Cellular roles of the human Obg-like ATPase 1 (hOLA1) and its YchF homologs.

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Abstract

P-loop NTPases comprise one of the major superfamilies of nucleotide binding proteins, which mediate a variety of cellular processes, such as mRNA translation, signal transduction, cell motility, and growth regulation. In this review, we discuss the structure and function of two members of the ancient Obg-related family of P-loop GTPases: human obg-like ATPase 1 (hOLA1) and its bacterial/plant homolog, YchF. After a brief discussion of nucleotide binding proteins in general and the classification of the Obg-related family in particular, we discuss the sequence and structural features of YchF and hOLA1. We then explore the various functional roles of hOLA1 in mammalian cells during stress response and cancer progression, and of YchF in bacterial cells. Finally, we directly compare and contrast the structure and function of hOLA1 with YchF before summarizing the future perspectives of hOLA1 research. This review is timely, given the variety of recent studies aimed at understanding the roles of hOLA1 and YchF in such critical processes as cellular stress-response, oncogenesis, and protein synthesis.

1.0 Introduction

1.1 Nucleotide binding proteins

Nucleotide binding proteins have vital roles in cellular processes including mRNA translation, signal transduction, cell motility, cell division, cell growth, chromatin remodeling, and cytoskeleton organization (Leipe et al. 2002; Milligan et al. 1990; Milon et al. 2006). Based on their structural and functional properties, these proteins are classified into the following superfamilies: (I) di-nucleotide binding folds (Rossman) and the related tubulin/FtsZ fold, (II) the protein kinase fold, (III) the HSP90 fold, (IV) the HSP70/RNase H fold, and (V) the mono-nucleotide binding folds (Phosphate loop nucleoside triphosphotases, or P-loop NTPases) (Leipe et al. 2002; Milner-White et al. 1991).

P-loop NTPases comprise the largest group of nucleotide binding proteins (10-18%) in most organisms (Leipe et al. 2002). Although most of the P-loop NTPases utilize ATP as an energy source, the superfamily of P-loop GTPases are an exception, utilizing GTP as a substrate to regulate a variety of critical cellular processes (Leipe et al. 2002). Based on structural features and

conserved sequences, P-loop GTPases can be sorted into two main classes: the translation factor related class (TRAFAC) and the signal recognition particle, MinD, and BioD (SIMIBI) NTPases (Caldon and March 2003; Leipe et al. 2002). These GTPases are grouped together by having a common core G-domain that contains the five conserved G-motifs (Leipe et al. 2002). These motifs are responsible for guanine nucleotide specificity and interacting with the phosphates, giving rise to conformational changes in the protein upon phosphate ester bond hydrolysis and phosphate release (Leipe et al. 2002). Examples of the functional importance of conformational changes are elongation factor G (EF-G; eEF2 in eukaryotes) inducing translocation of the ribosome along the mRNA and elongation factor Tu (EF-Tu; eEF1α in eukaryotes) delivering aminoacylated tRNA to the translating ribosome (Leipe et al. 2002). The TRAFAC class of P-loop GTPases can be further divided into four main families: Elongation factors (EF-G [eEF2 in humans], EF-Tu, IF2, and LepA), Era-related GTPases (Era, EngA, and ThdF/TrmE), protein secretion factors (FtsY and Ffh), and Obg-related proteins, discussed below (Berchtold et al. 1993; Bourne et al. 1991; Leipe et al. 2002).

1.2 Organization of Obg family members and identification of YchF/hOLA1

The Obg family consists of five ancient subfamilies based on several conserved sequence motifs—namely, Obg/CgtA, Drgl/Rbg, Nogl, Ygr210, and YchF/YyaF (Koller-Eichhorn et al. 2007; Leipe et al. 2002). Obg-related proteins contain the canonical G-domain found in P-loop GTPases and are defined by two sequence motifs and a second conserved domain. The first conserved motif (YxF(T/C)TxxxxxG) is found in all five subfamilies and is found within the switch I region (Walker A motif) (Koller-Eichhorn et al. 2007; Leipe et al. 2002). Except for Nog1, all Obg-related proteins share an additional glycine-rich motif (GAxxGxGxGxxx(I/L/V)) following the switch II region (Walker B motif) (Leipe et al. 2002). Additionally, the Obg-family proteins possess a C-terminal RNA-binding domain, the TGS (ThrRS, GTPase, SpoT) domain. YchF is unique within the Obg-family, as several homologs of YchF have been reported to preferentially bind and utilize ATP rather than GTP, making it the only G-protein known to use another energy pool in the cell (Becker et al. 2012b; Gradia et al. 2009; Koller-Eichhorn et al. 2007). The functional implications of this altered nucleotide specificity have not been determined, but it is likely due to the non-

canonical G4 motif in YchF homologs (NKxD in canonical GTPases; NVNE in *Escherichia coli*; NMSE in yeast; NLSE in humans) (Koller-Eichhorn et al. 2007).

Koller-Eichhorn et al. identified the human homolog of bacterial YchF by phylogenetic analysis of Obg-related proteins (Koller-Eichhorn et al. 2007). This uncharacterized protein was named human Obg-like ATPase 1 (hOLA1) (Hirano et al. 2006; Koller-Eichhorn et al. 2007). The same study reported another uncharacterized protein that is only found in higher eukaryotes: the human GTP-binding protein (GTPBP10) (Hirano et al. 2006; Koller-Eichhorn et al. 2007). However, the function of GTPBP10 has not yet been elucidated. hOLA1 is well conserved across all domains of life, sharing 45% sequence identity and 62% sequence similarity with the *E. coli* homolog (EcYchF) (Figure 1). This suggests functional conservation of YchF/hOLA1 across all domains of life, something normally characteristic of ribosomal proteins (Adekambi et al. 2011). Similar to EcYchF, hOLA1 preferentially utilizes ATP over GTP (Koller-Eichhorn et al. 2007), while the homolog in rice, OsYchF1, is able to utilize both ATP and GTP (Cheung et al. 2016). Accordingly, hOLA1 is a regulatory protein that likely changes conformation depending on whether ATP or ADP is bound, presumably while interacting with effector protein(s), to carry out its downstream function(s) (Koller-Eichhorn et al. 2007). It is not known if the effector protein(s) is/are able to trigger ATP hydrolysis, causing the ATP to ADP conformational change, apart from the bacterial ribosome, detailed further below.

1.3 Sequence and structural features of YchF/hOLA1

YchF/hOLA1 is comprised of three domains. The G domain of YchF/hOLA1 is separated in primary sequence by an inserted coiled-coil domain and followed by a TGS domain (Figure 2). The G-domain bridges the coiled-coil and TGS domains, forming a positively charged cleft that has been proposed to bind nucleic acids (Koller-Eichhorn et al. 2007). The nucleotide (i.e. GTP/ATP-) binding pocket is located opposite the cleft. Furthermore, YchF is a member of the hydrophobic amino acid substituted family of GTPases (HAS-GTPase) that have the canonical catalytic glutamine substituted with a hydrophobic amino acid, such as Isoleucine or Leucine in the case of YchF and hOLA1, respectively (Rosler et al. 2015). As such, HAS-GTPases require an alternative mode of catalysis. Recently, using molecular dynamics and biochemical analysis, Rosler and coworkers determined the catalytic residue of EcYchF to be histidine 114, located in a

flexible loop of the G-domain not resolved in the crystal structure (Rosler et al. 2015). This catalytic histidine is conserved across all homologs (Figure 1). To date, however, few investigations have been carried out to assess the phenotypes of catalytically inactive variants of YchF/hOLA1. Overall, based on the sequence identity and similarity, conservation of important residues, and similar structural fold, it is likely that YchF and hOLA1 have maintained analogous functional roles in bacterial and human cells.

2.0 Cellular roles of hOLA1

Several studies have implicated hOLA1 in the regulation of numerous cellular processes during stress conditions, such as oxidative stress response, heat shock, protein synthesis, integrated stress response (ISR), cell cycle regulation, and cancer metastasis (Ding et al. 2016; Koller-Eichhorn et al. 2007; Mao et al. 2013b; Xu et al. 2016; Zhang et al. 2009b). Sun *et al.* reported that hOLA1 is overexpressed in many different cancers, such as colon, rectum, stomach, lung, ovary and uterus (Sun et al. 2010). hOLA1 is also regulated by DNA damage, as the levels of hOLA1 were downregulated in cells treated with DNA damaging agents. However, ER stress-inducing agents did not affect the level of hOLA1 (Sun et al. 2010). Therefore, hOLA1 was initially dubbed DOC45 (DNA damage-regulated overexpressed in cancer 45) by Sun *et al*. However, the mechanism by which genotoxic compounds regulate the expression of hOLA1 is not yet clear (Sun et al. 2010). Additionally, Sun *et al.* (2010) reported that DOC45 is overexpressed in cells expressing oncogenic proteins, such as H-Ras and R-Ras2. These proteins are known to activate the phosphoinositide 3-kinase (PI3K) pathway, suggesting that expression of hOLA1 is linked to the PI3K pathway. Around the same time as the study of DOC45 by Sun *et al.* (2010), structural and functional aspects of hOLA1 were published by Koller-Eichhorn *et al*. (2007). Given the response of hOLA1 to DNA damaging agents, and its aberrant expression in various cancer cell lines, the roles of hOLA1 during cellular stress have been the focus of a large number of studies, as discussed below.

