Cell Prolif. 2000, **33**, 341–365

REVIEW ARTICLE

Chaperones in cell cycle regulation and mitogenic signal transduction: a review

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Received 29 *November* 1999*; revision accepted* 14 *April* 1999

Abstract. Chaperones/heat shock proteins (HSPs) of the HSP90 and HSP70 families show elevated levels in proliferating mammalian cells and a cell cycle-dependent expression. They transiently associate with key molecules of the cell cycle control system such as Cdk4, Wee-1, pRb, p53, p27/Kip1 and are involved in the nuclear localization of regulatory proteins. They also associate with viral oncoproteins such as SV40 super T, large T and small t antigen, polyoma large and middle S antigen and EpsteinBarr virus nuclear antigen. This association is based on a J-domain in the viral proteins and may assist their targeting to the pRb/E2F complex. Small HSPs and their state of phosphorylation and oligomerization also seem to be involved in proliferation and differentiation. Chaperones/HSPs thus play important roles within cell cycle processes. Their exact functioning, however, is still a matter of discussion.

HSP90 in particular, but also HSP70 and other chaperones associate with proteins of the mitogen-activated signal cascade, particularly with the Src kinase, with tyrosine receptor kinases, with Raf and the MAP-kinase activating kinase (MEK). This apparently serves the folding and translocation of these proteins, but possibly also the formation of large immobilized complexes of signal transducing molecules (scaffolding function).

INTRODUCTION

Proliferation of cells is a fundamental process which is highly regulated particularly in the course of development and maintenance of multicellular organisms. It is also subject to many external and internal perturbations against which several stabilizing and repair mechanisms have been developed. One of these stabilizing mechanisms involves chaperones, some isoforms of which (heat shock/stress proteins, HSPs) are increased upon exposure to stress (De Maio 1999). Evidence has accumulated that cell cycle components, regulatory proteins and members of the mitogenic signal cascade associate with chaperones and stress proteins for different periods of time. A short summary of this topic was recently published by Sato &

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Torigoe (1998). The intention of the present review is to give a more extended account of the evidence on the interactions between chaperones and cell cycle components, viral oncogene products and mitogenic cascades. We will also discuss the putative functions of these complexes and other functions of chaperones within the cell cycle.

Chaperones represent a group of highly conserved protein species which are localized in different cell compartments and assist newly synthesized proteins to fold or to translocate through membranes, stabilize certain protein conformations and help to eliminate denatured proteins by way of degradation (Becker & Craig 1994; Hartl 1996). Chaperones also play a role within the regulatory network of the cell cycle and within signal cascades. The cytosolic HSP90 in particular, acts as a dimer and forms complexes with protein kinases and transcription factors with the function of guiding the protein partner to its ultimate location, assisting in its conformational maturation and/or stabilizing its conformation (reviews: Scheibel & Buchner 1997; Beissinger & Buchner 1998). The HSP70 family whose members are found in the cytosol and in other compartments of the cell, is equally or even more involved in the functions described above. The constitutive members of this family (also called HSP70 cognates or HSC70) in the cytosol are particularly involved in folding and refolding of proteins, in maintaining their unfolded (transport) state and in the elimination of nonfunctional proteins (review: Becker & Craig 1994; Hartl 1996; De Maio 1999). They do this by cooperating with so called 'cochaperones' for example with HSP40, the eukaryotic homologue of DnaJ in *E. coli* (Laufen *et al*. 1999).

The cell cycle and its control processes can be upset by stress as induced by external factors such as heat shock, irradiation, toxic substances, by reactive oxygen species, viral infections and other perturbations. Stress also affects the mitogen-activated signal cascade and alters its signalling drastically. Cells counteract the effects of stress by increasing their stabilizing forces: among others they strongly increase the synthesis of HSPs. Many of the stress-induced HSPs, such as HSP90, HSP70 and the small HSPs, are isoforms of constitutive chaperones and fulfil similar or identical functions. With these increased amounts of HSPs many cellular functions become thermotolerant (review: Parsell & Lindquist 1993; De Maio 1999), which is also true for the heat-induced arrests of the cell cycle (Kühl, Kunz & Rensing 2000). This supports the notion of a protective function of chaperones and stress proteins in the maintenance of the cell cycle and signalling processes.

