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Hsp70 interactions with the p53 tumour suppressor protein

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Introduction

The heat shock proteins (HSPs) are encoded by genes whose expression is substantially increased during stress conditions, such as heat shock, alcohol, inhibitors of energy metabolism, heavy metals, oxidative stress, fever or inflammation. During these conditions, HSPs increase cell survival by protecting and disaggregating stress-labile proteins (Skowyra *et al*., 1990), as well as the proteolysis of the damaged proteins (Wickner *et al*., 1999). Under non-stress conditions, HSPs have multiple housekeeping functions, such as folding and translocating newly synthesized proteins, activation of specific regulatory proteins, including transcription factors, replication proteins and kinases, protein degradation, protein signalling, including steroid hormone activation and tumour immunogenicity, and antigen presentation (for reviews see Helmbrecht *et al*., 2000; Jolly and Morimoto, 2000).

This broad spectrum of functions gave rise to the term 'molecular chaperone', an entity that acts to assist other proteins folding and maturating in the cell. It should also be emphasized that not all HSPs are molecular chaperones and not all chaperones are HSPs (Ellis and Hartl, 1999).

HSPs are designated nomenclature according to their approximate molecular weight, e.g. the 70 kDa HSP is known as the molecular chaperone Hsp70. The 70 kDa heat shock-related proteins comprise a family of highly conserved molecular chaperones that regulate a wide variety of cellular processes during normal and stress conditions (Boorstein *et al*., 1994). Hsp70 is one of the most abundant of these proteins, accounting for as much as 1–2% of total cellular protein (Herendeen *et al*., 1979). In humans, there are at least 11 distinct genes that code for Hsp70 isoforms, which are located on several different chromosomes (Tavaria *et al*., 1996). The major, constitutively expressed *hsp70* isoform is called *hsc70* (gene product known as the clathrin-uncoating ATPase or Hsp73) (Welch, 1992). The transcription of inducible forms of *hsp70* or *hsp72* are under the control of the heat shock factor (HSF) (Morimoto, 1998), as well as a variety of physiological processes, such as cell cycle control, proliferation and differentiation (Helmbrecht *et al*., 2000; Jolly and Morimoto, 2000).

The ATPase activity and peptide binding properties (Zylicz *et al*., 1983) have been conserved amongst both the eukaryotic and prokaryotic Hsp70 homologues (McKay *et al*., 1994). Hsp70 co-chaperones, such as Hsp40 (Liberek *et al*., 1991a; Kelley, 1998), Bag-1 (Hohfeld and Jentsch, 1997; Luders *et al*., 2000; Sondermann *et al*., 2001), Hip (Hohfeld *et al*., 1995) and Hop (Dittmar *et al*., 1996; Chen *et al*., 1998) have been shown to play an important role in modulating Hsp70 activity, as well as protein substrate specificity.

In the absence of Hsp40 and ATP, Hsp70 preferentially binds to peptides (Fourie *et al*., 1994) and denatured protein (Liberek *et al*., 1991b). However, Hsp70 can also recognize and specifically bind to mature, folded proteins (for reviews see Wawrzynow and Zylicz, 1995; Mayer *et al*., 2000). In the presence of Hsp40, Hsp70 exhibits a broader range of substrate specificity (Wawrzynow *et al*., 1995; Misselwitz *et al*., 1998). The existence of several different isoforms of both Hsp70 and Hsp40 could result in the formation of multiple types of Hsp70–Hsp40 complexes that differ in substrate specificity (Zhen and Cyr, 1998).

Hsp70 and other chaperones are also known to be determinants of cell death and cell transformation processes. The elevated expression of Hsp70 and Hsp90 in tumour cells was detected in several cases (for reviews see Helmbrecht *et al*., 2000; Jolly and Morimoto, 2000). In breast cancer, the expression of Hsp70 was correlated with metastasis and poor prognosis (Ciocca *et al*., 1993; Lin *et al*., 1994; Vargas-Roig, 1998). The overexpression of Hsp70 in several cell types increased transformation (Jaattela, 1995; Seo *et al*., 1996; Volloch and Sherman, 1999). Consistent with these observations, the inhibition of Hsp70 expression by anti-sense Hsp70 cDNA resulted in the inhibition of tumour cell proliferation and the induction of apoptosis (Wei *et al*., 1995; Jaattela *et al*., 1998).

