoma cells and low normal cells cytotoxicity of heat shock protein 90 inhibitors, with special emphasis on 17-[2-(pyrrolidin-1-yl)ethyl]-aminno-17-demethoxygeldanamycin. J Physiol Pharmacol, 60, 161-6.

MALONEY, A. & WORKMAN, P. 2002. HSP90 as a new therapeutic target for cancer therapy: the story unfolds. Expert Opin Biol Ther, 2, 3-24.

MARCU, M. G. & NECKERS, L. M. 2003. The C-terminal half of heat shock protein 90 represents a second site for pharmacologic intervention in chaperone function. Curr Cancer Drug Targets, 3, 343-7.

MARISSEN, W. E., GUO, Y., THOMAS, A. A., MATTS, R. L. & LLOYD, R. E. 2000. Identification of caspase 3-mediated cleavage and functional alteration of eukaryotic initiation factor 2alpha in apoptosis. J Biol Chem, 275, 9314-23.

MATASSA, D. S., AMOROSO, M. R., AGLIARULO, I., MADDALENA, F., SISINNI, L., PALADINO, S., ROMANO, S., ROMANO, M. F., SAGAR, V., LORENI, F., LANDRISCINA, M. & ESPOSITO, F. 2013. Translational control in the stress adaptive response of cancer cells: a novel role for the heat shock protein TRAP1. Cell Death Dis, 4, e851.

MAURO, V. P. & EDELMAN, G. M. 2002. The ribosome filter hypothesis. Proc Natl Acad Sci U S A, 99, 12031-6.

MAYER, M. P. & LE BRETON, L. 2015. Hsp90: breaking the symmetry. Mol Cell, 58, 8-20.

MAZUMDER, B., SAMPATH, P., SESHADRI, V., MAITRA, R. K., DICORLETO, P. E. & FOX, P. L. 2003. Regulated release of L13a from the 60S ribosomal subunit as a mechanism of transcript-specific translational control. Cell, 115, 187-98.

MCCARTHY, M. M., PICK, E., KLUGER, Y., GOULD-ROTHBERG, B., LAZOVA, R., CAMP, R. L., RIMM, D. L. & KLUGER, H. M. 2008. HSP90 as a marker of progression in melanoma. Ann Oncol, 19, 590-4.

MESSAOUDI, S., PEYRAT, J. F., BRION, J. D. & ALAMI, M. 2008. Recent advances in Hsp90 inhibitors as antitumor agents. Anticancer Agents Med Chem, 8, 761-82.

MEYER, K. D., PATIL, D. P., ZHOU, J., ZINOVIEV, A., SKABKIN, M. A., ELEMENTO, O., PESTOVA, T. V., QIAN, S. B. & JAFFREY, S. R. 2015. 5' UTR m(6)A Promotes Cap-Independent Translation. Cell, 163, 999-1010.

MEYER, P., PRODROMOU, C., HU, B., VAUGHAN, C., ROE, S. M., PANARETOU, B., PIPER, P. W. & PEARL, L. H. 2003. Structural and functional analysis of the middle segment of hsp90: implications for ATP hydrolysis and client protein and cochaperone interactions. Mol Cell, 11, 647-58.

MILICEVIC, Z., BOGOJEVIC, D., MIHAILOVIC, M., PETROVIC, M. & KRIVOKAPIC, Z. 2008. Molecular characterization of hsp90 isoforms in colorectal cancer cells and its association with tumour progression. Int J Oncol, 32, 1169-78.

MORI, M., HITORA, T., NAKAMURA, O., YAMAGAMI, Y., HORIE, R., NISHIMURA, H. & YAMAMOTO, T. 2015. Hsp90 inhibitor induces autophagy and apoptosis in osteosarcoma cells. Int J Oncol, 46, 47-54.

MORIMOTO, R. I. 1998. Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes Dev, 12, 3788-96.

NECKERS, L. & IVY, S. P. 2003. Heat shock protein 90. Curr Opin Oncol, 15, 419-24.

NISHIZUKA, S., CHARBONEAU, L., YOUNG, L., MAJOR, S., REINHOLD, W. C., WALTHAM, M., KOUROS-MEHR, H., BUSSEY, K. J., LEE, J. K., ESPINA, V., MUNSON, P. J., PETRICOIN, E., 3RD, LIOTTA, L. A. & WEINSTEIN, J. N. 2003. Proteomic profiling of the NCI-60 cancer cell lines using new high-density reverse-phase lysate microarrays. Proc Natl Acad Sci U S A, 100, 14229-34.