ROLES OF CHAPERONES/STRESS PROTEINS OF HSP90 AND HSP70 FAMILIES IN THE CELL CYCLE

Growth-dependent expression

Many studies indicate that chaperones and stress proteins are involved in the regulation of cell growth and transformation. Generally, the level of chaperones/HSPs is increased in proliferating cells compared to those in the stationary state or differentiated cells (Wu *et al*. 1986; Hensold & Houseman 1988; Murakami *et al*. 1991; Sainis *et al*. 1994; Helmbrecht & Rensing 1999). However, in U937 human promonocytic leukaemia cells treated with the differentiation inducer sodium butyrate a transient increase of HSP70 was observed (García-Bermejo *et al*. 1995).

Some chaperones/HSPs are expressed in a cell-cycle dependent manner: Several mammalian cells show an increase of HSP70, or HSC70 mRNA and protein, in S-phase (Milarski & Morimoto 1986; Zeise *et al*. 1998; Helmbrecht & Rensing 1999). HSP90a mRNA accumulates during the G1/S transition (Jérôme *et al*. 1993). Moreover, a

translocation of HSP70, or HSC70, into the nucleus during S-phase has been observed (Zeise *et al*. 1998). Cytoplasmic HSP70 binds to the NLS of karyophilic proteins and is involved in the transport of proteins into the cell nucleus (Imamoto *et al*. 1992; Shi & Thomas 1992; Okuno, Imamato & Yineda 1993; Yang & DeFranco 1994; Shulga *et al*. 1996). It has been suggested that an enhanced requirement for nuclear transport occurs during S-phase. It is known that the nuclear import rate in proliferating cells is higher than that in quiescent cells (Feldherr & Akin 1990; 1993; Csermely, Schnaider & Szántó 1995). Milarski, Welch & Morimoto (1989) observed a cell cycle-dependent association of HSP70 with specific cellular proteins.

The expression of members of the HSP70 and HSP90 family is up-regulated by serum/mitogen/growth factor stimulation (Wu & Morimoto 1985; Ferris *et al*. 1988; Haire, Peterson & O'Leary 1988; Hickey *et al*. 1989; Hansen, Houchins & O'Leary 1991; Jérôme *et al*. 1991). Members of the HSP70 and HSP90 family are more commonly expressed in tumours than in noncancerous tissues (Yano *et al*. 1996, 1999; Ferrarini *et al*. 1992; Yufu, Nishimura & Nawata 1992); overexpression of HSP70 confers tumorigenicity in mouse fibrosarcoma cells (Jäättelä 1995) and induces a transformed phenotype in rat 1 fibroblasts (Volloch & Sherman 1999).

The expression of members of the HSP70 family is stimulated by infection with adenovirus (Nevins 1982; Kao & Nevins 1983; Wu *et al*. 1986; Simon *et al*. 1987; Phillips, Abravaya & Morimoto 1991), herpes simplex virus (Phillips, Abravaya & Morimoto 1991), human cytomegalovirus (Santomenna & Colberg-Poley 1990), HTLV-I (D'Onofrio *et al*. 1995), SV40 and polyoma virus (Khandjian & Türler 1983), HIV (Wainberg *et al*. 1997) and EpsteinBarr virus (Cheung & Dosch 1993). Elevated HSP90 expression is observed after infection with SV40 and polyoma virus (Khandjian & Türler 1983) and adenovirus (Simon *et al*. 1987). Expression of members of the HSP70 family is under the control of a number of viral and cellular protooncogenes including adenovirus product E1A (Herrmann, Dery & Matthews 1987), SV40 T antigen (Sainis *et al*. 1994), c-myc (Kingston, Baldwin & Sharp 1984; Taira *et al*. 1992), myb (Foos, Natour & Klempnauer 1993), and tumour-suppressor protein p53 (Agoff *et al*. 1993).

HSP70 overexpression provides protection against the cytotoxicity of doxorubicin and allows proliferation to restart in doxorubicin-mediated G2 arrest (Karlseder *et al*. 1996). Overexpression of human HSP72 stimulated the growth rate and appearance of S-phase cells in the SHOK cell line (Suzuki & Watanabe 1994) whereas overexpression of HSC70 suppressed transformation of rat embryo fibroblasts (Yehiely & Oren 1992). Evidence for a role of HSP70 in cell proliferation has also been obtained from *hsp70* antisense application, which abolished progress through G1 and S phase in human tumour cells (Wei *et al*. 1995).