2.1 hOLA1 in antioxidant and heat-shock responses

Oxidative stress is associated with human diseases, and is the result of an imbalance between the production of Reactive Oxygen Species (ROS) and their breakdown by the body to prevent their harmful effects (Pham-Huy et al. 2008). Zhang *et al.* (2009) reported that hOLA1 is upregulated in the presence of H_2O_2 in yeast and human cells, leading to further studies on the biological functions of hOLA1 during these oxidative stresses (Lee et al. 1999; Zhang et al. 2009a). Zhang *et al.* reported that hOLA1-depleted cells are more resistant to peroxides and thiol-depleting agents (Zhang et al. 2009a). However, no morphological or growth defects were apparent in hOLA1-depleted cells (Zhang et al. 2009a). Additionally, reduced expression of hOLA1 in the presence of oxidants resulted in decreased production of ROS. Accordingly, hOLA1 is a negative regulator of the antioxidant response. Downregulation of hOLA1 also affected the expression of additional genes, yet none of these genes are known to encode antioxidant or cytoprotective enzymes. Furthermore, inhibition of protein synthesis by cycloheximide did not reverse the improved resistance to oxidative stress in hOLA1-depleted cells, implying that hOLA1 acts as an antioxidant suppressor by a post-translational mechanism (Schneider-Poetsch et al. 2010; Zhang et al. 2009a; Zhang et al. 2009c).

Heat shock response is a cellular defense mechanism that has been evolutionarily conserved, and is characterized by the expression of molecular chaperones called heat shock proteins (HSP), including HSP70 (Mayer and Bukau 2005). HSP70 is required to maintain protein homeostasis and thermo-tolerance in the cell, and is found to be highly expressed in various malignant tumors (Mao et al. 2013b; Mayer and Bukau 2005; Wu et al. 2017). HSP70 has also been associated with the molecular pathogenesis of several neurodegenerative diseases (Mayer and Bukau 2005). Depletion of hOLA1 increased heat shock-induced cell death, whereas overexpression of hOLA1 made the cells more resistant to heat shock (Mao et al. 2013b). Notably, knockdown of hOLA1 in either noncancerous or breast cancer cell lines had no effect on the cellular levels of HSPs (including HSP27, HSP90, and HSP110) except HSP70, which decreased in expression. Moreover, it was shown that hOLA1 does not regulate HSP70 at the transcriptional level. A direct interaction between hOLA1 and HSP70 was confirmed by co-immunoprecipitation, revealing that hOLA1 binds with HSP70 both *in vitro* and *in vivo* and furthermore that hOLA1 binding stabilizes HSP70 (Mao et al. 2013b). It is through the carboxyl terminal variable domain of HSP70 that hOLA1 interacts, impeding ubiquitination by CHIP (C-terminal of HSP70-binding protein, an E3 ligase of HSP70), and thereby reducing proteasomal turnover of HSP70 (Figure 3)

(Mao et al. 2013b). Additionally, hOLA1 and HSP70 localized together in the cytoplasm in non-cancerous, breast cancer, and cervical cancer cells. Together, these findings suggest that hOLA1 is a unique type of heat-shock regulator that regulates HSP70 protein stability (Mao et al. 2013b). Since hOLA1 has critical roles in both antioxidant and heat-shock responses, hOLA1 is a crucial factor for intracellular homeostasis (Mao et al. 2013b; Zhang et al. 2009a; Zhang et al. 2009c).

2.2 hOLA1 as a regulator of protein synthesis and Integrated Stress Response (ISR)

Protein synthesis is a fundamental cellular process carried out over three different phases: initiation, elongation, and termination. Translation initiation is highly regulated and the rate-limiting step in this process (Aitken and Lorsch 2012). hOLA1 interacts with the 60S and 40S ribosomal subunits, the 80S ribosome, and translating ribosomes, suggesting a function for hOLA1 in the regulation of protein synthesis (Chen et al. 2015). Regulation of gene expression and maintenance of cell homeostasis is critical for cellular survival. Under stress conditions, such as nutrient deprivation, cells use alternative mechanisms to control gene expression and conserve energy. The Integrated Stress Response (ISR) is one such mechanism (Harding et al. 2003; Pakos-Zebrucka et al. 2016). The ISR is a cellular response that is activated in response to different pathophysiological or extrinsic stresses (e.g. hypoxia, nutrient deprivation and viral infection). However, intrinsic stresses, such as Endoplasmic Reticulum (ER) stress, can also activate the ISR (Pakos-Zebrucka et al. 2016). The central node for translation regulation during the ISR is phosphorylation of the alpha subunit of eukaryotic initiation factor 2 (eIF2α) by four different kinases: GCN2 (general control non-derepressible 2), HRI (heme-regulated inhibitor), PERK (PKR-like endoplasmic reticulum kinase), and PKR (protein kinase RNA activated). These kinases are activated during diverse cellular stresses. The consequence of eIF2α-phosphorylation is to prevent reformation of the ternary complex (TC; eIF2•GTP•Met-tRNAi^{Met}), thus blocking delivery of Met-tRNAi^{Met} to the 40S ribosomal subunit and attenuating translation initiation (Aitken and Lorsch 2012; Sharma et al. 2016). hOLA1 was found to bind to eIF2α and prevent TC formation, leading to the inhibition of protein synthesis and promotion of ISR (Chen et al. 2015). This suggested that hOLA1 provides a novel mechanism of TC inhibition, and thereby inhibition of protein synthesis.

Inhibition of protein synthesis through the inhibition of TC formation was the first evidence that hOLA1 is a suppressive factor of global protein synthesis, demonstrated both *in vitro* and *in vivo* (Chen et al. 2015). Remarkably, a bi-cistronic reporter assay revealed that hOLA1 had a suppressive effect on cap-dependent translation but did not affect cap-independent translation of the Hepatitis C virus (HCV) Internal Ribosome Entry Site (IRES) (Chen et al. 2015). IRESs employ a cap-independent mechanism of translation and are not dependent on eIF2 (Terenin et al. 2013). GTP hydrolysis by the TC is critical for the formation of the 48S preinitiation complex and placement of the initiator Met-tRNAi^{Met} into the 40S ribosomal A-site. eIF2 dissociates from the 48S preinitiation complex in the GDP-bound state and binds to eIF2B, which catalyzes the exchange of GDP to GTP on eIF2, allowing the regeneration of the eIF2 ternary complex for subsequent rounds of translation initiation (Aitken and Lorsch 2012; Sharma et al. 2016). The two nucleotide-bound states of eIF2 (GTP- and GDP-bound, respectively) provide a critical role in translational control during cellular stress. Phosphorylation of the α subunit of eIF2 at serine 51 sequesters eIF2B bound to eIF2, forming an inactive complex (Aitken and Lorsch 2012; Pakos-Zebrucka et al. 2016). Preventing the regeneration eIF2•GTP results in decreased TC availability, thus inhibiting overall translation (Chen et al. 2015). Chen *et al.* (2015) proposed a mechanism wherein hOLA1 binds with the eIF2•GTP complex and facilitates the hydrolysis of GTP, and the resulting eIF2α•GDP complex is unable to bind to Met-tRNA$_i^{Met}$ thereby preventing further rounds of translation initiation (Chen et al. 2015). Hence, in addition to eIF2α-phosphorylation, hOLA1 provides an alternative mechanism to inhibit TC formation (Figure 4) (Chen et al. 2015).

TC availability has a crucial role in the regulation of ISR signaling (Chen et al. 2015; Pakos-Zebrucka et al. 2016). A class of genes that are particularly sensitive to TC availability encodes upstream open reading frames (uORFs) in the 5' UTR of the mRNA. One example of a uORF-regulated gene encodes activating transcription factor 4 (ATF4) (Barbosa et al. 2013; Sharma et al. 2016). During normal conditions (when TC is abundant), translation initiation occurs from start codons of uORFs and is more likely to re-initiate at a uORF than the start codon of the main coding ORF, inhibiting translation initiation of ATF4 (Barbosa et al. 2013; Pakos-Zebrucka et al. 2016; Sharma et al. 2016). Under stress conditions, eIF2α phosphorylation causes TC deficiency, which leads to delayed ribosome re-initiation and increases the translation of ATF4 (Barbosa et al. 2013; Sharma et al. 2016). As hOLA1 suppresses TC formation and regeneration

of active TC (Chen et al. 2015), hOLA1 depletion would lead to increased TC levels and thereby decrease ATF4 translation.

