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Association of HSP90 with the heme-regulated eukaryotic initiation factor 2α kinase—A collaboration for regulating protein synthesis

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Abstract. Among the various heat shock proteins (HSPs), members of the HSP70 and HSP90 families have drawn particular attention due to their heat shock-unrelated functions. HSP90, an ubiquitous and abundant member of the HSP90 family has been shown to be associated with a large array of protein factors. These proteins reside in the nucleus as well as in the cytoplasm and are involved in various physiological processes, such as, regulation of chromatin structure, cell cycle, cytoskelelal architecture, protein trafficking and protein synthesis. In this article, we focus our interest on the role of HSP90 in protein synthesis. Recent data obtained from a few laboratories strongly suggest that HSP90 interacts with the heme-regulated eukaryotic initiation factor 2α (elF-2α) kinase, also called the heme-regulated inhibitor, and causes its activation which leads to inhibition of protein synthesis. On the basis of data reported from various laboratories, including our own, we propose a possible model on the mechanism of HSP90-mediated activation of heme-regulated inhibitor and regulation of protein synthesis.

Keywords. HSP90; chromatin structure; actin polymerization; cell cycle regulation; activation of HRI; regulation of translation.

1. Introduction

Both prokaryotes and eukaryotes, and their cells cultured *in vitro,* when exposed to heat or similar other stresses, synthesize a small group of specific proteins, called the heat shock proteins (HSPs) or more generally stress proteins. This phenomenon which is accompanied by rapid changes in the pattern of gene expression is known as heat shock or stress response (reviewed in Lindquist 1986; Morimoto *et al* 1990). The universality of heat shock response and the high degree of conservation of HSPs suggest that they play important roles in cell physiology and metabolism. Indeed the induction of HSPs is correlated with the induction of thermotolerance, protecting organisms both from lethality and from heat-induced developmental defects. However, more recent research has shown that HSPs and closely related proteins are produced at normal temperature and play major roles in a wide variety of normal cellular processes. Therefore, in the past few years, HSPs have been the focus of investigations in many areas of cell biology, including protein trafficking, signal transduction, DNA replication, RNA transcription, protein synthesis and in the assembly of diverse protein structures (reviewed in Maresca and Lindquist 1991). They have also generated an intense research interest in the areas of developmental biology, immunology, infectious diseases, host-parasite interactions and cancer biology. Since a number of excellent

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reviews on various aspects of HSPs, and their functions, are available (Schleisinger *et al* 1982; Nover 1984; Pelham 1986; Lindquist and Craig 1988; Morimoto *et al* 1990; Schlesinger 1990), we have chosen to focus our discussion on the role of HSP90 in protein synthesis. HSP90 has been shown to associate with the heme-regulated eukaryotic initiation factor 2α(eIF-2α) kinase (also called the heme-regulated inhibitor, HRI), a key regulator of protein synthesis, and may thereby modulate regulation of translation (Rose *et al* 1989; Matts *et al* 1992; Pal 1994), We believe that research in this area has a great potential in understanding regulation of translation not only during stress response but also in normal cell functioning and embryonic development.

2. HSP90

HSP90, the most abundant of the HSP90 family, is an ubiquitous and versatile cytoplasmic protein. Although it is constitutively expressed, it is also induced during heat shock and other kinds of stress. However, its abundance and inducibility vary from one tissue to another (Morange *et al* 1984). HSP90 proteins appear to be essential for viability at all temperatures. In *Saccharomyces cerevisiae,* two genes encode HSP90 isotypes. It has been demonstrated that disruption of either gene compromised the organism's ability to grow at elevated temperatures, whereas simultaneous disruption of both genes was lethal (Borkovich *et al* 1989). HSP90 is methylated at lysine and arginine residues (Wang and Lazarides 1984) and it has an isoelectric point of about pH 5·5. It binds to ATP and undergoes autophosphorylation at serine and threonine residues (Hackett and Lis 1983). Mouse and human HSP90 have negligible and significant levels of ATPase activity, respectively (Nadeau *et al* 1993). It has been reported to have a protease activity as well (Schnaider *et al* 1993). Although it is localized predominantly in the cytoplasm, it has also been localized in the nucleus in normal and heat shocked cells (Schleisinger 1990). Its localization in the nucleus and its association with various nuclear proteins suggest that HSP90 may also function as a nuclear chaperone.