Antisense-inhibition of HSP90 decreased the rate of cell division of U937 human monoblastoid cells, and HSP90 overexpression stimulated the appearance of cells in S-phase (Galea-Lauri, Latchman & Katz 1996). A growth stimulating factor, which reacts with anti-HSP90a antibody, was purified from the culture supernatant of human hybridoma SH-76 cells in serum-free medium. The protein stimulated growth of some but not all lymphoid cell lines (Kuroita *et al*. 1992).

Potential role in DNA replication

In some cell lines the cell cycle-dependent expression of HSP70 was shown to be DNA synthesis-dependent and suppressed by treatment with the inhibitor of DNA synthesis Ara C (Milarski & Morimoto 1986; Hang & Fox 1995) indicating a potential involvement of HSP70 in DNA replication. These results are supported by observations that UV irradiated human fibroblasts show high nuclear HSP72 levels during recovery of S phase, suggesting a function

during the recovery of DNA replication inhibited after UV irradiation. (Suzuki & Watanabe 1992). Recently, structures have been determined which link class II topoisomerases, the DNA repair enzyme MutL and HSP90 (Stock 1999).

A specific function of heat shock proteins is known for the initiation of bacteriophage replication: The *E. coli* heat shock proteins DnaK (the HSP70 homologue), DnaJ and GrpE bind to the nucleoprotein complex consisting of the λ O protein, λ P protein and DnaB that assemble at the λ origin of replication. DnaJ, DnaK and GrpE act together with ATP to release λP protein from this complex, thereby freeing the DnaB helicase for its DNA-unwinding reaction (Liberek, Georgopoulos,& Zylicz 1988; Alfano & McMacken 1989a, 1989b; Dodson, McMacken & Echols 1989; Zylicz *et al*. 1989; Hoffman *et al*. 1992; Wyman *et al*. 1993). The three stress proteins are also involved in the replication of plasmid P1 and F (Wickner & Chattoraj 1987; Ezaki *et al*. 1989; Tilly & Yarmolinsky 1989; Kawasaki, Wada & Yura 1990) yet their role is totally different. The P1 replication initiator protein RepA exists in a stable complex with DnaJ. In an ATP-dependent reaction repA dimers dissociate to monomers by means of DnaK with the help of DnaJ and GrpE which is a prequisite for DNA binding of RepA to the origin of replication of plasmid P1 (Wickner, Hoskins & McKenney 1991a, 1991b; Wickner *et al*. 1992).

Association of chaperones with components of the eukaryotic cell cycle

Several key players in cell cycle control systems are presently known to transiently associate with chaperones: complexes are found among the cyclins and cyclin-dependent kinases (Cdks), among the kinases which control Cdk activity and among the Cdk inhibitors of the Cip/Kip family. For recent overviews over the general functioning of these key regulatory molecules see Morgan (1997), Johnson & Walker (1999), with regard to cyclins, Cdks and their control by phosphorylation and see Peter (1997) and Endicott, Noble & Tucker (1999) with regard to Cdk inhibitors. One of the main functions of the active G1-Cdks is to phosphorylate the retinoblastoma protein (pRb) and thus release the transcription factors (E2F, DP) which in turn activate S-phase specific genes (reviews: Johnson & Schneider-Broussard 1998; Black & Azizkhan-Clifford 1999; Lavia & Jansen-Durr 1999; Lipinski & Jacks 1999). The function of the tumour suppressor p53 as guardian of the genome is based on its ability to arrest cell growth, or to promote apoptosis, in response to DNA damage, nutrient depletion, hypoxia, heat shock, ribonucleotide depletion, etc. (Sionor & Haupt 1999) and to promote DNA repair. pRb as well as p53 also belong to the class of proteins known to transiently associate with chaperones.