Hsp70 chaperone activity may influence tumorigenesis by regulating the activity of proteins that are involved in cell cycle machinery. Hsp70 family members and/or Hsp90 transiently associate with key molecules of the cell cycle control systems, including p53, Cdk4, Wee-1, c-Myc, pRb and p27/Kip1 (for reviews see Helmbrecht *et al*., 2000; Jolly and Morimoto, 2000). Hsp70 and/or Hsp90 also interact with important kinases of the mitogen-activated signal cascades, such as Src kinases, tyrosine receptor kinases, Raf and MAP-kinases (for reviews see Gabai *et al*., 2000; Helmbrecht *et al*., 2000; Song *et al*., 2001). Many recent, interesting observations have also been made with regards to Hsp70's ability to negatively regulate various stages of the p53-dependent or independent apoptotic pathways (Beere *et al*., 2000; Gabai *et al*., 2000; Li *et al*., 2000; Mosser *et al*., 2000; Nylandsted *et al*.,

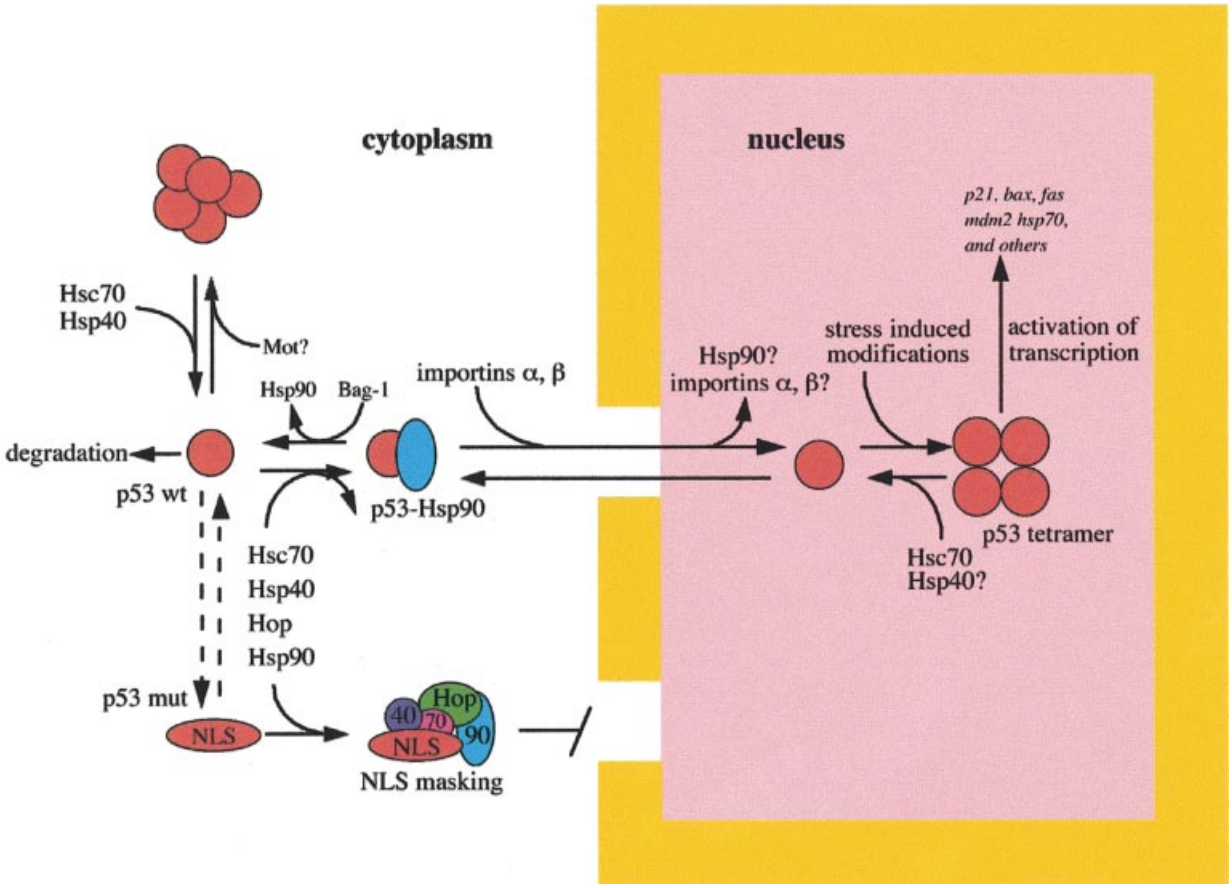

Fig. 1. Hypothetical model for the involvement of molecular chaperones in the import and export of wild-type and mutant p53. Under non-stress conditions, there exists equilibrium between the import and export of wild-type p53 in and out of the nucleus. We propose that wild-type p53 may be escorted to the nucleus by chaperones, such as Hsp90. The binding of Hsp90 to wild-type p53 inhibits the formation of multiple chaperone complexes with wild-type p53. Bag-1 may dissociate the Hsp90–wtp53 complex facilitating the p53 degradation by proteosome or calpain-mediated pathways. During stress conditions, wild-type p53 is post-translationally modified (e.g. phosphorylation), which leads to the formation of active p53 tetramers. In the nucleus, activated wild-type p53 acts as a transcription factor, regulating the expression of genes involved in cell arrest, DNA repair and apoptosis. p53 also induces the transcription of other genes including *hsp70*. Mutant p53, which does not interact directly with Hsp90, can form a multi-chaperone complex in the cytoplasm, resulting in the increased stability and the cytoplasmic sequestration of mutant p53. We propose that during stress conditions, wild-type p53 located in the cytoplasm could temporarily adopt the mutant conformation, which initiates the formation of a multichaperone complex that could partially stabilize the wild-type p53 and prevent nuclear import by masking the p53 NLS. During recovery from stress exposure, Hsp90 and other proteins may be able to rescue