2.3 hOLA1 in cell cycle regulation and cancer

2.3.1 hOLA1 in CHOP signalling and apoptosis

Interestingly, it was shown that hOLA1-depleted cells grew to a much larger size and into late-stage tumors with more extensive metastasis in vivo (Chen et al. 2015). This phenotype is likely caused by inhibition of apoptosis (programmed cell death) and greatly reduced levels of CHOP (CCAAT-enhancer-binding protein homologous protein) (Oyadomari and Mori 2004; Schultz and Harrington 2003). CHOP is a transcription factor that regulates apoptosis and is a direct target of ATF4 (Oyadomari and Mori 2004). It regulates apoptosis by repressing anti-apoptotic proteins, such as Bcl-2, and inducing pro-apoptotic proteins, such as DOC1, DOC4, and DOC6 (Oyadomari and Mori 2004; Schwartzman and Cidlowski 1993). Accordingly, under tumor stress conditions, hOLA1 interacts with eIF2, subsequently inhibiting TC formation and promoting activation of the ISR (Chen et al. 2015). However, when hOLA1 is depleted, decreased ISR-CHOP signaling leads to increased cellular survival (Chen et al. 2015). Accordingly, hOLA1-depleted cells displayed advanced tumor growth due to the decreased apoptosis in response to intra-tumoral stress (Chen et al. 2015). Hence, hOLA1 could be an essential factor in deciding cell fate in solid tumors. Moreover, in orthotopic xenograft breast cancer models, hOLA1-depleted cells demonstrated higher invasiveness, possibly through increased survival and metastasis in unfavorable conditions (Chen et al. 2015). Interestingly, a higher chance of cancer relapse in patient-based studies was correlated with a lower expression of hOLA1 in these individuals (Chen et al. 2015). Conversely, the same study revealed that hOLA1 mRNA levels were higher in tumors than those in the adjacent noncancerous tissues (Chen et al. 2015).

2.3.2 hOLA1 in cell cycle regulation

Most recently, hOLA1-null mouse embryos were demonstrated to be small for their gestational stage and to have high perinatal lethality due to delayed development and organ immaturity—in particular, they displayed lung immaturity (Ding et al. 2016). hOLA1 knockout mouse embryos showed a growth retardation phenotype compared to control mice of the same gestational age as a result of decreased cell cycle progression (Ding et al. 2016). The cell cycle is highly regulated to prevent mutations and uncontrolled cell growth, which can lead to such consequences as tumor formation. Cyclins and cyclin dependent kinases (CDKs) are required for the transition from one phase of the cell cycle to the next in mammalian cells (Bertoli et al. 2013; Nurse 2017). The G1 to S phase transition requires the activation of the cyclin D-cdk4/6 and cyclinE-cdk2 complexes that phosphorylate Rb (Retinoblastoma protein) and that release transcription factor E2F, allowing cells into the S phase. The G2 to M transition requires the formation of the cyclin B-Cdk1 complex by dephosphorylation of CDK1. These phase transitions are negatively regulated by cyclin-dependent kinase inhibitors: p21 and p27, both up-regulated by p53 (Nurse 2017).

Notably, hOLA1 knockout MEFs demonstrated both G1/S and G2/M delay due to lower levels of cyclin D1 and cyclin E1 but had no change in cyclin B1 levels (Ding et al. 2016). Lower levels of cyclins D1 and E1 were the result of increased p21 and p53 levels, known cell cycle inhibitors (Ding et al. 2016). Moreover, it is shown that increased p53 levels were due to enhanced protein stability, and the accumulation of p21 resulted from increased mRNA translation. In addition, it is known that hOLA1 suppresses protein synthesis by interacting with eIF2, thus inhibiting TC formation (Chen et al. 2015). Knockout of hOLA1 leads to baseline ATF4 expression without a change in eIF2α phosphorylation, suggesting that increased translation of p21 could be due to increased levels of eIF2 in the absence of its suppressor, hOLA1 (Chen et al. 2015; Ding et al. 2016). Accordingly, the impaired cell cycle progression resulted from reduced G1/S-specific cyclins and translationally increased cyclin-dependent kinase inhibitor p21 in the absence of hOLA1, suggesting that hOLA1 is a translational regulator of p21 (Chen et al. 2015; Ding et al. 2016).

2.3.3 hOLA1 as a GSK-3-PP1 regulator

Further studies to define a mechanism for aggressive tumor growth in xenograft models of colon cancer (using H116 cells) and ovarian cancer (using SKOV3 cells) revealed hOLA1 as a

GSK-3/PP1 regulator (Beurel et al. 2015; Xu et al. 2016). GSK-3 (glycogen synthase kinase 3) is a multifunctional serine/threonine kinase that regulates various signaling pathways by phosphorylating serine/threonine and has also been implicated in cancer (Beurel et al. 2015; Xu et al. 2016). The activity of GSK-3 itself is controlled by phosphorylation along with protein-protein interactions and intracellular localization influencing its phosphorylation activity (Beurel et al. 2015). In opposition to GSK-3 is phosphatase protein 1 (PP1) which acts by dephosphorylating serines/threonines of many GSK-3 substrates. PP1 itself is regulated by two different subunits: inhibitor-1 and inhibitor-2. Multiple phosphoproteins found in both ISR and non-ISR pathways demonstrated hypo-phosphorylation in hOLA1-depleted cells, such as eIF2α, GSK-3β, I-2, and Pin1 (Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, an enzyme that regulates the cell cycle) and reduced levels of snail (a transcription factor). When hOLA1 was ectopically expressed in hOLA1-knockdown cells, the phosphorylation levels were restored, implicating hOLA1 directly or indirectly in the phosphorylation of Ser/Thr (Xu et al. 2016). Interestingly, hOLA1-depleted cells were reported to show increased PP1 activity (Xu et al. 2016). Further studies revealed that hOLA1 regulates PP1 activity indirectly through its interaction with GSK3β, thus blocking its phosphorylation of I-2 in an ATP-dependent manner (Xu et al. 2016). Hence, hOLA1 was characterized as a new GSK3β inhibitor. Additionally, the GSK3β-I-2-PP1 positive feedback loop was found to stimulate both PP1 and GSK3β in hOLA1 depleted cells, resulting in decreased Ser/Thr phosphorylation (Xu et al. 2016). Accordingly, suppression of the GSK3β-I-2-PP1 positive feedback loop by hOLA1 could lead to less aggressive tumor growth phenotypes (Figure 5).

2.3.4 hOLA1 in cancer metastasis

Cell adhesion plays a key role in cancer metastasis. On the inner surface of the cell membrane, clustered integrin (induced to form by the activation of cell membrane receptors) recruits various adaptor and signaling proteins. Overexpression of focal adhesion kinases (FAKs) leads to the formation of FAs (focal adhesions), cell spreading, and migration (Albelda 1993). An enhanced cell adhesion phenotype was demonstrated in hOLA1-depleted cells, due to increased expression of FAKs (Jeyabal et al. 2014). Additionally, hOLA1 depletion resulted in over-activation of cofilin (an actin binding protein that regulates assembly and disassembly of actin filaments) by decreased

phosphorylation (Jeyabal et al. 2014). Conversely, increased expression of hOLA1 resulted in inactive cofilin by phosphorylation (Jeyabal et al. 2014). hOLA1 depletion and reduced phosphorylation resulted in enhanced actin polymerization and dynamics (Jeyabal et al. 2014). Accordingly, hOLA1 regulates cell adhesion *via* FAK signaling and a specific enzyme activity that modulates cofilin phosphorylation. Notably, hOLA1 interaction with BRCA1 and its inhibitory effects on centrosome regulation could explain why hOLA1 depleted cells acquire genetic instability required for tumor progression (Gomez and Hergovich 2014; Jeyabal et al. 2014).

2.3.5 hOLA1 in breast cancer progression

Breast cancer is one of the leading causes of cancer mortality worldwide. 5-10% of breast cancer cases, and some ovarian cancer cases, have hereditary causes (Takahashi et al. 2017) and have been aptly termed hereditary breast and ovarian cancer (HBOC) (Matsuzawa et al. 2014). Approximately 30% of these individuals have germline mutations in either the breast cancer associated gene 1 or 2 (BRCA1 or BRCA2). hOLA1 has been shown to interact with BRCA1 in complex with BRCA1-associated RING domain protein (BARD1) and γ-tubulin, a component of the centrosome (Chiba 2015; Gomez and Hergovich 2014; Matsuzawa et al. 2014). It is this interaction with γ-tubulin that implicated BRCA1 and hOLA1 in centrosome biology. Overall, it is the C-terminus of hOLA1 that interacts directly with the C-terminus of BARD1, and yet hOLA1 does not require BARD1 to bind to BRCA1's N-terminus and γ-tubulin. Additional studies revealed that BRCA1/BARD1/hOLA1 complex is formed through the interaction of the N-terminal domains of both BRCA1 and BARD1 (Chiba 2015; Gomez and Hergovich 2014; Matsuzawa et al. 2014). As a result, hOLA1 could regulate various centrosome functions together with BRCA1 (Gomez and Hergovich 2014; Matsuzawa et al. 2014). A mutation in the hOLA1 gene (E168Q) has been reported to impair its ability to bind BRCA1 and failed to rescue centrosome regulation (Gomez and Hergovich 2014). Additionally, BRCA2 localizes to the centrosome and downregulation of BRCA2 resulted in centrosome amplification. Mutations in BRCA2 would then lead to aberrant regulation of centrosomes and may be a key dysfunction leading to carcinogenesis in HBOC (Chiba 2015; Gomez and Hergovich 2014; Matsuzawa et al. 2014). Interestingly, mutations in hOLA1 were observed in the germline of patients with HBOC

without BRCA1 or BRCA2 mutations (Takahashi et al. 2017). Additionally, there could be large insertions, deletions, or epigenetic alterations in hOLA1 that contribute to tumorigenesis in HBOC patients (Takahashi et al. 2017).