OBRIG, T. G., CULP, W. J., MCKEEHAN, W. L. & HARDESTY, B. 1971. The mechanism by which cycloheximide and related glutarimide antibiotics inhibit peptide synthesis on reticulocyte ribosomes. J Biol Chem, 246, 174-81.

OKAMOTO, J., MIKAMI, I., TOMINAGA, Y., KUCHENBECKER, K. M., LIN, Y. C., BRAVO, D. T., CLEMENT, G., YAGUI-BELTRAN, A., RAY, M. R., KOIZUMI, K., HE,

B. & JABLONS, D. M. 2008. Inhibition of Hsp90 leads to cell cycle arrest and apoptosis in human malignant pleural mesothelioma. J Thorac Oncol, 3, 1089-95.

OZAWA, K., MURAKAMI, Y., EKI, T., SOEDA, E. & YOKOYAMA, K. 1992. Mapping of the gene family for human heat-shock protein 90 alpha to chromosomes 1, 4, 11, and 14. Genomics, 12, 214-20.

PACE, D. A. & MANAHAN, D. T. 2007. Cost of protein synthesis and energy allocation during development of antarctic sea urchin embryos and larvae. Biol Bull, 212, 115-29.

PAEPE, B. D., CREUS, K. K., WEIS, J. & BLEECKER, J. L. 2012. Heat shock protein families 70 and 90 in Duchenne muscular dystrophy and inflammatory myopathy: balancing muscle protection and destruction. Neuromuscul Disord, 22, 26-33.

PERON, M., BONVINI, P. & ROSOLEN, A. 2012. Effect of inhibition of the ubiquitin-proteasome system and Hsp90 on growth and survival of rhabdomyosarcoma cells in vitro. BMC Cancer, 12, 233.

PICARD, D. 2002. Heat-shock protein 90, a chaperone for folding and regulation. Cell Mol Life Sci, 59, 1640-8.

PICARD, D., KHURSHEED, B., GARABEDIAN, M. J., FORTIN, M. G., LINDQUIST, S. & YAMAMOTO, K. R. 1990. Reduced levels of hsp90 compromise steroid receptor action in vivo. Nature, 348, 166-8.

PICK, E., KLUGER, Y., GILTNANE, J. M., MOEDER, C., CAMP, R. L., RIMM, D. L. & KLUGER, H. M. 2007. High HSP90 expression is associated with decreased survival in breast cancer. Cancer Res, 67, 2932-7.

PIRKKALA, L., NYKANEN, P. & SISTONEN, L. 2001. Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. FASEB J, 15, 1118-31.

POWERS, M. V., CLARKE, P. A. & WORKMAN, P. 2009. Death by chaperone: HSP90, HSP70 or both? Cell Cycle, 8, 518-26.

PRATT, W. B. 1998. The hsp90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. Proc Soc Exp Biol Med, 217, 420-34.

PRINCE, T., SUN, L. & MATTS, R. L. 2005. Cdk2: a genuine protein kinase client of Hsp90 and Cdc37. Biochemistry, 44, 15287-95.

PRINCIOTTA, M. F., FINZI, D., QIAN, S. B., GIBBS, J., SCHUCHMANN, S., BUTTGEREIT, F., BENNINK, J. R. & YEWDELL, J. W. 2003. Quantitating protein synthesis, degradation, and endogenous antigen processing. Immunity, 18, 343-54.

PRINSLOO, E., KRAMER, A. H., EDKINS, A. L. & BLATCH, G. L. 2012. STAT3 interacts directly with Hsp90. IUBMB Life, 64, 266-73.

PROVOST, E., BAILEY, J. M., ALDRUGH, S., LIU, S., IACOBUZIO-DONAHUE, C. & LEACH, S. D. 2014. The tumor suppressor rpl36 restrains KRAS(G12V)-induced pancreatic cancer. Zebrafish, 11, 551-9.

RAJASEKHAR, V. K. & HOLLAND, E. C. 2004. Postgenomic global analysis of translational control induced by oncogenic signaling. Oncogene, 23, 3248-64.

RAMSEY, A. J., RUSSELL, L. C. & CHINKERS, M. 2009. C-terminal sequences of hsp70 and hsp90 as non-specific anchors for tetratricopeptide repeat (TPR) proteins. Biochem J, 423, 411-9.