such wild-type p53 protein that has acquired the mutant conformation. During recovery from stress, wild-type p53 tetramers located in the nucleus could be dissociated in the presence of Hsp70 and Hsp40, thus inhibiting p53 transcriptional activity. In addition, chaperones may participate in regulating the localization of wild-type p53 in some cancer cells. The Mot-2 protein, in the absence of an appropriate Hsp40 co-chaperone, may induce the aggregation of wild-type p53 in cytoplasm, which results in the inhibition of apoptosis in cancer cells. We propose that such an effect of Mot-2 could be reversed by the presence of Hsc70 and Hsp40 chaperones.

2000b). However, this review will focus only on the role of Hsp70 and its co-chaperones in the regulation of p53 function.

Molecular chaperones are potential mediators of p53 conformation

The p53 tumour suppressor protein functions as a transcriptional activator that binds specifically to double-stranded DNA. p53 is normally expressed at low levels in a latent form that is unable to bind specifically to DNA. Exposure to many stress signals, such as ionizing and non-ionizing radiation, hypoxia, anti-metabolites that inhibit ribonucleotide biosynthesis, heat shock, radiation and low extracellular pH, and DNA damage by chemicals, activates p53 protein and promotes cell cycle arrest. However, in response to certain oncogenic changes or when the stress is excessive, p53 can induce tumour-suppressive apoptotic cell death. This implies that there is strong selection for tumour cells to lose p53 function (for reviews see Hupp *et al*., 2000; Zhao *et al*., 2000; Evan and Vousden, 2001). The inactivation of wild-type p53 function can occur by proteolysis, chemical or protein modification, nuclear exclusion (cytoplasmic sequestration), or mutations in the p53 gene. The Mdm2 protein has been shown to inhibit p53 activity by binding to the p53 transactivation domain and interfering with the recruitment of basal transcription machinery components (for review see Hupp *et al*., 2000). In addition, Mdm2 can mediate p53 degradation by targeting its ubiquitylation. Wild-type p53 can stimulate the transcription of the *mdm2* gene, which creates a negative feedback loop for p53 deactivation (for review see Hupp *et al*., 2000). Point mutations in the p53 gene are detected in >50% of the most common cancers (for reviews see Greenblatt *et al*., 1994; Lin *et al*., 1994). Mutated p53 protein exhibits a decreased