In addition to breast cancer, somatic mutation of hOLA1 has been reported in other types of malignancies: colorectal, uterus, ovary, skin, and liver cancers (Friedenson 2005). Further, overexpression of hOLA1 has been observed in ovarian, colorectal, and lung cancers, which suggests that the dysregulation of hOLA1—and the resulting dysregulation of centrosome dynamics—is associated with carcinogenesis in numerous organs (Chiba 2015; Friedenson 2005; Gomez and Hergovich 2014).

2.3.6 hOLA1 in lung cancer progression

hOLA1 plays many critical roles in diverse intrinsic cellular stresses: heat-shock, changes in cell migration and invasiveness, ISR, and DNA damage stresses (Chen et al. 2015; Mao et al. 2013b; Zhang et al. 2009c). However, it is important to study the clinical relevance of hOLA1 and its underlying mechanisms (Bai et al. 2016). It was demonstrated that hOLA1 expression negatively correlates with that of E-cadherin. E-cadherin is a transmembrane protein involved in cell adhesion and is the epithelial-mesenchymal transition (EMT) marker in lung cancer (Bai et al. 2016). Cancers where hOLA1 is over-expressed generally have a poor prognosis, particularly in lung carcinoma (Bai et al. 2016). Furthermore, high levels of hOLA1 are associated with advanced TNM staging, which is a notation system that describes the size of the original tumour (T), the regional lymph nodes that are involved (N), and the distant metastisis (M) of a given cancer (Bai et al. 2016). An inverse correlation between hOLA1 and E-cadherin protein levels can be seen in all types of lung cancers, which is mediated through GSK-3 and Snail (Bai et al. 2016). hOLA1 blocks GSK-3β activity (as described in Section 2.5) which, in turn, prevents the GSK-3β phosphorylation of Snail for degradation. Snail is a transcriptional suppressor of E-cadherin; therefore, at high levels of hOLA1, as seen in lung cancer, E-cadherin is repressed by the higher levels of Snail (Bai et al. 2016). Moreover, the TGS domain of hOLA1 is required for the binding of GSK3β. This was the first finding that the TGS domain of hOLA1 is essential for its overall function (Bai et al. 2016; Koller-Eichhorn et al. 2007). Accordingly, hOLA1 has a negative correlation with GSK3β activity and E-cadherin protein levels, and a stabilizing effect on snail,

thus regulating EMT (Bai et al. 2016). Altered activity of GSK3β and/or snail could be the reason for decreased cell migration and invasiveness along with the increased cell-matrix adhesion in hOLA1-depleted cells (Bai et al. 2016).

3.0 Cellular roles of YchF in bacteria and plants

3.1 Involvement in Oxidative Stress

Bacterial YchF has been implicated in a number of cellular processes, ranging from protein synthesis (Teplyakov et al. 2003; Tomar et al. 2011b) and ribosome biogenesis (Teplyakov et al. 2003) to regulation of iron usage (Cheung et al. 2016; Danese et al. 2004) and oxidative stress response (Hannemann et al. 2016). Of these, oxidative stress response and the interaction with translation components are regulatory functions that YchF has in common with hOLA1 (Chen et al. 2015; Zhang et al. 2009c). In both *E. coli* and human cells (HeLa and BEAS-2B cell lines), overexpression of YchF or hOLA1 respectively confers a greater sensitivity of cells to oxidative stress (Wenk et al. 2012; Zhang et al. 2009a). It was recently shown in *E. coli* that cysteine-35 of EcYchF allows for dimerization under oxidative conditions, which reduces the ATPase activity of EcYchF (Hannemann et al. 2016). This cysteine is conserved in most other species, including hOLA1 (Figure 1), suggesting that dimerization of hOLA1 is also possible, but this has not yet been confirmed. Furthermore, *E. coli* expressing an ATPase-deficient variant of YchF do not display hypersensitivity to hydrogen peroxide, suggesting that ATP hydrolysis is required for the inhibition of oxidative stress response (Wenk et al. 2012).

It has been shown that EcYchF interacts with antioxidant enzymes KatG, KatE, and AhpCF (Table 1) under non-stress conditions (Wenk et al. 2012), potentially inhibiting their function until needed. Under oxidative stress, the dimerization of YchF presumably results in the release of bound antioxidant enzymes, allowing for these enzymes, such as catalase KatG, to detoxify the cell of hydrogen peroxide. This would suggest that YchF can regulate endogenous hydrogen peroxide concentrations through its control of these antioxidant enzymes. The inactivated YchF homodimer can then interact with thioredoxin 1 (TrxA) or other redox controlling enzymes (Table 1) which reduce the cysteines involved in dimerization, returning YchF to its monomeric state where it can once again inhibit oxidative stress response (Hannemann et al. 2016). Interestingly,

the Koch lab proposes that the reason that YchF and homologs have evolved to utilize ATP over GTP may be due to guanine being particularly sensitive to oxidation (Hannemann et al. 2016). Additionally, the cellular level of ATP raises upon oxidative stress, most-likely due to the inhibition of processes using ATP (Akhova and Tkachenko 2014). This raises the question of the organisms where the YchF/hOLA1 homolog unambiguously uses ATP or GTP. The rice homolog, OsYchF1, is one such homolog where ATP binding has been shown to be important for biotic stress response but not abiotic stress, while the opposite is true for GTP (Cheung et al. 2016).

3.2 Interaction of YchF with the Ribosome

The interaction between YchF and the bacterial ribosome is the most extensively characterized of its proposed functional roles *in vivo*. In *E. coli*, YchF has been shown to interact with the ribosome, suggesting a role in translation and/or ribosome biogenesis (Becker et al. 2012a), while in *Arabidopsis thaliana* the YchF homolog (AtYchF) has been confirmed to be involved in ribosome biogenesis (Olinares et al. 2010). Polysome profile analysis found that YchF co-fractionates with the 50S ribosomal subunit and the 70S ribosome in *E. coli* (Tomar et al. 2011a) and with the 60S/40S ribosomal subunits, the 80S ribosome, and polysomes in *T. cruzi* (Gradia et al. 2009). This interaction suggests a functional role for YchF with the ribosome during protein synthesis or potentially during ribosome biogenesis. Furthermore, an unknown component of the 70S ribosome stimulates the ATPase activity of YchF, indicating that, in bacteria, the 70S ribosome acts as a GTPase activating factor (GAP) for YchF (Becker et al. 2012a). Currently, it is not known at what stage of protein synthesis or ribosome biogenesis YchF is involved. Further work is needed to elucidate where on the ribosome YchF binds and the functional ribosome complex(es) on which YchF acts.

Table 1 – Confirmed protein or RNA interacting partners of hOLA1 and its homologs.

Interacting Partner	**Organism(s) in which interaction confirmed**	**Functional role of the interacting partner**	**Proposed functional interaction with YchF/hOLA1**	**Reference(s)**

Ribosomal Protein L26	*T. cruzi*	Ribosomal protein in the large subunit	Potential YchF binding site on the ribosome	(Gradia et al. 2009)
Ribosomal Protein S7	*T. cruzi*	Ribosomal protein in the small subunit	Potential YchF binding site on the ribosome	(Gradia et al. 2009)
RPN10	*T. cruzi*	Non-ATPase subunit of the proteasome	Potential binding site on the proteasome	(Gradia et al. 2009)
80S (70S) ribosome	*T. cruzi*, *E. coli, H. sapiens* (A549 cells)	Monomer of the full ribosomal complex	Undetermined functional role during protein synthesis, ribosome biogenesis, stress response	(Becker et al. 2012a; Gradia et al. 2009; Tomar et al. 2011a)
Polysomes	*T. cruzi*, *E. coli, H. sapiens* (A549 cells)	Multiple ribosomes actively translating along one mRNA	Undetermined functional role during protein synthesis, ribosome biogenesis, stress response	(Gradia et al. 2009; Tomar et al. 2011a)
60S (50S) large ribosomal subuntis	*T. cruzi*, *E. coli, H. sapiens* (A549 cells)	Large subunit of the ribosome	Undetermined functional role during protein synthesis, ribosome biogenesis, stress response	(Becker et al. 2012a; Gradia et al. 2009; Tomar et al. 2011a)
40S (30S) small ribosomal subunit	*T. cruzi*, *E. coli, H. sapiens* (A549 cells)	Small subunit of the ribosome	Undetermined functional role during protein synthesis, ribosome biogenesis, stress response	(Becker et al. 2012a; Gradia et al. 2009)
26 S proteosome	*S. cerevisiae*	26 S proteasome involved in protein degradation	Unclear; confirmed Proteasome interacting protein	(Guerrero et al. 2006)
KatG	*E. coli*	Catalase	YchF negatively regulates antioxidant activity through inhibition of KatG	(Hannemann et al. 2016; Wenk et al. 2012)
KatE	*E. coli*	Catalase	YchF negatively regulates antioxidant activity potentially through inhibition of KatE	(Hannemann et al. 2016; Wenk et al. 2012)
AhpCF	*E. coli*	Alkyl hydroperoxide reductase	YchF negatively regulates antioxidant activity	(Hannemann et al. 2016; Wenk et al. 2012)