One of the most important properties of HSP90 is its association with various biologically important molecules such as, steroid hormone receptors (Sanchez *et al* 1985; Catelli *et al* 1985), actin (Koyasu *et al* 1986; Nishida *et al* 1986), tubulin (Sanchez *et al* 1988), 18 kDa cyclophilin (Nadeau *et al* 1993), calmodulin (Minami *et* al 1993) yeast protein kinase c, heme-regulated eukaryotic initiation factor 2*α* kinase (Rose *et al* 1989; Matts *et al* 1992; Pal 1994), casein kinase II (Miyata and Yahara 1995), various viral transforming proteins bearing tyrosine protein kinase activity, namely, $pp60^{v\text{-}src}$, $ppl40^{fps}$ and $pp94^{Yes}$ (Lipsich *et al* 1982) and weel tyrosine kinase of yeast (Aligue *et al* 1994). However, significance of some of these associations is yet to be established.

2.1 *Various members of the HSP90 family*

HSP90 is known by its subunit molecular weight. The precise molecular weight of HSP90 in higher vertebrates varies slightly among different organisms. The various members of this multigene family which consists of closely related protein isoforms, reported from various organisms are listed in table 1. Based on the expression patterns of their genes, they are broadly classified as follows: HSC90 (cognate) comprising of the genes which are constitutively expressed and are either not or only weakly expressed

Table 1. Different forms of HSP90 in various organisms and their homology to *Pleurodeles waltl* HSC90*.

Species	Nomenclature		Homology %
	MW	Form	
Homo sapiens	HSP86	α	82·7
	HSP84	β	92·1
Mus musculus	HSP86	α	82·4
	HSP84	β	92·1
Gallus gallus	HSP90	α	83·1
	HSC90	β	91·0
Drosophila melanogaster	HSP83		75·0
Arabidopsis thaliana	HSP81		66·1
Trypanosoma brucci	HSP83		59·7
Plasmodium falciparum	HSP90		56·0
Saccharomyces cerevisiae	HSP82		59·7
	HSC82		60·0
Escherichia coli	C62·5		35·2

*Adapted from Coumailleau *et al* 1995.

during heat shock, and HSP90 (heat-inducible) comprising of the genes which may be expressed at low level at normal temperature but are expressed in higher quantity during heat shock. In higher vertebrates, there are two isoforms of these species, *α* and *ß.* In mammals, the *ß*-form of HSP90 is strongly expressed under normal physiological temperature, as compared to the *α*-form (Barnier *et al* 1987; Legagneux *et al* 1989). A considerable homology between these proteins at the level of amino acid sequences and immuno-crossreactivity across species have been reported (Schlesinger *et al* 1982; Lindquist 1986; Coumailleau *et al* 1995).

Among other members, a glucose-regulated protein, GRP94, is an abundant member of the HSP90 family. It is present in the endoplasmic reticulum and is also called endoplasmin. It has 50% homology to HSP90. It has two ATP binding sites and it undergoes Ca^{2+}-dependent autophosphorylation at serine and threonine residues (Csermely and Kahn 1991; Csermely *et al* 1995).

2.2 *Expression during oogenesis and embryonic development*

HSP90 has been reported to be expressed in oocytes and embryos of a variety of species. HSP89 is constitutively expressed in the ectoderm of 8-day old mouse embryos and also in embryonal carcinoma cells (Bensaude and Morange 1983). HSC90 (Pw90) has recently been shown to be constitutively expressed in the oocytes of *Pleurodeles waltl* (Coumailleau *et al* 1995). The messenger RNA of Pw90, starts to accumulate from very early oogenesis, and is expressed at every stage of oogenesis. Although the protein accumulates in the cytoplasm in oocytes, it translocates to the nucleus during oogenesis, reaching a concentration equilibrium between cytoplasm and nucleus at stage VI (Coumailleau *et al* 1995). In *Drosophila,* HSP83 is induced at high levels in ovarian nurse cells and subsequently passed into the developing oocytes. In yeast, HSP83

mRNA accumulates to a high level as the cells approach stationary phase and enter sporulation (Kurtz and Lindquist 1984; Kurtz *et al* 1986).

During oogenesis and early embryogenesis, regulation of gene expression occurs predominantly at the level of translation. In most of the eukaryotes studied so far. maternal mRNAs which are synthesized during oogenesis are stored as translationally repressed mRNPs until their protein products are required for development and morphogenesis (Davidson 1986). Thus, there is a need of global negative regulation of translation, besides regulation of translation of specific mRNAs in time and space (Pal 1991; Pal *et al* 1988, 1994; J K Pal, A Kar and N S More, unpublished results). As we will describe later, it has been demonstrated that HSP90 interacts with HRI and regulates translation in mammalian reticulocyte lysates. It has been postulated that HRI-like eIF-2α kinases may exist in non-erythroid cell types (Pal *et al* 1991; Chen and London 1995). Although it remains to be determined if such a kinase is expressed in embryos, it is tempting to speculate that HSP90 may regulate such a kinase and thereby regulate protein synthesis to a large extent. Further research will certainly illuminate this aspect of HSP90-mediated developmental regulation.