RESCHKE, M., CLOHESSY, J. G., SEITZER, N., GOLDSTEIN, D. P., BREITKOPF, S. B., SCHMOLZE, D. B., ALA, U., ASARA, J. M., BECK, A. H. & PANDOLFI, P. P. 2013. Characterization and analysis of the composition and dynamics of the mammalian riboproteome. Cell Rep, 4, 1276-87.

RIVERA, M. C., MAGUIRE, B. & LAKE, J. A. 2015. Dissociation of ribosomes into large and small subunits. Cold Spring Harb Protoc, 2015, 363-7.

SAHIN, F., QIU, W., WILENTZ, R. E., IACOBUZIO-DONAHUE, C. A., GROSMARK, A. & SU, G. H. 2005. RPL38, FOSL1, and UPP1 are predominantly expressed in the pancreatic ductal epithelium. Pancreas, 30, 158-67.

SASS, J. B., MARTIN, C. C. & KRONE, P. H. 1999. Restricted expression of the zebrafish hsp90alpha gene in slow and fast muscle fiber lineages. Int J Dev Biol, 43, 835-8.

SASS, J. B., WEINBERG, E. S. & KRONE, P. H. 1996. Specific localization of zebrafish hsp90 alpha mRNA to myoD-expressing cells suggests a role for hsp90 alpha during normal muscle development. Mech Dev, 54, 195-204.

SAXENA, A. K., SAXENA, S. & CHAUDHAERY, S. S. 2010. Molecular modelling and docking studies on heat shock protein 90 (Hsp90) inhibitors. SAR QSAR Environ Res, 21, 1-20.

SCHAFER, S., ADAMI, E., HEINIG, M., RODRIGUES, K. E., KREUCHWIG, F., SILHAVY, J., VAN HEESCH, S., SIMAITE, D., RAJEWSKY, N., CUPPEN, E., PRAVENEC, M., VINGRON, M., COOK, S. A. & HUBNER, N. 2015. Translational regulation shapes the molecular landscape of complex disease phenotypes. Nat Commun, 6, 7200.

SCHNEIDER-POETSCH, T., JU, J., EYLER, D. E., DANG, Y., BHAT, S., MERRICK, W. C., GREEN, R., SHEN, B. & LIU, J. O. 2010. Inhibition of eukaryotic translation elongation by cycloheximide and lactimidomycin. Nat Chem Biol, 6, 209-217.

SCHWANHAUSSER, B., BUSSE, D., LI, N., DITTMAR, G., SCHUCHHARDT, J., WOLF, J., CHEN, W. & SELBACH, M. 2011. Global quantification of mammalian gene expression control. Nature, 473, 337-42.

SHAKOORI, A. R., OBERDORF, A. M., OWEN, T. A., WEBER, L. A., HICKEY, E., STEIN, J. L., LIAN, J. B. & STEIN, G. S. 1992. Expression of heat shock genes during differentiation of mammalian osteoblasts and promyelocytic leukemia cells. J Cell Biochem, 48, 277-87.

SHAMOVSKY, I., IVANNIKOV, M., KANDEL, E. S., GERSHON, D. & NUDLER, E. 2006. RNA-mediated response to heat shock in mammalian cells. Nature, 440, 556-60.

SHARMA, K., VABULAS, R. M., MACEK, B., PINKERT, S., COX, J., MANN, M. & HARTL, F. U. 2012. Quantitative proteomics reveals that Hsp90 inhibition preferentially targets kinases and the DNA damage response. Mol Cell Proteomics, 11, M111 014654.

SHEN, Y., LIU, J., WANG, X., CHENG, X., WANG, Y. & WU, N. 1997. Essential role of the first intron in the transcription of hsp90beta gene. FEBS Lett, 413, 92-8.

SHI, Z. & BARNA, M. 2015. Translating the Genome in Time and Space: Specialized Ribosomes, RNA Regulons, and RNA-Binding Proteins. Annu Rev Cell Dev Biol, 31, 31-54.

SHIMAMURA, T. & SHAPIRO, G. I. 2008. Heat shock protein 90 inhibition in lung cancer. J Thorac Oncol, 3, S152-9.