			potentially through inhibition of AhpCF	
Dps	*E. coli*	Iron scavenging protein	Unclear	(Wenk et al. 2012)
Gag polyprotein	*H. sapiens* (Jurkat and HEK293) + HIV-1	Virion assembly, Plasma membrane interaction, Packaging of viral RNA	Unclear	(Jager et al. 2011)
HSP70	*H. sapiens* (HEK-293T cells)	Heat-shock protein 70	hOLA1 stabilizes and protects HSP70 degradation	(Mao et al. 2013a)
OsGAP1	*O. satvia*	GTPase activating protein for OsYchF1 in plants	OsGAP1 regulates OsYchF1 GTPase activity	(Cheung et al. 2008)
BARD1	*H. sapiens* (HEK-293T cells)	BRCA1-associated RING domain protein; ubiquitin ligase inconjuction with BRCA1	hOLA1 interacts with the BARD1:BRCA1:γ-Tubulin complex to regulate centromeres	(Matsuzawa et al. 2014)
BRCA1	*H. sapiens* (HEK-293T cells)	Breast cancer-associated gene 1; ubiquitin ligase inconjuction with BARD1	hOLA1 interacts with the BARD1:BRCA1:γ-Tubulin complex to regulate centromeres	(Matsuzawa et al. 2014)
γ-Tubulin	*H. sapiens* (HEK-293T cells)	Microtubule protein found in centromeres and spindle pole bodies	hOLA1 interacts with the BARD1:BRCA1:γ-Tubulin complex to regulate centromeres	(Matsuzawa et al. 2014)
eIF2α	*H. sapiens* (HEK-293T cells)	Protein synthesis initiation factor	hOLA1 binds eIF2α and hydrolyzes all local GTP preventing eIF2α•GTP•Met-tRNAi ternary complex formation thus blocking initiation of translation	(Chen et al. 2015)
GSK3β	*H. sapiens* (HEK-293T cells)	Serine/threonine kinase	Unclear	(Xu et al. 2016)
TrxA	*E. coli*	Thioredoxin 1; redox homeostasis controlling protein	Binds to YchF homodimers and dissociates them by reducing the disulfide bridge	(Hannemann et al. 2016)

TrxC	*E. coli*	Thioredoxin 2; redox homeostasis controlling protein	Presumably reduces the disulfide bridge of YchF homodimers like TrxA; unconfirmed role	(Hannemann et al. 2016)
Bcp	*E. coli*	Peroxiredoxin; redox homeostasis controlling protein	Presumably reduces the disulfide bridge of YchF homodimers like TrxA; unconfirmed role	(Hannemann et al. 2016)
Glutaredoxin 4	*E. coli*	redox homeostasis controlling protein	Presumably reduces the disulfide bridge of YchF homodimers like TrxA; unconfirmed role	(Hannemann et al. 2016)

4.0 Comparing YchF and mammalian hOLA1

4.1 Structural comparison of hOLA1 and its YchF homologs

YchF and hOLA1 have similar overall structures (Figure 2). Structural information for YchF from microorganisms is available from crystal structures of *Haemophilus influenzae* (HiYchF), *Schizosaccharomyces pombe* (SpYchF), and two of *Thermus thermophilus* (TtYchF) (Teplyakov et al. 2003). The TtYchF crystal structure was solved in both the *apo*-state and the GDP-bound state, for YchF was initially thought to be a GTPase based on sequence homology. Additionally, the structure of the rice homolog (OsYchF1) has been solved in the *apo*, GDPNP-, and ADPNP-bound states (Cheung et al. 2016). Although the sequences are highly conserved, the sequence differences that do exist between YchF homologs allow for some species-specific interactions, such as the interaction between OsYchF1 and the membrane associating GTPase activating protein 1, OsGAP1, in rice (Cheung et al. 2013; Cheung et al. 2016). Recently, the catalytic residue responsible for ATP hydrolysis was identified as the conserved histidine 114 within a flexible extended loop in the G-domain (Rosler et al. 2015), which corresponds to histidine 134 in hOLA1. Interestingly, the serine residue in EcYchF (S16; *E. coli* numbering) involved in phosphorylation is conserved across species while the cysteine residue (C35; *E. coli* numbering) implicated in YchF dimerization is missing in some organisms but present in both *E. coli* and humans (Figure 1).

4.2 Oxidative stress response

Like hOLA1, EcYchF has been shown to negatively regulate oxidative stress response (see sections 2.1 and 3.1, respectively) (Wenk et al. 2012; Zhang et al. 2009a). Direct involvement in oxidative stress response can be found for EcYchF as EcYchF has been shown to co-purify with several catalases (Table 1). EcYchF overexpression inhibits KatG and other catalase activity under normal growth conditions, preventing unnecessary catalase activity, presumably to help maintain homeostasis (Wenk et al. 2012). These functional interactions are relevant with respect to the phenotypic results from overexpression and knockdown/knockout of hOLA1/YchF. Overexpression leads to hypersensitivity to oxidative stress, which would be explained by the presence of excess hOLA1/YchF inhibiting the function of catalases and peroxidases, allowing the accumulation of ROS. In the absence of YchF/hOLA1, cells are more resistant to oxidizing agents and thiol-depleting compounds (Hannemann et al. 2016; Zhang et al. 2009a), likely due to the lack of catalase inhibition. To date, there have been no studies that show hOLA1 interacting directly with any catalases or other antioxidant enzymes in other organisms.

4.3 Protein Synthesis Involvement

hOLA1 has been implicated in translation initiation in humans (Chen et al. 2015). In bacteria, however, translation initiation occurs by a mechanism that primarily entails the binding of the 30S ribosomal subunit to the three bacterial initiation factors (IF1-3) and the initiator tRNA fMet•tRNA^{fMet} (Milon et al. 2012). Following the 30S-initiation factor complex formation, the 30S subunit binds to the Shine-Dalgarno sequence in the 5' untranslated region of the mRNA before selection of the start codon. This mechanism differs greatly from that employed by mammalian cells. Notably, the eIF2α-initiator tRNA complex—the formation of which hOLA1 represses in humans—differs significantly from its bacterial counterpart. To date, there is no evidence suggesting that YchF interacts with IF2 or fMet•tRNA^{fMet} to regulate translation initiation in bacteria. The fact that only the 70S ribosome stimulates the ATPase function of YchF suggests that, in bacteria, YchF may be involved in a step of translation after initiation or perhaps a late stage in ribosome biogenesis, when the near-mature ribosomal subunits are tested for functional

70S ribosome formation before being added into the pool of actively translating ribosomes. Furthermore, the observation that both YchF and hOLA1 interact with the 70S/80S ribosome and polysomes, respectively, supports the hypothesis that both factors are involved in a step(s) downstream of translation initiation. However, no exact functional role outside of translation initiation has been postulated as of yet. Currently, it is not known if the human cytosolic or mitochondrial ribosomes can act as a GAP for hOLA1.

Previous studies in *T. cruzi* have also shown that TcYchF interacts with the proteasome and go on to speculate that YchF bridges the ribosome and proteasome, allowing for degradation of proteins damaged during synthesis (Gradia et al. 2009). Furthermore, the yeast homolog of YchF (YBR025c) was also implicated in interacting with the 26 S proteasome via *in vivo* protein-protein cross-linking followed by mass spectroscopic analysis (Guerrero et al. 2006). The functional implications for YchF interacting with the proteasome have not been further characterized to date, but would provide an interesting link between protein synthesis and degradation. One potential mechanism follows: during oxidative stress, damage could occur to the translation machinery or the protein being synthesized. YchF would bind to the damaged ribosome, or to the ribosome with the damaged-polypeptide, and recruit the proteasome to degrade the polypeptide before it can cause any negative downstream effects and/or allow repair of the ribosome.

