This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2001)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2001 Term

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Heat Shock Protein 90: A Family Affair

by

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For 77:222, Spring 2001

8. March 2001

Abbreviations:

FeSOD, Iron Superoxide Dismutase
GA, Geldonamycin
GR, Glucocorticoid Receptor
Hop, heat shock protein co-chaperone
hsp, heat shock protein
MnSOD, Manganese Superoxide Dismutase
ROS, Reactive Oxygen Species
TPR, tetratricopeptide repeat

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Abstract:

Shortly after their discovery, heat shock proteins or hsp were classified into families. These proteins have been studied to understand their structure, however, research is still going on to understand their functions in the cell. There are many ways that heat shock proteins are initiated in cells and then are used by those cells. There are many interactions that go on with hsp and many ways in which they are used. Understanding these heat shock proteins and learning more about their function will be helpful in many scientific endeavors.

Introduction:

In the 1960's, genetics researchers started looking at *drosophila* for information. It was during one of these experiments that heat shock proteins were discovered, although they did not have a name at that time. It was discovered that *drosophila* genomes induced chromosomal puffing in the salivary gland when there was an increase above the normal developmental temperature [2]. This puffing was easy to visualize in the *drosophila* chromosome with a light microscope. It was noticed that this puffing in the chromosome led to an increase in transcription of those "puffed" sections of the genome. This increased transcription led to an increase in translation of what would become known as heat shock proteins [2]. This decision to call them heat shock proteins was made in 1974 because the increase in translation of these proteins was initiated by an increase in temperature [2]. This paper will discuss the 90-kDa-heat shock protein family and what kinds of interactions they undergo.

Heat Shock Protein Background:

Heat shock proteins are created in the cell when the cell perceives a stress. These stresses could be due to heavy metals, alcohol, xenobiotics, ROS and of course heat. It has been shown that heat shock proteins protect cells against damage to the critical biomolecules by oxidative stress in a type of anti-oxidant defense [2].

There are three classes of heat shock proteins that have been discovered. These proteins may play a role in protecting cells when exposed to the above-mentioned stress [8]. The first class is a small molecular weight class from about 15-30 kDa, the second class is the hsp 70 family. This family contains highly conserved

proteins with molecular weight around 70 kDa. The third class is the hsp 90 family with molecular weight around 90 kDa. When researchers started to study these hsp families they ran them out on gels and found that these different molecular weights were in the stress induced cells. These proteins were named because of their molecular weights. Heat shock protein 90 is located on human chromosome 1 on the q arm around the 21st and 22nd position as Figure 1 shows.

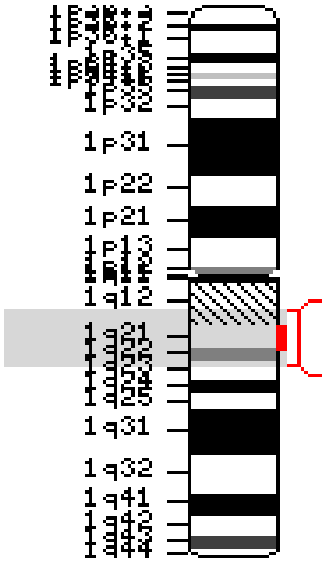

Figure 1: The placement of hsp 90 on chromosome 1 arm q position 21 and 22. There are other homologous sequences for hsp but this is the chromosomal placement for hsp 90-α [adapted from 8].

Hsp 90 is a chaperone protein, which means that it helps with the proper folding and assembly of other structural proteins [1,2]. As a chaperone hsp 90 also helps move proteins from one place in the cell to another place where they can be more useful. To understand how hsp 90 accomplished these tasks it is good to find out what hsp 90 looks like. From studies of heat shock proteins across different species it has been noted that there is little divergence in homology of hsp 90 across the mammalian and plant species [5]. Hsp 90 is a protein that is located in the cytoplasm with a very small

amount showing up in the nucleus. Hsp 90 is also found to be present in unstressed cells and accounts for 1-2% of the measurable cytosolic proteins [5]. This however does not imply that hsp 90 is not increased when stress is induced. This heat shock protein family is involved in renaturation of proteins that were unfolded during the stressful event [4]. There is little known about the functional properties or the mechanistic details of hsp 90, however it is the most abundant hsp in eukaryotes [7].