3. Association of HSP90 with a variety of protein factors

HSP90 is known to interact with various protein factors having extraordinarily diverse functions. A number of reviews are available on this aspect (Lindquist 1986; Pelham 1986; Morimoto *et al* 1990; Schlesinger 1990; Maresca and Lindquist 1991). However, in order to facilitate our discussion on its association with the heme-regulated eIF-2α kinase, a brief account of its association with some specific protein factors and possible functional significance of such associations which are not extensively reviewed earlier, will be presented here.

3.1 *With histones and alteration of chromatin structure*

It has been shown that histones, histone H1 in particular, are able to modulate the autophosphorylation of HSP90 (Csermely and Kahn 1991). More recently, it has been demonstrated that HSP90 binds to histone-agarose and enhances the binding of histones to DNA (Csermely *et al* 1994). Interestingly, HSP90 has numerous negatively charged residues in the central domain. These are the possible sites through which it interacts with histones. Csermely *et al* (1994) have also demonstrated that HSP90-histone interactions result in a tighter, condensed state of the chromatin in rat liver, such that the chromatin is resistant to high salt treatment. Based on these data, the authors postulated that HSP90 may modulate the structure of chromatin and regulate transcriptional activity of steroid receptors and other transcription factors. These suggest an important role of HSP90 in functional alteration of chromatin structure.

3.2 *With actin filaments and actin polymerization*

HSP90 has been shown to bind actin filaments *in vivo* (Koyasu *et al* 1986). Purified HSP90 co-precipitates with polymerized actin. Each dimeric (native) form of HSP90 binds to a maximum of 10 actin molecules existing in polymerized form (Nishida *et al* 1986). Further experimental evidences suggest that HSP90 crosslinks actin filaments

(Koyasu *et al* 1986). Recent data suggest that the C-terminal region of HSP90, which is involved in dimerization of the molecule (Minami *et al* 1993), is perhaps the binding site for F-actin (Kellermayer and Csermely 1995). These authors have also shown that ATP causes dissociation of HSP90 from F-actin, which seems to be in fair agreement with the observation that HSP60 (Gao *et al* 1992) and HSP70 (Margulis and Welsh 1991; Haus *et al* 1993) also dissociate from actin in presence of ATP.

3.3 *With Weel tyrosine kinase and cell cycle regulation*

The role of Cdc2-cyclin B protein kinase in the regulation of cell cycle is well established (reviewed in Nurse 1990; Murray and Hunt 1993; Dunphy 1994). Details of this mechanism have been worked out in the fission yeast, *Schizosaccharomyces pombe.* The Cdc2-cyclin B complex is inactive during interphase due to phosphorylation of Cdc2 on Tyr15 (Gould and Nurse 1989). Dephosphorylation of this Tyr15 is a prerequisite for the expression of MPF activity of Cdc2-cyclin kinase which permits the cells to enter mitosis. In fission yeast, the Tyr15 of Cdc2 is phosphorylated and dephosphorylated by Wee1, a 107kDa protein kinase and Cdc25 protein phosphatase, respectively. Therefore, in *S. pombe,* the decision to enter mitosis is largely controlled by relative activities of these two regulatory molecules.

Recently, it has been shown that a specific gene, *swo1*$^{+}$ which encodes an HSP90 homologue, is necessary for the formation of active Weel tyrosine kinase (Aligue *et al* 1994). It has also been demonstrated that Swo1 is essential for growth at all temperatures. Co-immunoprecipitation of Weel and Swo1 under *in vivo* conditions suggests that active Wee1 requires physical interaction with Swo1 (Aligue *et al* 1994). Previously demonstrated interaction of HSP90 in activating a number of other tyrosine kinases, such as, pp60^{v-src}, along with this observation, possibly suggest the existence of a general regulatory mechanism for activation of tyrosine protein kinases by HSP90.

3.4 *With the heme-regulated eIF-2α kinase* (*HRI*) *and regulation of protein synthesis*

3.4a *HRI and initiation of protein synthesis:* In mammalian reticulocytes and their lysates, protein synthesis is dependent on the availability of heme. During heme-deficiency, protein synthesis is inhibited due to activation of an inhibitor, the HRI or the heme-controlled repressor (HCR), which is a cAMP-independent protein kinase. During heme-deficiency, HRI undergoes autophosphorylation and specifically phosphorylates the α-subunit (38 kDa) of the eukaryotic initiation factor 2 (eIF-2). Therefore, it is also called the heme-regulated eIF-2α kinase (reviewed in Ochoa 1983; London *et al* 1987; Chen 1993; Chen and London 1995). The details of the mechanism of inhibition, which is at the level of initiation, are described below.