5.0 Future Perspectives

Cells are often challenged by diverse stresses, such as hypoxia, oxidative stress, and nutrient deprivation. A failure of cells to respond to such stresses can lead to severe disease states, such as cancer. hOLA1 plays crucial roles in tumorigenesis, antioxidant suppression, inhibition of global protein synthesis, cell cycle regulation, cell migration, invasiveness, and cell adhesion (Bai et al. 2016; Chiba 2015; Jeyabal et al. 2014; Xu et al. 2016; Zhang et al. 2009a). However, the regulation of translation by hOLA1 is poorly understood, as *in vitro* assays to study the direct effect of hOLA1 in eIF2α phosphorylation or de-phosphorylation have failed. The interaction of hOLA1 with eIF2 may not be the only mechanism for the regulation of translation, or hOLA1 might not interact directly with eIF2. It is necessary to further study the hOLA1-eIF2 interaction both *in vitro* and *in*

vivo. For instance, further studies are required to determine the binding site of hOLA1 on eIF2. Moreover, the mechanism by which hOLA1 might facilitate hydrolysis of eIF2-bound GTP is unclear. It would be interesting to study whether hOLA1 directly hydrolyzes GTP bound to eIF2, stimulates eIF2s GTPase activity, or functions as an exchange factor removing the GTP from eIF2 and then allowing GDP to bind eIF2α. Alternatively, hOLA1 may prevent either nucleotide exchange (GDP to GTP on eIF2) or hydrolyze all the local GTP prior to GTP binding to eIF2. Additionally, both suppression and promotion of the ISR have been reported to inhibit tumor growth *in vivo*, suggesting a dual role of the ISR and hOLA1 in promoting cellular survival or apoptosis. Moreover, quantitative studies of translation by polysome profiling and ribosome profiling (i.e. RiboSeq) may reveal if there is a specific mRNA, or subset of mRNAs, that hOLA1 targets *in vivo*. For instance, it would be interesting to study whether hOLA1 specifically regulates non-canonical translation of mRNAs encoding IRESes or uORFs. The mechanism underlying decreased levels of apoptosis in hOLA1-depleted cells also remains unclear. Further studies are needed to elucidate whether hOLA1 affects translational elongation and termination, as shown in yeast. hOLA1 downregulation is known to cause cancer relapse (Chen et al. 2015), yet the underlying mechanism is still unclear. Moreover, hOLA1 was found to regulate the centrosome through its interaction with BRCA1, BARD1, and γ-tubulin. Subsequently, depletion of hOLA1 caused centrosome amplification. However, further studies are required to determine if the loss of hOLA1 could result in delayed mitosis or centrosome abnormalities. The interaction between hOLA1 and BRCA1 implicates hOLA1 as a DNA-damage regulating agent, for BRCA1 is a major regulator of DNA repair (Chiba 2015; Gomez and Hergovich 2014; Matsuzawa et al. 2014). Therefore, further characterization of hOLA1 in DNA damage repair is required. HSP70 is upregulated in several malignancies, and hOLA1 is known to interact with HSP70 to prevent its degradation. The interaction of hOLA1 with HSP70 was revealed by co-immunoprecipitation, and it is important to study whether hOLA1 acts as a molecular chaperone or a specific co-chaperone for HSP70, as hOLA1 failed to co-immunoprecipitate with the HSP70-CHIP complex (Mao et al. 2013b). An important hallmark of cancer cells is persistent survival under various stress conditions. As hOLA1 is implicated in the cellular response to such conditions, hOLA1 may represent a novel therapeutic target for cancer treatment.

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Figure Captions:

Figure 1: Sequence alignment of hOLA1/YchF homologs. Each homolog with a solved crystal structure (*H. influenzae*, *S. pombe*, *T. thermophilus*, *O. sativa*, and *H. sapiens*) was aligned against other well-studied YchF homologs (*E. coli* and *T. cruzi*). Identical amino acid residues are shaded black; similar residues are shaded grey. The conserved serine residue that is phosphorylated, the conserved cysteine residue implicated in dimerization, the conserved catalytic histidine, and the altered G4 motif are highlighted in green, red, orange, and blue, respectively. The sequence alignment was generated using T-coffee and Boxshade.

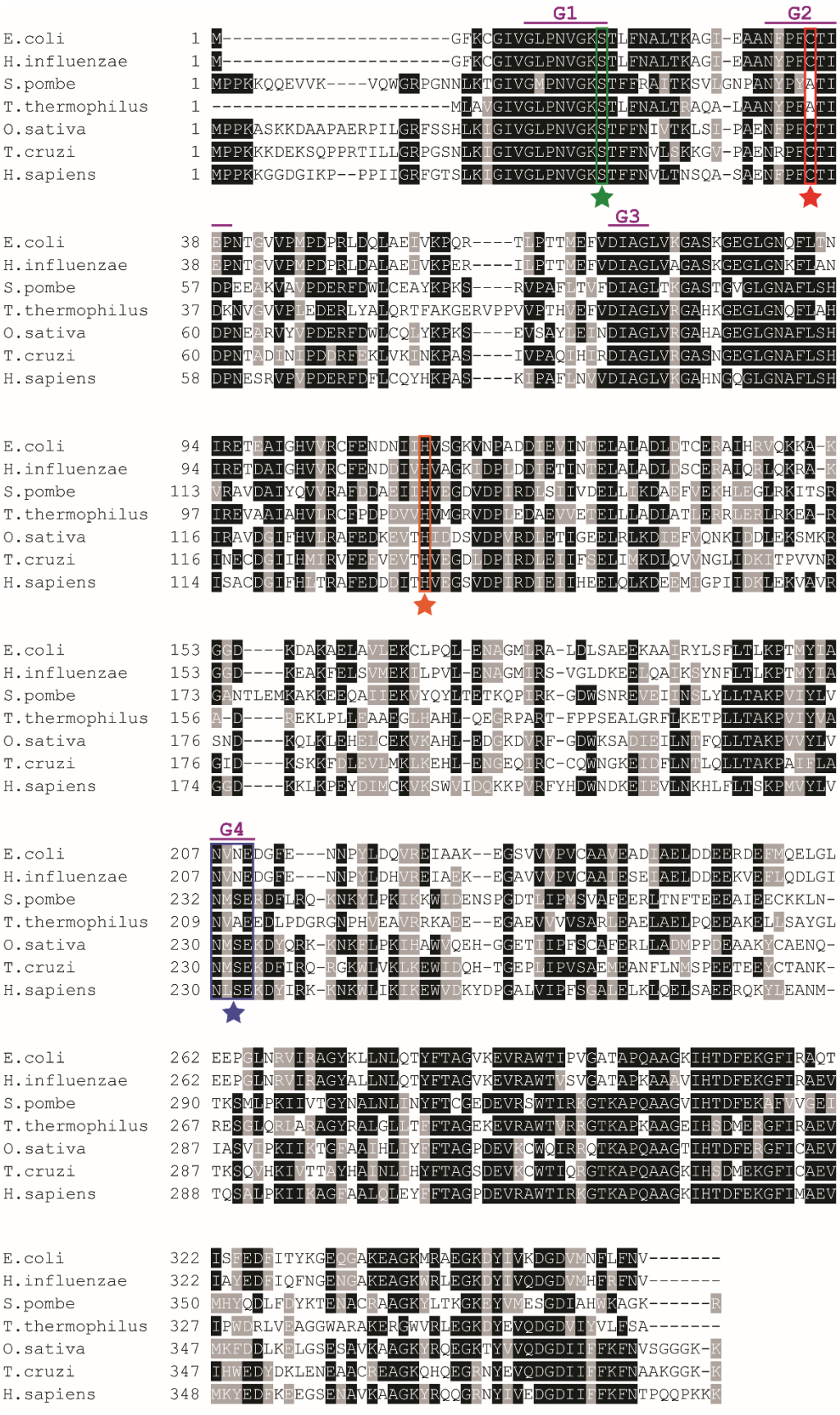
Figure 2: Structural alignment of hOLA1 and *H. influenzae* (Hi)YchF. Crystal structures of the hOLA1-AMPPCP (green/teal/blue; PDB 2OHF) and HiYchF (red/orange/yellow; PDB 1JAL) were aligned using pymol. No nucleotide was present in the crystallization of HiYchF. Both structures can be superimposed with minimal deviation. The three domains that are found in all YchF homologs are highlighted as follows: the G-domain is orange in HiYchF and teal in hOLA1, the alpha-helical domain is red in HiYchF and green in hOLA1, and the TGS-domain is yellow in HiYchF and blue in hOLA1. Differences in nucleotide bound states have previously been shown to induce structural changes within the G-domain of YchF (Rosler et al. 2015), and it is presumed that these nucleotide-dependent changes give rise to the slight deviations in the aligned structures. The bound AMPPCP from the hOLA1-AMPPCP crystal structure is shown as black sticks bound to the G-domain.

Figure 3: Model of HSP70-stabilization by hOLA1 to protect cells from heat shock. hOLA1 competes with CHIP (an E3 ligase) to bind at the C-terminal variable domain of HSP70 and protect it from CHIP-mediated ubiquitination, thus decreasing proteolytic degradation of HSP70.

Figure 4: Schematic diagram of translational control by hOLA1: In addition to the eIF2α phosphorylation mechanism to inhibit the regeneration of eIF2-GTP, hOLA1 facilitates the hydrolysis of GTP to eIF2-GDP (an inactive complex) *via* GTPase activity, thus limiting the

availability of TC. This leads to decreased global translation and increased selective translation. TC: Ternary Complex.