Heat Shock Protein Structure:

Heat shock protein 90 functions as a homodimer and can be separated into three functional domains; the NH_2-terminal domain, the COOH-terminal domain and a central charged domain [1,7]. The N-terminal domain is a common site for ATP binding [1]. When ATP binds to hsp 90 there is a conformational change in the N-terminal domain that leads to a decrease in substrate binding affinity and a release of peptides [7]. The C-terminal domain is important for ATP independent chaperone function and also for dimerization. This dimerization occurs in the C-terminal domain around the 50-100 amino acid range [1,7]. The C-terminal domain, where this ATP independent chaperone activity takes place, is also a binding site for non-native proteins [7]. The central charged domain provides elements for stability of the functional dimeric form [1]. The confirmation state and chaperone function of hsp 90 is controlled by nucleotide binding and dimerization [1,5]. Wild type hsp 90 have highly conserved N and C terminal domains that are connected by a charged linker. Comparison of all known hsp 90 sequences reveals that this linker is of variable size and may be involved in the gain of function in hsp 90 [7]. The N-terminal domain where the ATP binds decreases the

affinity for substrates. ADP, however, shows no influence on the chaperone activity *in vitro* [7]. The visualization of ATP binding to hsp 90 was shown through biochemical research and also by x-ray crystallography as seen in Figure 2.

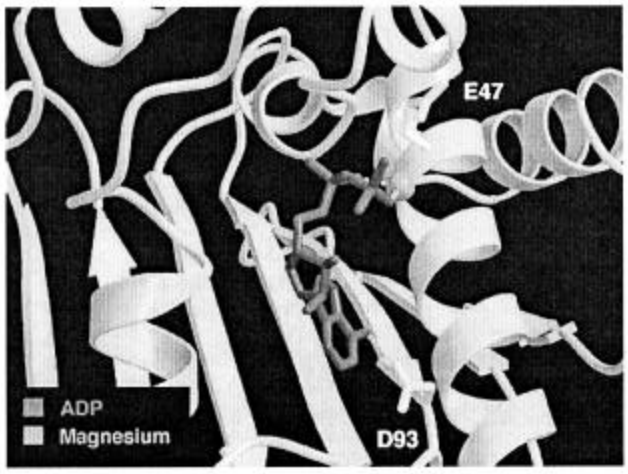

Figure 2: Structure of the N-terminal domain of yeast hsp 90 with bound ADP and Mg^{2+} [4]. The contacts between the nucleotide and hsp 90 are similar to yeast hsp 90. This ADP nucleotide binds to the D93 and E47 regions. This D93 binding seems to be the most critical for the nucleotide to stay bound [adapted from 4].

Heat Shock Protein Interactions:

Heat shock proteins are assembled into a heterocomplex as seen in Figure 3. This complex is assembled in a particular order. The hsp 90 and hsp 70 do not complex together without the presence of protein p60. p60 is a 60-kDa protein that is found to join the hsp 90 and hsp 70 [5]. This p60 protein is up regulated during viral transformations in human cells. The p60 in Figure 3 is called the TPR Domain Proteins. TPR (tetratricopeptide repeats) domains are actually in a heterocomplex of six to eight. These six or eight, the actual number is yet unknown, bind to the TPR site in the hsp 90 and make up the p60 protein [5]. This leads to the ATP dependent reaction of the glucocorticoid receptor (GR) and is the next step in assembly. This GR is the unlabeled

part of Figure 3. This complex of GR-hsp90-p60-hsp70 is very unstable and requires the addition of p23 or a molybdate (MoO_4^{2-}) [5]. Either p23 (23 kDa protein) or molybdate can be used to stabilize this complex but both are shown in the figure to help understand their location in the complex. The fourth part of the assembly is the cycling out of the p60 and an abundance of the hsp 70 from the complex, which is not shown in the representation in Figure 3. The final part of complex assembly is the binding of the chaperoned proteins with the C-terminal domain. The complex assembly requires an ATP/Mg^{2+} and a monovalent cation (K^+, NH_4^+ and Rb^+) for assembly, also not shown in the Figure.