SILVA, A., SAMPAIO-MARQUES, B., FERNANDES, A., CARRETO, L., RODRIGUES, F., HOLCIK, M., SANTOS, M. A. & LUDOVICO, P. 2013. Involvement of yeast HSP90 isoforms in response to stress and cell death induced by acetic acid. PLoS One, 8, e71294.

SIMPSON, N. E., LAMBERT, W. M., WATKINS, R., GIASHUDDIN, S., HUANG, S. J., OXELMARK, E., ARJU, R., HOCHMAN, T., GOLDBERG, J. D., SCHNEIDER, R. J., REIZ, L. F., SOARES, F. A., LOGAN, S. K. & GARABEDIAN, M. J. 2010. High levels of Hsp90 cochaperone p23 promote tumor progression and poor prognosis in breast cancer by increasing lymph node metastases and drug resistance. Cancer Res, 70, 8446-56.

SINGH, N. P. 2000. A simple method for accurate estimation of apoptotic cells. Exp Cell Res, 256, 328-37.

SLAVOV, N. & DAWSON, K. A. 2009. Correlation signature of the macroscopic states of the gene regulatory network in cancer. Proc Natl Acad Sci U S A, 106, 4079-84.

SLAVOV, N., SEMRAU, S., AIROLDI, E., BUDNIK, B. & VAN OUDENAARDEN, A. 2015. Differential Stoichiometry among Core Ribosomal Proteins. Cell Rep, 13, 865-73.

SONG, M. J., JUNG, C. K., PARK, C. H., HUR, W., CHOI, J. E., BAE, S. H., CHOI, J. Y., CHOI, S. W., HAN, N. I. & YOON, S. K. 2011. RPL36 as a prognostic marker in hepatocellular carcinoma. Pathol Int, 61, 638-44.

STANKIEWICZ, M. & MAYER, M. P. 2012. The universe of Hsp90. Biomol Concepts, 3, 79-97.

STEPHANOU, A. & LATCHMAN, D. S. 1999. Transcriptional regulation of the heat shock protein genes by STAT family transcription factors. Gene Expr, 7, 311-9.

TAKAGI, M., ABSALON, M. J., MCLURE, K. G. & KASTAN, M. B. 2005. Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell, 123, 49-63.

TANG, D., KHALEQUE, M. A., JONES, E. L., THERIAULT, J. R., LI, C., WONG, W. H., STEVENSON, M. A. & CALDERWOOD, S. K. 2005. Expression of heat shock proteins and heat shock protein messenger ribonucleic acid in human prostate carcinoma in vitro and in tumors in vivo. Cell Stress Chaperones, 10, 46-58.

TEBALDI, T., RE, A., VIERO, G., PEGORETTI, I., PASSERINI, A., BLANZIERI, E. & QUATTRONE, A. 2012. Widespread uncoupling between transcriptome and translatome variations after a stimulus in mammalian cells. BMC Genomics, 13, 220.

TENG, T., THOMAS, G. & MERCER, C. A. 2013. Growth control and ribosomopathies. Curr Opin Genet Dev, 23, 63-71.

TREPEL, J., MOLLAPOUR, M., GIACCONE, G. & NECKERS, L. 2010. Targeting the dynamic HSP90 complex in cancer. Nat Rev Cancer, 10, 537-49.

USMANI, S. Z., BONA, R. & LI, Z. 2009. 17 AAG for HSP90 inhibition in cancer--from bench to bedside. Curr Mol Med, 9, 654-64.

VOLTA, V., BEUGNET, A., GALLO, S., MAGRI, L., BRINA, D., PESCE, E., CALAMITA, P., SANVITO, F. & BIFFO, S. 2013. RACK1 depletion in a mouse model causes lethality, pigmentation deficits and reduction in protein synthesis efficiency. Cell Mol Life Sci, 70, 1439-50.

VOSS, A. K., THOMAS, T. & GRUSS, P. 2000. Mice lacking HSP90beta fail to develop a placental labyrinth. Development, 127, 1-11.

WALLIN, R. P., LUNDQVIST, A., MORE, S. H., VON BONIN, A., KIESSLING, R. & LJUNGGREN, H. G. 2002. Heat-shock proteins as activators of the innate immune system. Trends Immunol, 23, 130-5.

WALSH, D. & MOHR, I. 2014. Coupling 40S ribosome recruitment to modification of a cap-binding initiation factor by eIF3 subunit e. Genes Dev, 28, 835-40.