Figure 5: A representative model of hOLA1 regulation of the GSK-3β-I-2-PP1 signaling circuit. PP1 is inhibited by its inhibitory subunit I-2. This can be reactivated through phosphorylation of I-2 by GSK-3. Activated PP1 in turn dephosphorylates I-2 and GSK-3, promoting GSK-3 and PP1 activity in a positive feedback loop. hOLA1 acts as a suppressor of this loop by inhibiting GSK-3 activity.


G1
G2
G3
G4
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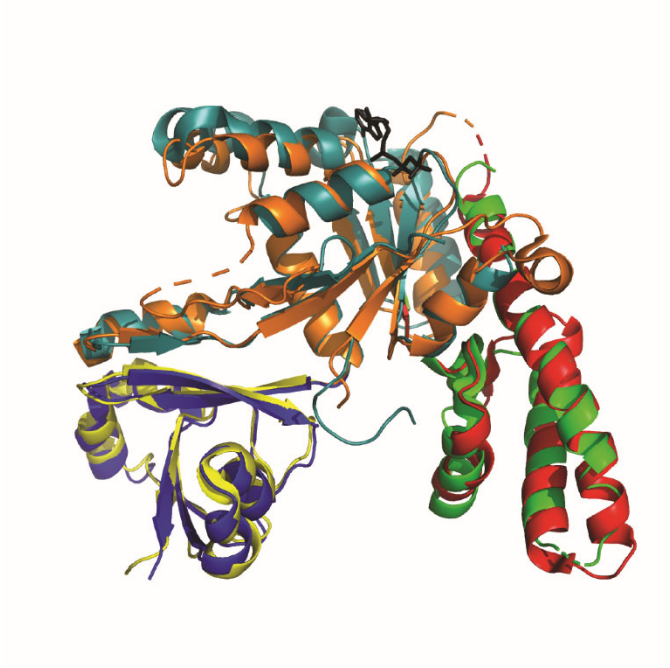
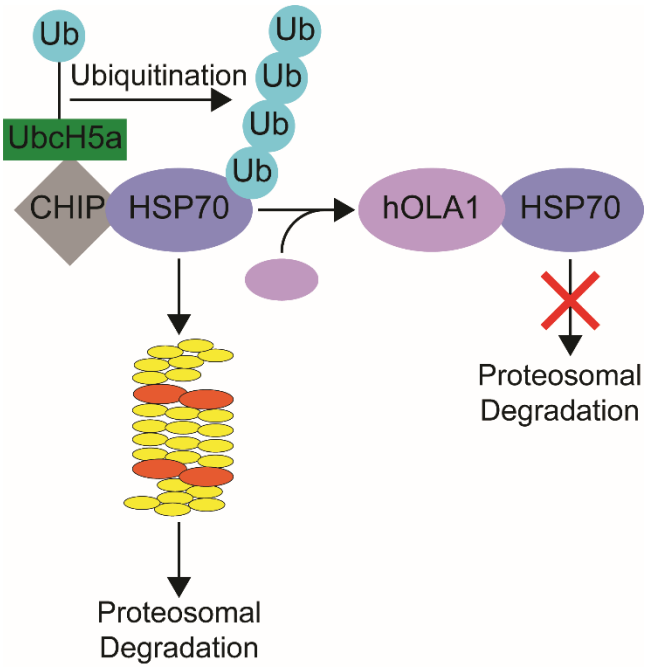
H.influenzae
S.pombe
T.thermophilus
O.sativa
T.cruzi
H.sapiens

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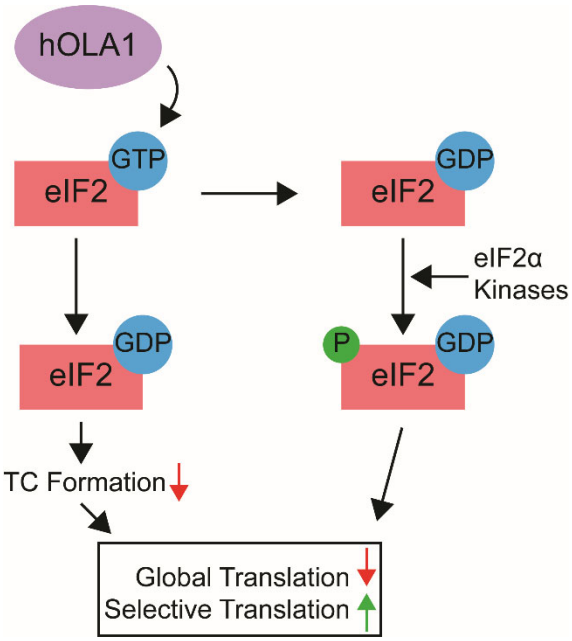
Ubiquitination
Ub
Ub
Ub
Ub
UbcH5a
CHIP
HSP70
hOLA1
HSP70
Proteosomal
Degradation
Proteosomal
Degradation


hOLA1
GTP
eIF2
GDP
eIF2
eIF2α
Kinases
GDP
eIF2
P
GDP
eIF2
TC Formation
Global Translation
Selective Translation

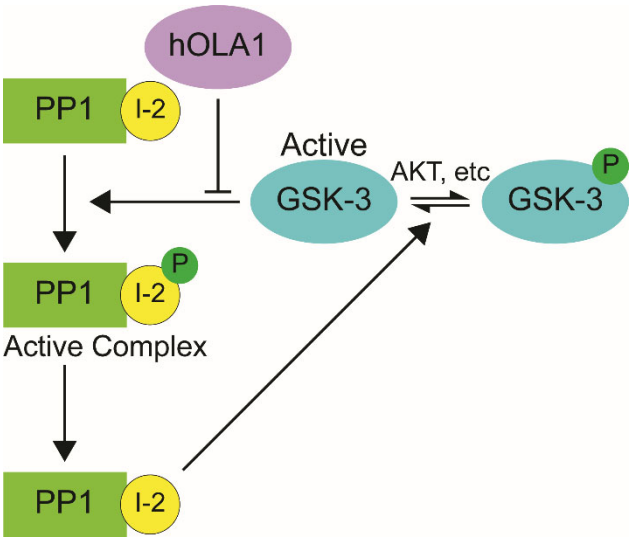
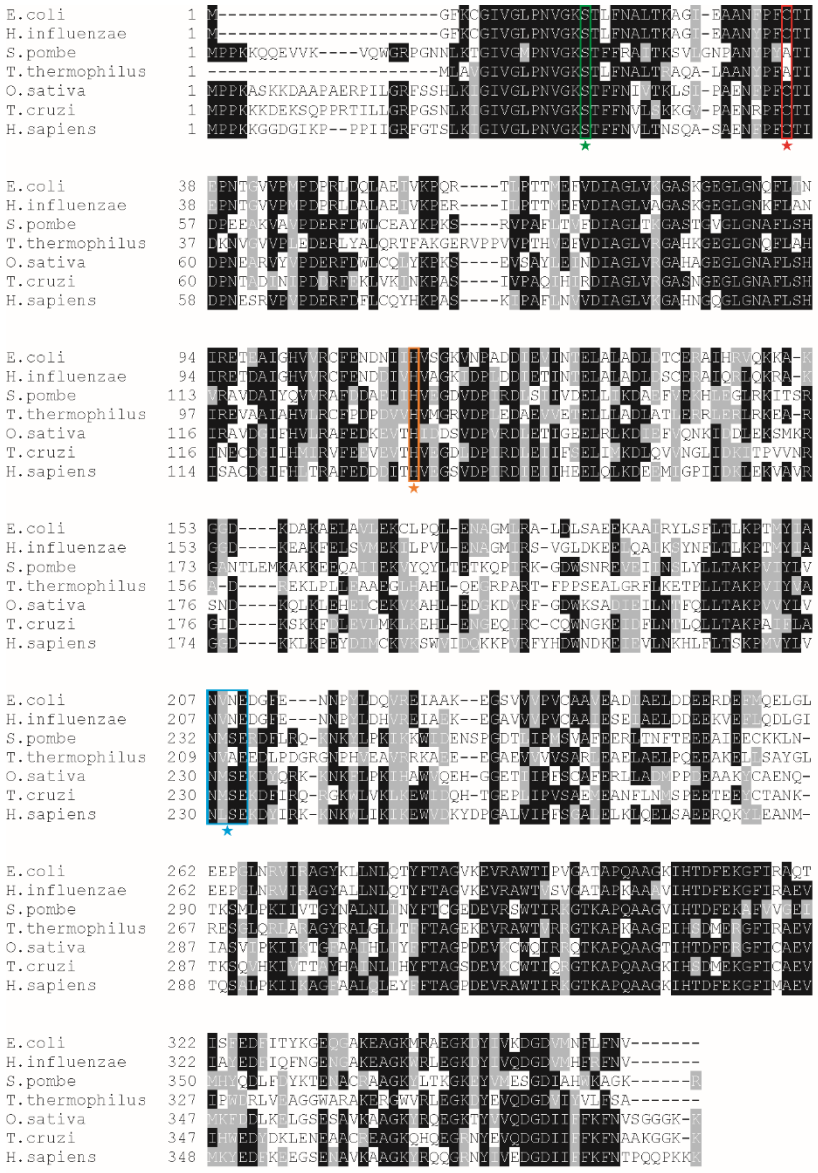

hOLA1
PP1
I-2
Active
GSK-3
AKT, etc
GSK-3
P
PP1
I-2
P
Active Complex
PP1
I-2

Figure 1: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T.thermophilus
O.sativa
T.cruzi
H.sapiens