Cdk4. Src and Raf heterocomplexes with HSP90 contain a 50-kDa protein (Brugge, Yonemoto & Darrow 1983; Whitelaw *et al*. 1991; Hutchison *et al*. 1992; Stancato *et al*. 1993; Wartmann & Davis 1994; Silverstein *et al*. 1998) that was identified as the mammalian homologue of the yeast cell cycle control protein Cdc37 (Perdew *et al*. 1997). Cdc37 interacts with Cdk4 (Dai, Kobayashi & Beach 1996), preferentially with the kinase molecules not bound to D-type cyclins (Stepanova *et al*. 1996) (Fig. 1). Geldanamycin-induced disruption of the Cdc37/HSP90 complex with Cdk4 decreases the half-life of newly synthesized Cdk4, indicating a role for Cdc37/HSP90 in Cdk4 stabilization (Stepanova *et al*. 1996). Cdc37 is coexpressed with cyclin D1 during cell division (Stepanova *et al*. 1996). Mutations in yeast Cdc37 also impair v-src function (Dey, Lightbody & Boschelli 1996).

Wee 1. A member of the HSP90 family in *Schizosaccharomyces pombe* (SWO1) interacts with the Wee1 kinase (Fig. 1). Wee1 regulates the length of the G2 phase by catalysing the

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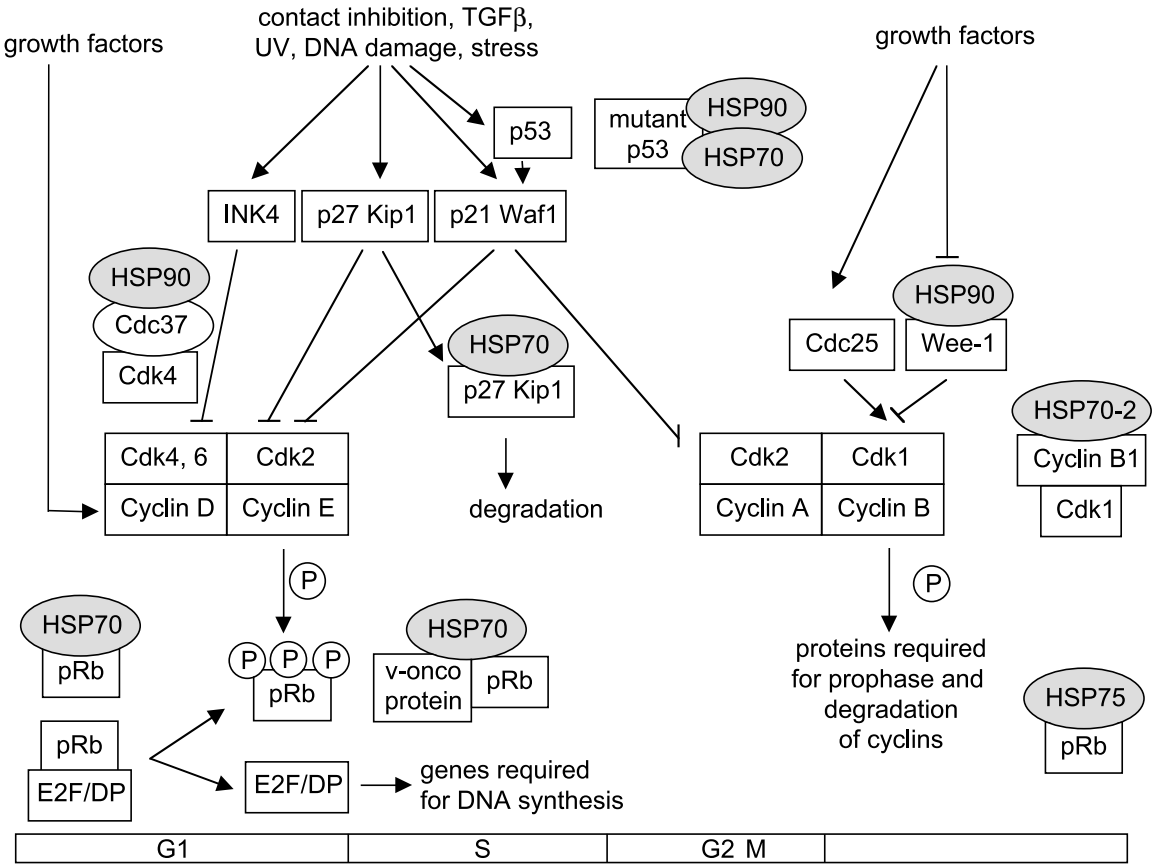