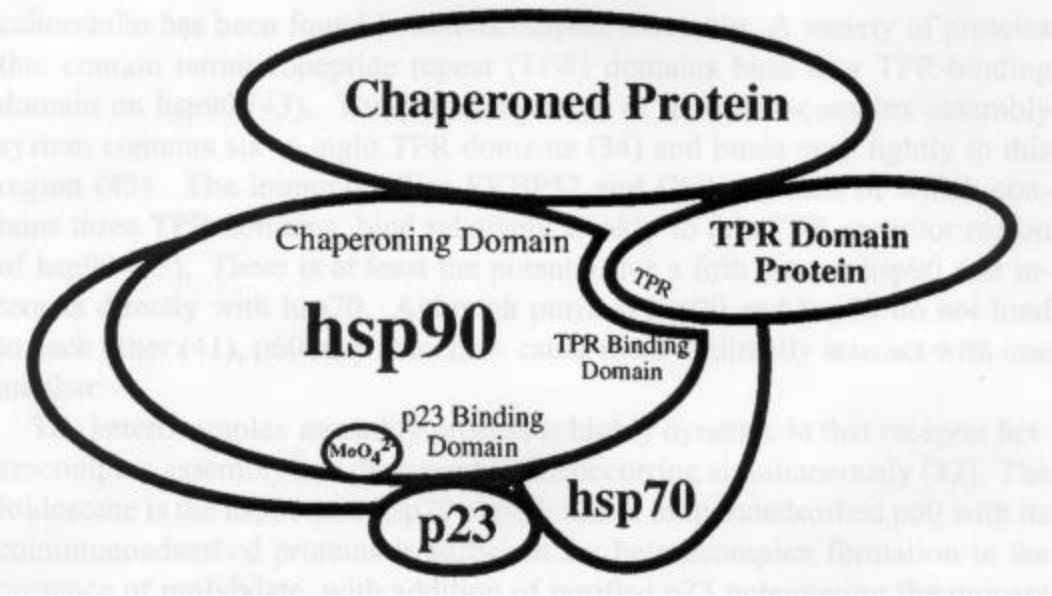

Figure 3: Protein binding domains on hsp 90. Hsp 90 and hsp 70 are the heat shock proteins. TPR Domain Proteins are tetratricopeptide receptor domains, which make up p60. MoO_4^{2-} is molybdate and p23 is another stabilization protein. The empty fragment is the GR. The chaperoned protein is the protein that the complex will be moving [adapted from 5].

Hsp 90 function can be inhibited by antibiotics and anti-cancer drugs such as geldonamycin (GA) which causes the misfolding and degradation of other co-proteins [4]. GA binds to the ATP binding site of the N-terminal domain and causes a sensitivity

in the ATPase activity that usually occurs when ATP is bound [6]. The ATP binding constants of the different N-terminal hsp 90 fragments were figured out by titration calorimetry and are shown in Table 1. It has been shown that ATP binding to the N-terminal domain can cause switching in the conformational state of hsp 90 and thereby releasing substrates for the complex in Figure 3 [7].

Table 1: Dissociation constants of hsp 90 fragments for ADP and ATP [7].

	K_d, μM	
Fragment	ADP	ATP
In the absence of peptide GR1		
N210	40 ± 2	98 ± 4
N272	43 ± 3	94 ± 1
In the presence of peptide GR1		
N210	ND	101 ± 6
N272	ND	287 ± 27

The dissociation constants of ADP/ATP and N210/N272 (parts of the N terminal domain) were determined by titration calorimetry. These experiments were done in the absence and the presence of GR which will change the affinity for the ATP [adapted from 7].

It was shown in this example that the GR is important for the stability and that the binding of ATP can increase the function of the complex to chaperone [7].

Because of these different binding constants hsp 90 is involved in the folding and conformational regulation of many different medically relevant signal transduction molecules such as nuclear receptors, *e.g.* dioxin and steroid receptors, and proto-oncogenic kinases [4,5].

Hop is a heat shock co-chaperone that also provides a link between hsp 70 and hsp 90. Hop is removed from the complex after assembly and the ATP binding creates an hsp 90-ATP complex, which recruits the p23 protein that helps generate mature

receptor complexes [1]. There are other co-chaperones that bind to the hsp 90 complex as well.

Summary:

Heat shock proteins have many different families and many different functions. The multiple reactions and chaperone activities help with stressed cells as well as normal cells. The difference in families seems to cause some different functions but these families seem to be able to work together to accomplish their chaperone functions. Although designated heat shock proteins they are very useful for stresses of all kinds that the cell will undergo. With little understanding so far on the complete functions of these proteins it will be beneficial to keep studying them to understand all they can do for the cell and for research.

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