Figure 2:

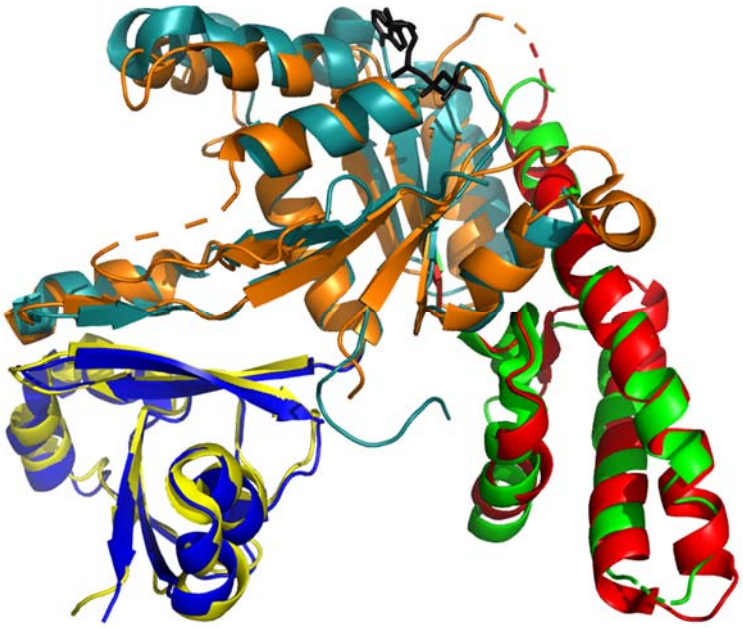


Figure 3

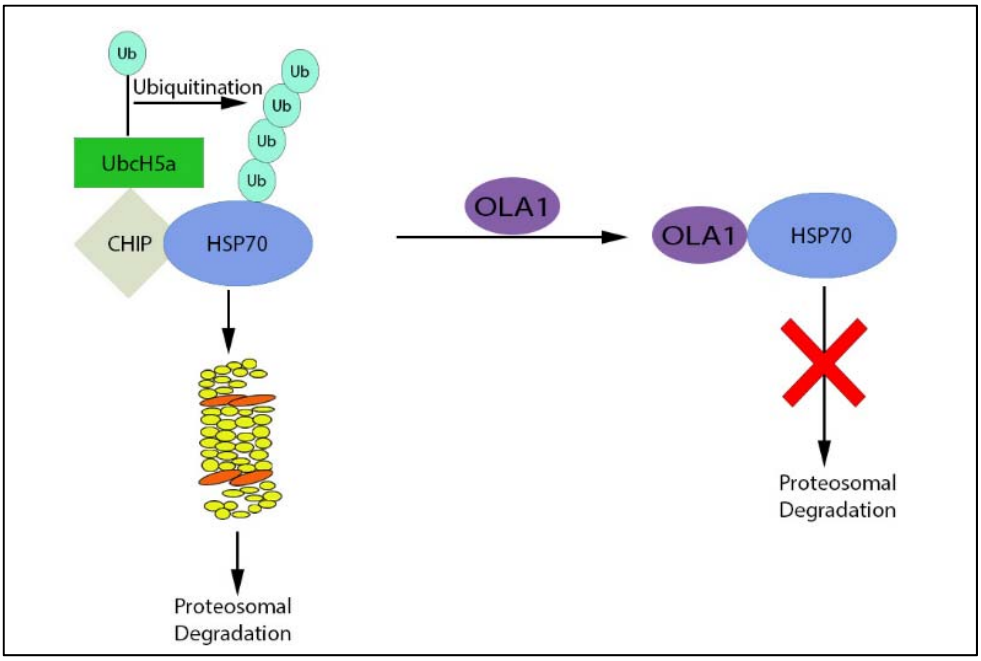

Ub
Ubiquitination
Ub
Ub
Ub
Ub
UbcH5a
CHIP
HSP70
Proteosomal
Degradation
OLA1
OLA1
HSP70
Proteosomal
Degradation

Figure 4

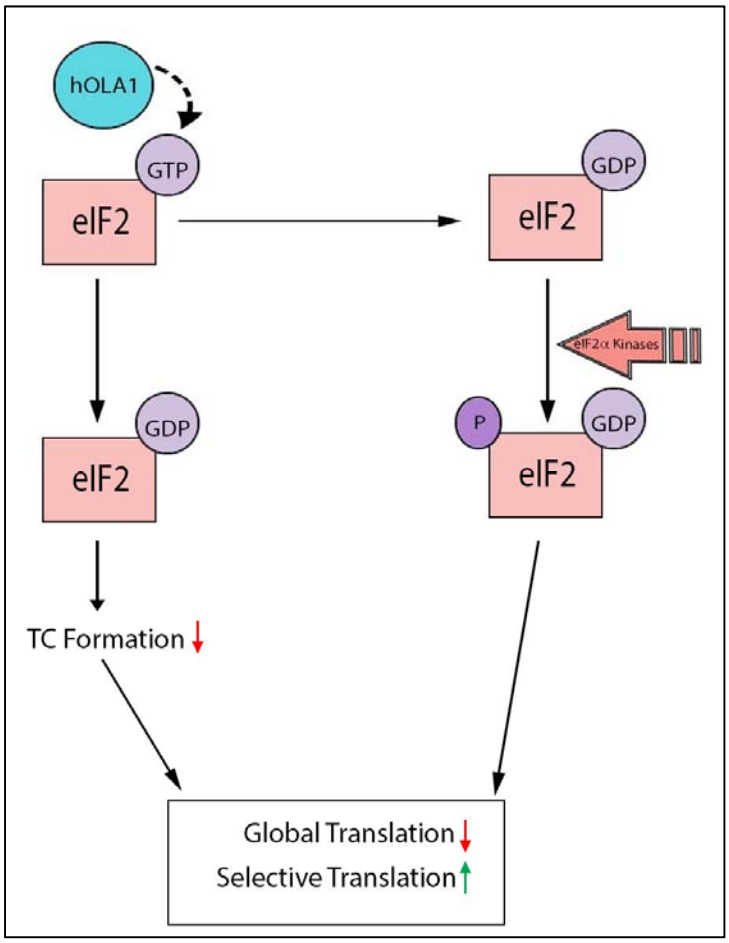
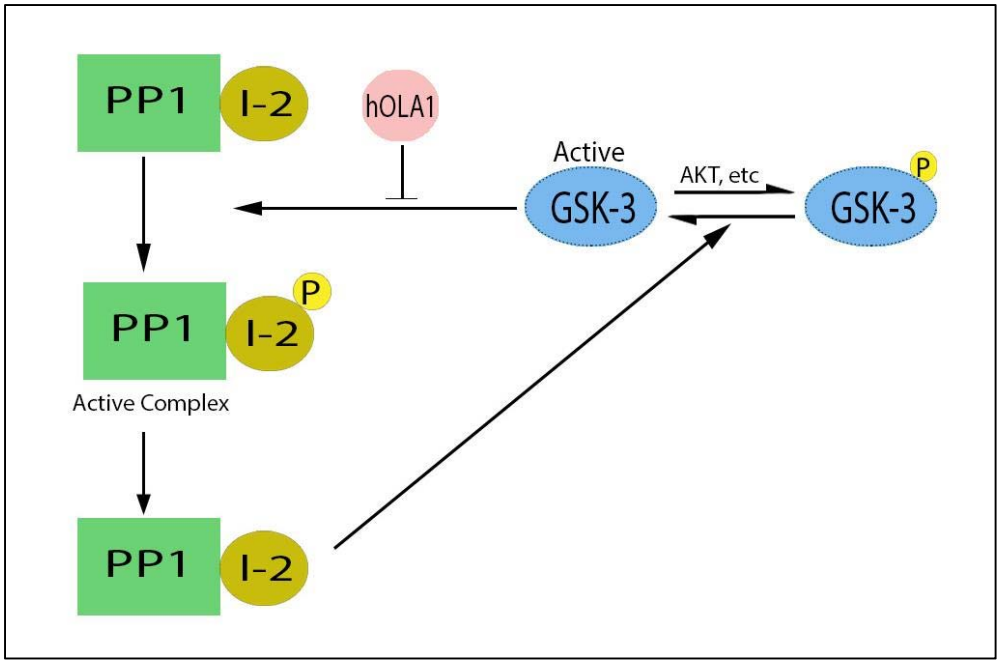

hOLA1
GTP
eIF2
GDP
eIF2
eIF2α Kinases
GDP
eIF2
P
GDP
eIF2
TC Formation
Global Translation
Selective Translation

Figure 5:


PP1
I-2
hOLA1
Active
GSK-3
AKT, etc
GSK-3
P
PP1
I-2
P
Active Complex
PP1
I-2