WAN, K., YABUKI, Y. & MIZUTA, K. 2015. Roles of Ebp2 and ribosomal protein L36 in ribosome biogenesis in Saccharomyces cerevisiae. Curr Genet, 61, 31-41.

WANG, J., CUI, S., ZHANG, X., WU, Y. & TANG, H. 2013. High expression of heat shock protein 90 is associated with tumor aggressiveness and poor prognosis in patients with advanced gastric cancer. PLoS One, 8, e62876.

WARNER, J. R. & MCINTOSH, K. B. 2009. How common are extraribosomal functions of ribosomal proteins? Mol Cell, 34, 3-11.

WAYNE, N., MISHRA, P. & BOLON, D. N. 2011. Hsp90 and client protein maturation. Methods Mol Biol, 787, 33-44.

WEGELE, H., MULLER, L. & BUCHNER, J. 2004. Hsp70 and Hsp90--a relay team for protein folding. Rev Physiol Biochem Pharmacol, 151, 1-44.

WHITESELL, L. & LINDQUIST, S. L. 2005. HSP90 and the chaperoning of cancer. Nat Rev Cancer, 5, 761-72.

WILLIAMS, N. E. & NELSEN, E. M. 1997. HSP70 and HSP90 homologs are associated with tubulin in hetero-oligomeric complexes, cilia and the cortex of Tetrahymena. J Cell Sci, 110 (Pt 14), 1665-72.

WILSON, D. N. & DOUDNA CATE, J. H. 2012. The structure and function of the eukaryotic ribosome. Cold Spring Harb Perspect Biol, 4.

WORKMAN, P. & POWERS, M. V. 2007. Chaperoning cell death: a critical dual role for Hsp90 in small-cell lung cancer. Nat Chem Biol, 3, 455-7.

WU, L. M., WANG, S. Y., WANG, S. X., HUANG, Y. M. & LI, J. G. 2010. [Proliferation promotion and apoptotic inhibition effects of ribosomal protein RPL36A small interference RNA on U937 cells]. Zhongguo Shi Yan Xue Ye Xue Za Zhi, 18, 344-9.

XUE, S. & BARNA, M. 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. Nat Rev Mol Cell Biol, 13, 355-69.

XUE, S. & BARNA, M. 2015. Cis-regulatory RNA elements that regulate specialized ribosome activity. RNA Biol, 12, 1083-7.

XUE, S., TIAN, S., FUJII, K., KLADWANG, W., DAS, R. & BARNA, M. 2015. RNA regulons in Hox 5' UTRs confer ribosome specificity to gene regulation. Nature, 517, 33-8.

YANO, M., NAITO, Z., YOKOYAMA, M., SHIRAKI, Y., ISHIWATA, T., INOKUCHI, M. & ASANO, G. 1999. Expression of hsp90 and cyclin D1 in human breast cancer. Cancer Lett, 137, 45-51.

ZAGOURI, F., BOURNAKIS, E., KOUTSOUKOS, K. & PAPADIMITRIOU, C. A. 2012. Heat shock protein 90 (hsp90) expression and breast cancer. Pharmaceuticals (Basel), 5, 1008-20.

ZAGOURI, F., SERGENTANIS, T. N., CHRYSIKOS, D., PAPADIMITRIOU, C. A., DIMOPOULOS, M. A. & PSALTOPOULOU, T. 2013. Hsp90 inhibitors in breast cancer: a systematic review. Breast, 22, 569-78.

ZHANG, S. L., YU, J., CHENG, X. K., DING, L., HENG, F. Y., WU, N. H. & SHEN, Y. F. 1999. Regulation of human hsp90alpha gene expression. FEBS Lett, 444, 130-5.

ZHANG, X., GAO, X., COOTS, R. A., CONN, C. S., LIU, B. & QIAN, S. B. 2015. Translational control of the cytosolic stress response by mitochondrial ribosomal protein L18. Nat Struct Mol Biol, 22, 404-10.

ZHOU, J., WAN, J., GAO, X., ZHANG, X., JAFFREY, S. R. & QIAN, S. B. 2015. Dynamic m(6)A mRNA methylation directs translational control of heat shock response. Nature, 526, 591-4.

ZIMMERMANN, R. A. 2003. The double life of ribosomal proteins. Cell, 115, 130-2.

ZUEHLKE, A. D., BEEBE, K., NECKERS, L. & PRINCE, T. 2015. Regulation and function of the human HSP90AA1 gene. Gene, 570, 8-16.