During initiation of protein synthesis, eIF-2 mediates the binding of the initiator tRNA (Met-tRNA$_i$) to the 40S ribosomal subunit. It binds to Met-tRNA and GTP and forms a eIF-2·Met-tRNA·GTP ternary complex. This complex subsequently joins the 40S subunit of ribosome leading to the formation of a 43S pre-initiation complex. Upon binding of mRNA and joining of 60S subunit of ribosome, eIF-2·GTP is hydrolyzed to eIF-2·GDP which is then released as an inactive complex from the 80S initiation complex (figure 1). For eIF-2 recycling, eIF-2·GDP binds to another initiation factor, eIF-2B, which catalyzes the exchange of GDP for GTP to form eIF-2·GTP complex.

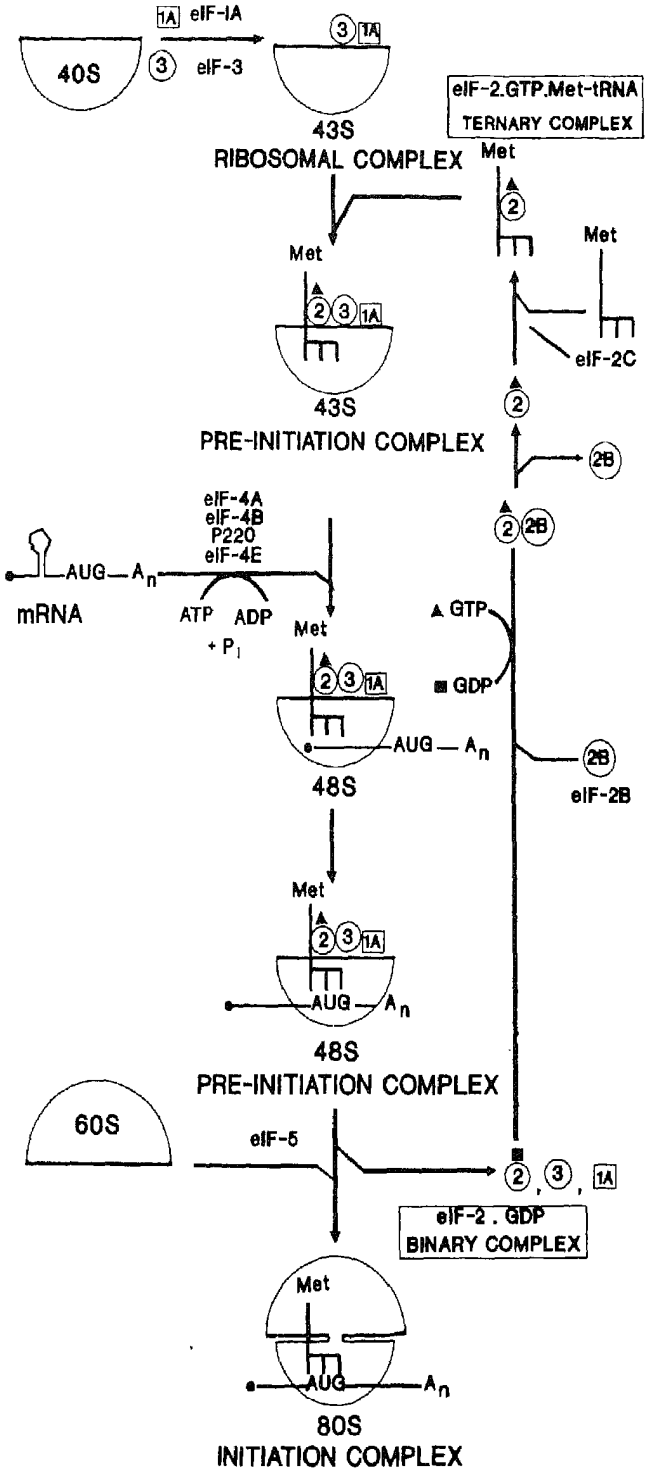

Figure 1. Summary of initiation of protein synthesis in eukaryotes.