ability to bind DNA and/or an altered protein conformation (Cho *et al*., 1994). Conformational mutants are also called class II or dominant-negative p53 mutants, and they display no wild-type conformation as detected by monoclonal antibodies (Gannon *et al*., 1990). Temperature-sensitive mutants, such as p53Ala135Val and p53Val143Ala, can revert back and forth between wild-type and mutant conformations in a temperature-dependent manner (Michalovitz *et al*., 1990; Zhang *et al*., 1994).

The results from several *in vivo* experiments also suggest that wild-type p53 may exist in a constant state of equilibrium between wild-type and mutant conformations. In murine fibroblasts, which contained genetypically wild-type p53, a change from wild-type to mutant p53 conformation was observed on serum starvation (Milner and Watson, 1990). Wild-type p53 could also be converted to the mutant conformation in the presence of Cu(II) (Hainaut *et al*., 1995). In addition, mutant p53 conformation was shifted to wild-type conformation through interactions with p53 C-terminal peptides and the mutant p53 core domain (Selivanova *et al*., 1997, 1999). When one wild-type dimer of a tetramer p53 protein binds to a DNA half-site, the other non-bound dimer can possess wild-type or mutant conformation (McLure and Lee, 1999). Proteins that stabilize p53 DNA binding conformation may, indeed, stabilize wild-type p53 DNA complexes by facilitating a conformational shift towards wild-type conformation. Recently, we have demonstrated that Hsp90 can bind to wild-type p53 and stabilize wild-type p53–DNA complexes at 25°C, as well as partially protect wild-type p53 DNA binding conformation during long-term exposures at 37°C (F.W.King *et al*., in preparation). Chaperones such as Hsp90 may play a role in regulating the shift between wild-type and mutant p53 conformations (Figure 1).

Hsc70, Hsp90 and co-chaperones play a role in the stabilization and localization of mutant p53

Several different laboratories have identified either Hsc70 or Hsp70 in stable immunocomplexes with the conformational mutant form of p53 protein, but not wild-type p53 protein (for review see Hupp *et al*., 2000). In addition to Hsc/p70, Hsp90 and various co-chaperones have also been found to be associated with the p53 protein. In mammary tumour cell lines, both Hsc70 and Hsp90 were found associated with mutated p53 in the cytoplasm (Blagosklonny *et al*., 1996). Hsp40 has also been found associated with a mutant p53 immunocomplex (Sugito *et al*., 1995). Whitesell and co-workers utilized the temperature-sensitive murine p53 mutant p53Ala135Val, expressed in A1-5 fibroblasts, to demonstrate that chaperones Hsc70, Hsp90, p23 and cyclophilin 40 exclusively co-immunoprecipitate with the mutant form of p53 at 37°C. At lower temperatures favouring wild-type conformation (30°C), multiple chaperones complexes were not detected and p53 transcriptional activity was restored (Whitesell *et al*., 1998). A reconstituted *in vitro* system was recently used to demonstrate that the formation of a stable Bag-1-resistant complex between Hsp90 and mutant p53Arg175His requires the presence of Hsp40, Hsc70, Hop and ATP (F.W.King *et al*., in preparation). On the other hand, Hsp90 alone can bind directly to wild-type p53 and compete against both Hsc70 and Hsp40 for binding to wild-type p53 (F.W.King *et al*., in preparation).