Therefore, eIF-2B is also known as guanine-nucleotide-exchange factor (GEF) or reversing factor (RF). In case eIF-2 is phosphorylated at ser-51 residue by HRI, RF cannot catalyze the exchange reaction and it gets sequestered (figure 2). RF being rate limiting in the lysate, recycling of eIF-2 is impaired leading to inhibition of protein synthesis at the level of initiation. Two other eIF-2α kinases which phosphorylate the

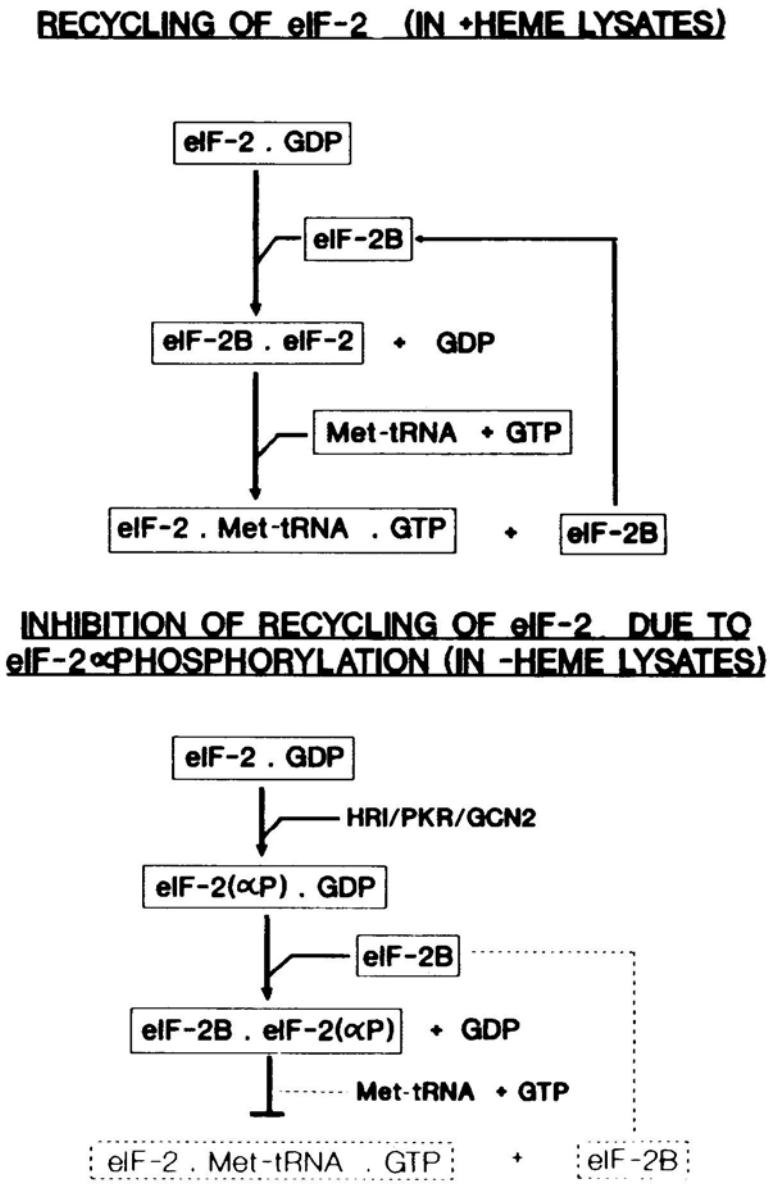

Figure 2. Schematic illustration of recycling of eIF-2 in normal reticulocyte lysates and its inhibition due to eIF-2α phosphorylation under conditions of either heme-deficiency (HRI), ng concentration of double stranded RNA (dsl/PKR) in reticulocyte lysates or amino acid starvation (GCN2) in yeasts.

same ser-51 residue of eIF-2 are known. They are: (i) interferon-induced double-stranded (ds) RNA-dependent eIF-2α kinase which is also called the ds RNA-dependent inhibitor (dsI) or PKR, and (ii) GCN2 kinase of yeast which is activated during amino acid starvation. Thus HRI, dsI and GCN2 which regulate this process in various systems are of immense importance in the initiation of protein synthesis (reviewed in London *et al* 1987; Hershey 1991; Chen and London 1995).

HRI has been purified from rabbit reticulocyte lysates and has been extensively characterized (Ranu and London 1976; Kramer *et al* 1976; Farrell *et al* 1977). Highly purified HRI specifically phosphorylates the α-subunit of eIF-2 and when added to reticulocyte lysates, produces characteristic biphasic kinetics of shut off of protein synthesis as well as disaggregation of polyribosomes. HRI is a dimer of a 92 kDa polypeptide and has an apparent native molecular size of 150–180 kDa as estimated by sucrose-gradient centrifugation (Ranu and London 1976; Trachsel *et al* 1978) and also by non-denaturing polyacrylamide gel electrophoresis (Anand *et al* 1995). HRI is present in the hemin-supplemented reticulocyte lysates as an inactive form and it is activated in heme-deficient lysates. However, it has been recently demonstrated that removal of HRI by immunoadsorption with a HRI-specific monoclonal antibody from lysates, makes the lysate suitable for undertaking protein synthesis at a normal rate even in the absence of hemin (Pal *et al* 1991). This observation along with previous data (reviewed in London *et al* 1987) suggests that HRI is the principal regulator of inhibition of protein synthesis in heme-deficient lysates. HRI-like inhibitors have also been purified from murine erythroleukemia (MEL) cells (Mellor *et al* 1993). Although HRI is present in MEL cells as well as K562 human erythroid cells, its expression increases during induction of differentiation *in vitro* (Crosby *et al* 1994). Two eIF-2α kinases have also been purified from Ehrlich ascites cells. However, they seem to be distinct from both HRI and PKR (Olmsted *et al* 1993). Furthermore, inhibitors with HRI-like properties have been reported from Human K562 erythroleukemic cells (Pal *et al* 1991), and a few non-erythroid cells, namely, Krebs ascites cells (Ranu 1980a), rat liver cells (Delaunay *et al* 1977), HeLa cells (De Benedetti and Baglioni 1986), freshly isolated hepatocytes depleted of heme with allyliosopropylacetamide (Fagard and Guguen-Guillouzo 1983), *Plasmodium* (Surolia and Padmanaban 1991) and wheat germ extracts (Ranu 1980b).

Rabbit reticulocyte HRI cDNA has been cloned and it recognizes a 3·1 kb mRN in reticulocytes (Chen *et al* 1991a). More recently, rat brain HRI cDNA has also been cloned and it shows 82% homology to rabbit reticulocyte HRI cDNA (Mellor *et al* 1994). The other two eIF-2α kinases that are cloned are, mouse and human PKR and yeast GCN2. All of these eIF-2α, kinases share extensive homology in the kinase catalytic, domains (Chen *et al* 1991a, b). However, the regulatory mechanisms and molecular sizes of these are very different (Chen and London 1995). HRI cDNA has significant homology with a number of yeast cell cycle regulatory proteins (Chen *et al* 1991b). The significance of this homology remains to be determined.

3.4b *Expression of HRI:* HRI is abundant in reticulocytes and its level decreases significantly in the mature erythrocytes (Pal *et al* 1991; Crosby *et al* 1994). However, no data is yet available on its expression in the precursor cells of the erythroid lineage (reviewed in Chen 1993; Chen and London 1995). Using HRI-specific monoclonal antibody, HRI was detected only in reticulocytes and bone marrow of anemic rabbits, and therefore it was assumed that HRI is erythroid specific (Pal *et al* 1991; Crosby *et al*

1994). However, recent data described below suggest that HRI is also expressed in other tissues. Mellor *et al* (1994), using rat brain HRI cDNA, detected HRI expression in a variety of rat tissues although to a much less extent (10 times) as compared to that in reticulocytes. Crosby *et al* (1994), on the other hand, using rabbit reticulocyte HRI cDNA, could detect HRI expression in non-erythroid tissues only under conditions of low stringency of hybridization. Interestingly, they detected a few more transcripts of various sizes ranging from 2·5 to 4·2 kb in addition to the 3·1 kb HRI mRNA in other tissues. It remains to be determined if any of the low molecular weight mRNAs detected by Crosby *et al* (1994) would translate into different proteins, presumably as isoforms of HRI.

3.4c *Activation of HRI and eIF-2α phosphorylation:* Activation of HRI is monitored by determining its autophosphorylation as well as phosphorylation of eIF-2*α*. Activation of HRI was first demonstrated in rabbit reticulocytes and their lysates both *in vivo* and *in vitro* during heme-deficiency anemia (reviewed in London *et al* 1987). Subsequently it has been found that a variety of other reagents and conditions, such as, oxidized glutathione, N-ethylmaleimide (NEM) and other sulphydryl-reactive reagents, ethanol, low partial pressure of oxygen, heavy metal toxicity, heat shock, addition of oxidants and denatured proteins, nutritional starvation and viral infection, cause a substantial activation of HRI (reviewed in Chen 1993; Rhoads 1993). We will discuss here activation of HRI during heat shock and interaction of HSP90 with HRI.