Cellular levels of wild-type p53 are ultimately regulated by either the proteasome degradation pathway (Maki *et al*., 1996) or the calcium-dependent protease calpain (Pariat *et al*., 1997). Hsp90-associated multiple chaperone complexes with mutant p53 protein have been linked to the impairment of mutant p53 ubiquitylation. Treatment of cells that express mutant p53 protein with the antibiotic geldanamycin results in an increase of mutant p53 ubiquitylation and proteosome-mediated degradation (Whitesell *et al*., 1997). In addition, geldanamycin was also shown to reduce the levels of Hsp90 bound to mutant p53 and increase mutant p53 translocation into the nucleus (Whitesell *et al*., 1998). Recent studies demonstrated that multiple chaperone complexes play an important role in masking the p53 nuclear localization signal (NLS) sequence while in a complex with the conformational mutant p53 (Akakura *et al*., 2001). Hsc70 in a complex with mutant p53 and other chaperones, such as Hsp90, prevented the NLS from accessing the nuclear import receptor, whereas nuclear import of p53 possessing wild-type conformation was not affected (Akakura *et al*., 2001). These combined results clearly demonstrate that multiple chaperone complexes with mutant p53 are the factors mediating the stabilization and cytoplasmic sequestration of conformational mutant p53 (Figure 1).

Hsp70 family members participate in the cytoplasmic sequestration of wild-type p53 in cancer cells

In addition to mutant p53 retention in the cytoplasm, events involving the accumulation of wild-type p53 have been discovered in various tumour cells. In the absence of stress, human tumour cells, including neuroblastoma, breast cancer, colon cancer, retinoblastoma, as well as normal embryonic stem cells, have displayed the accumulation of high levels of wild-type p53 protein in the cytoplasm (for review see Helmbrecht *et al*., 2000). In colon carcinomas, cytoplasmic accumulation of wild-type p53 correlates with poor prognosis (Bosari *et al*., 1995). Cytoplasmically sequestered wild-type p53 protein is resistant to Mdm2-mediated degradation (Zaika *et al*., 1999) and it displays a high degree of aggregation that impairs the G_1 checkpoint following DNA damage (Moll *et al*., 1996).

Three potential NLSs have been proposed to reside in the C-terminal domain of p53 and are required for p53 entry into the nucleus (Zerrahn *et al*., 1992). The truncated form of importin α, identified in breast cancer cells, is deficient in its ability to import wild-type p53 into the nucleus, resulting in the cytoplasmic sequestration of p53 (Kim *et al*., 2000). The *mot-2* gene product, originally cloned from immortal murine cells, was shown to interact with sequestered wild-type p53 in the cytoplasm of immortal cells (Wadhwa *et al*., 1998). Interestingly, mot-2 is a member of the Hsp70 family that perform chaperone functions in the mitochondria (Wadhwa *et al*., 1998).

The nuclear export signal (NES), located in the p53 tetramerization domain, is required for subcellular local-

ization of p53 (Stommel *et al*., 1999). The leucine-rich NES sequence of p53 is masked upon nuclear p53 tetramerization, which inhibits the export of p53 from the nucleus and p53 degradation (Stommel *et al*., 1999). Cellular stress, such as ionizing radiation, has long been known to stabilize p53 and its nuclear accumulation (for review see Jimenez *et al*., 1999). Peptides derived from the p53 C-terminal tetramerization domain can mask the p53 NES sequence (but not the NLS), which facilitates nuclear localization of wild-type p53 in neuroblastoma cells (Stommel *et al*., 1999). These results together imply that proteins interacting with the p53 C-terminal domain may play a role in regulating the equilibrium of p53 translocation between the nuclear and cytoplasmic compartments. We propose that molecular chaperones could be involved in the regulation of p53 localization in either stressed or normal cellular conditions (Figure 1).

Conclusions

Cell survival during stress requires induction of the heat shock response. The expression of heat shock genes provides an adaptive mechanism for stress tolerance. However, the same adaptive mechanism can ultimately harm healthy cell growth by negatively influencing pathways that regulate cell growth control and apoptosis. It still remains unclear how HSPs, such as Hsp70, interfere with normal growth processes. These unknown effects of Hsp70 can be potentially problematic for new therapeutic applications, such as the regulation of Hsp70 expression as a tool to induce apoptosis in cancer cells (Nylandsted *et al*., 2000a), and the use of Hsp70 protein to create new types of adjuvants for vaccines that specifically target tumour cells or increase cell resistance to viral infections (Janetzki *et al*., 2000; Menoret *et al*., 2000; Srivastava and Jaikaria, 2001), which are part of a new era in molecular medicine.

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