3.4d *Activation of HRI during heat shock and involvement of HSP90:* Inhibition of protein synthesis in hemin-supplemented reticulocyte lysates or intact reticulocytes incubated at elevated (heat shock) temperatures (40–42°C) was observed in the mid 1970s (Mizuno 1975). This inhibition showed biphasic kinetics and was accompanied by polyribosome disaggregation and depletion of 40S-Met-tRNA$_i$ initiation complexes. However, the primary cause of this inhibition which is due to phosphorylation of HRI and eIF-2*α* in reticulocytes as well as in HeLa cells was demonstrated by Ernst *et al* (1982). Interestingly, in HeLa cells, inhibition of protein synthesis occurs within 10–15 min during heat shock and unlike reticulocytes, prolonged incubation of cells at 42°C results in restoration of protein synthesis by 60–120 min (McCormic and Penman 1969). It was also observed that in reticulocyte lysates (Petryshyn *et al* 1979) as well as in HeLa cells (De Benedetti and Baglioni 1986), this heat shock-induced activation of eIF-2α kinase was blocked by anti-HRI polyclonal antibodies. Phosphorylation of eIF-2α during heat shock has also been shown in Ehrlich ascites cells (Scorsone *et al* 1987) and more recently in *Drosophila* cells cultured *in vitro* (Duncan *et al* 1995). These data, therefore, establish that HRI gets activated during heat shock. The above results have initiated a great deal of interest among researchers working on regulation of protein synthesis. Recent data from a few laboratories, as described below, unequivocally indicated that HSP90 interacts with HRI both *in vivo* and *in vitro* in reticulocyte lysates to activate HRI, although the mechanism of this activation remains to be determined.

HSP90 has been shown to co-purify with HRI in reticulocyte lysates (Rose *et al* 1989). Furthermore, purified phosphorylated form of HSP90 from HeLa cells has been demonstrated to activate HRI and inhibit protein synthesis in rabbit reticulocyte lysates (Szyszka *et al* 1989; Rose *et al* 1989). Based on these results they postulated

that HSP90 may have a regulatory role in protein synthesis, via its interaction with HRI and phosphorylation of eIF-2α. On the other hand, Malts and Hurst (1989) reported an association of HSP90 with latent HRI in hemin-supplemented reticulocyte lysates. They also showed that in reticulocyte lysate preparation with different levels of HSP90, the restoration of protein synthesis by the delayed addition of hemin is greater in lysates with higher levels of HSP90. Similarly, Mendez and de Haro (1994) in their model on regulation of HRI activity involving HSP90-HRI-casein kinase II, proposed an inactive HRI-HSP90 heterodimer complex in which NEM promotes the dissociation of HSP90. However, our data (Pal 1994: J K Pal, Z Xu, R L Matts, H P Hahn, I M London, J-J Chen, unpublished results) are in agreement with those of Rose *et al* (1989).

It was shown earlier by immunoadsorption analysis using both anti-HRI and anti-HSP90 antibodies that HSP90 co-precipitates with HRI (Malts *et al* 1992). In these immunoprecipitates, two other proteins, namely, HSP70 and EC1 antigen were also observed. These data supported the notion that HSP90 may have a role in the regulation of HRI activity. More recently, it has been observed that HSP90 is co-precipitable with HRI from reticulocyte lysates under protein synthesizing conditions treated with NEM, but not from either hemin-supplemented or hemin-deficient reticulocyte lysates (Pal 1994). HRI in the NEM-treated lysates is most active. Furthermore, HSP90 was also co-precipitated from lysates heat shocked at 42°C. The quantity of HSP90 in such immunecomplexes increased as a function of duration of heat shock. Interestingly, the quantity of HSP90 complexed with HRI in heat shocked lysates increased several fold in presence of NEM (Pal 1994: J K Pal, Z Xu, R L Matts, H P Hahn, I M London. J.J Chen, unpublished results). These results indicate that NEM and heat shock have synergistic effect on the association of HSP90 with HRI. Since it is known that NEM also induces stress response, these results are not unexpected. Thus, it is conceivable that at normal temperature, when the level of HSP90 in the lysates is low, very little, if any, HRI-HSP90 complex formation takes place. During heat shock when more and more HSP90 is synthesized, more of it is available for complex formation with HRI or other protein factors. These data also suggest that the mechanism of regulation of HRI activity in reticulocyte lysates under heme-deficiency may be different from that under heat shock or NEM-treatment.

Based on these observations, we propose a model suggesting the possible mechanism of activation of HRI in reticulocyte lysates under various conditions (figure 3). Under normal physiological condition, when reticulocytes are enriched with heme, heme promotes intersubunit disulphide bond formation in HRI (Yang *et al* 1992). Such a disulphide-linked dimer of HRI is inactive. However, during heme-deficiency, HRI is active due to inhibition of disulphide bond formation, Under conditions of heat shock or NEM-treatment, when more of HSP90 is available. HRI tends to heterodimerize with HSP90 which is originally a dimer. HRI in such a HSP90-HRI heterodimer complex is highly active. Thus, in normal physiological condition, there is an equilibrium between a HRI–HRI disulphide-linked dimer and a HRI-HSP90 heterodimer. During stress, such an equilibrium is shifted towards the formation of more of HRI-HSP90 heterodimer, thereby resulting in activation of HRI which causes inhibition of protein synthesis. However, in the light of the recent observation that HSP90 interacts with F-actin in the form of a dimer (Kellermayer and Csermely 1995). the possibility of formation of a tetramer complex of HRI and HSP90 cannot he excluded.

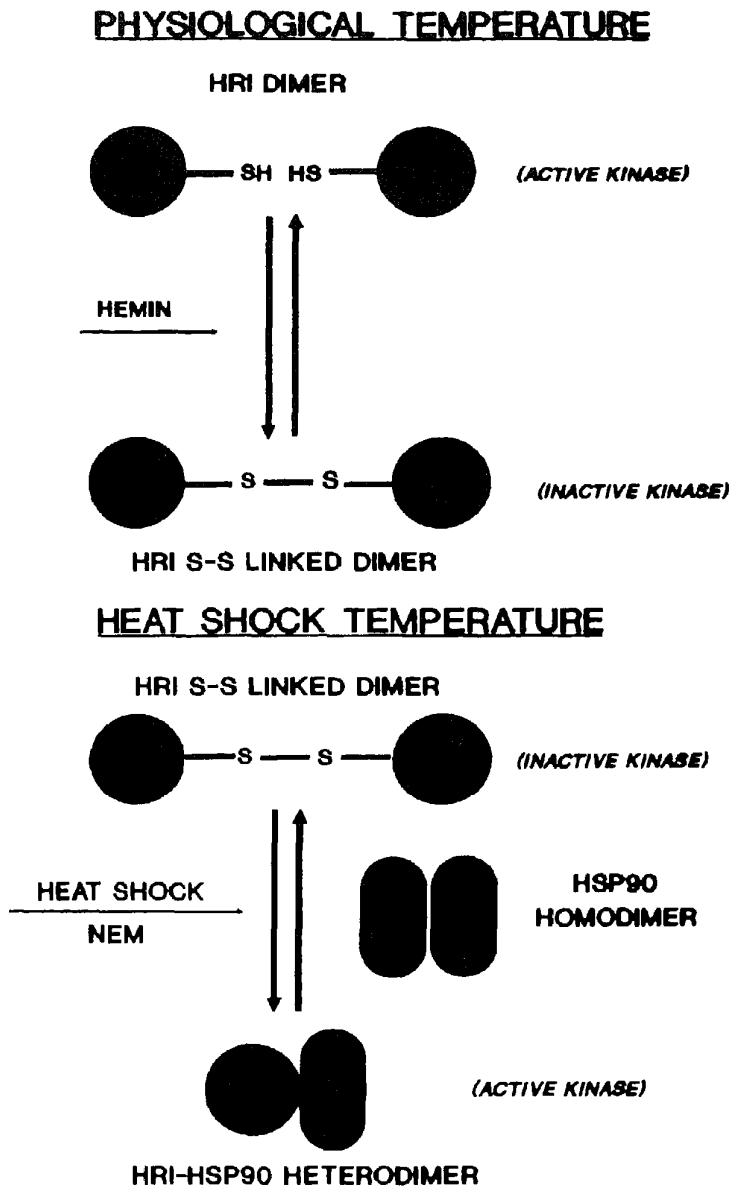

Figure 3. A model for HSP90-mediated translational control in reticulocytes. Mechanisms of activation of HRI during heme-deficiency and heat shock or NEM-induced stress are illustrated.

4. Conclusion

The role of HSPs in thermotolerance is well known. However, recent discoveries on the roles of HSP70 and HSP90 families in an astonishingly large number of cellular physiological processes are noteworthy. Their roles in recently emerging areas, namely,

cell cycle regulation and regulation of protein synthesis are particularly fascinating Interestingly, significant levels of expression of HSP70 and HSP90 in the oocytes and early embryos in a variety of species have opened up the possibility of further investigations on their role as important modulator in developmental regulation Perhaps the most intriguing aspect of these studies is how a protein like HSP90 (or HSP70) is structurally so versatile that it can interact with such a wide array of protein factors with diverse functions, and presumably also with RNAs. Indeed, in *Drosophila* HSP83 (a member of the HSP90 family) has been shown to interact with *hsrω* (93D), a non-protein coding locus important in developmental regulation (Morcillo et al 1983; Lakhotia and Ray 1996). Although it is speculated that HSP83 may interact with the transcripts of this gene, the exact nature of interaction remains to be determined Thus, this area merits further investigations on the heat shock proteins which will generate significant information on protein–protein, protein-RNA and protein-DNA interactions and their impact on various biological processes.

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