Figure 1. Association of chaperones (HSPs) with cell cycle control proteins. Cdk, cyclin-dependent kinase; Cdc25, cell division cycle protein 25 (phosphatase); Cdc37, cell division cycle protein 37, with similar function to HSP90; E2F/DP, heterodimer of transcription factor; INK4, p21Waf1 and p27Kip1, inhibitors of Cdks; p53, tumour suppressor; pRb, retinoblastoma protein (tumour suppressor); TGFβ, transforming growth factor β. Wee1, inhibitory kinase. Heat shock proteins (HSPs), grey; →, activation; —|, inhibition (for further explanation see text).

inhibitory tyrosyl phosphorylation of Cdk1 (Cdc2). SWO1 minus mutants undergo premature mitosis when grown at a semipermissive temperature. It is assumed that formation of active Wee1 kinase requires interaction with the HSP90 homologue, perhaps in a manner analogous to the interaction between HSP90 and v-Src (Aligue, Akhaven-Niak & Russell 1994) (see below).

c-Myc. HSP70 colocalizes with c-Myc and v-Myc in the nucleus of Myc-overexpressing CV-1, COS, HeLa and 293 cells (Koskinen *et al*. 1991; Henriksson *et al*. 1992). The Myc-induced distribution of HSP70 to nuclear bodies is distinct from the cell-cycle dependent and stress-induced nuclear translocation of HSP70 (Koskinen *et al*. 1991).

pRb. Constitutive HSC70 binds to a specific sequence in the N-terminal region of the retinoblastoma protein (pRb) (Fig. 1; Nihei *et al*. 1993b). This complex can be dissociated in an ATP-dependent manner. HSC70 binds selectively to nonphosphorylated pRb. When associated with HSC70 pRb is not susceptible to in vitro phosphorylation (Inoue *et al*. 1995).

An interaction of pRb with a 75-kDa protein (named HSP75) was observed, which shows sequence homology to members of the HSP90 family. A unique motif in HSP75 but not in other HSP90 family members appears to be important for binding to the SV40 T-antigen-binding domain of hypophosphorylated pRb (Fig. 1). In mammalian cells, pRb forms complexes with HSP75 during M phase, when the nuclear envelope breaks down, as well as after heat shock, when HSP75 moves from its normal cytoplasmic location into the nucleus. *In vitro*, HSP75 can faciliate refolding of denatured pRb into its native conformation. It is assumed that the circumstances of the interaction of pRb and HSP75 are characterized by the potential need for pRb to refold or renature (Chen *et al*. 1996).

p53. Wild type p53 is a sequence-specific DNA-binding protein that regulates transcription. It is a short-lived protein which turns over very rapidly in the ubiquitin-proteasome pathway. In mutant p53 the normal processing is impaired, which results in the marked accumulation of dysfunctional molecules with a prolonged half-life.

There is evidence that wild-type p53 protein is conformationally flexible (Milner 1995). Growth stimulation of normal human lymphocytes was shown to convert wild-type p53 to a mutated conformation (Zhang *et al*. 1992). Addition of fresh serum resulted in loss of wild-type conformation of murine p53 (Milner, Cook & Mason 1990). A loss of mutated p53 conformation has been reported to occur during DMSO-induced differentiation/apoptosis of erythroleukaemia cells (Ryan & Clark 1994).

Many transforming p53 mutants form stable complexes with constitutive and/or inducible members of the HSP70 familiy under constitutive as well as stress conditions (Fig. 1, Pimhasi-Kimhi *et al*. 1986; Hinds *et al*. 1987; Sturzbecher *et al*. 1987; Finlay *et al*. 1988; Gannon *et al*. 1990; Hinds *et al*. 1990; Davidoff *et al*. 1992; Hainaut & Milner 1992; Nihei *et al*. 1993a; Ohnishi *et al*. 1995; Merrick *et al*. 1996; Selkirk *et al*. 1996; Whitesell *et al*. 1998). HSP40, the mammalian homologue of bacterial DnaJ interacts with HSP70 and p53 (Sugito *et al*. 1995). Complexes dissociated *in vitro* when incubated with ATP (Hainaut & Milner 1992; Nihei *et al*. 1993a). A shift of a temperature-sensitive mutant to the wild-type form requires ATP hydrolysis. This suggests a role for HSP70 in regulating p53 conformation (Hainaut & Milner 1992). The interaction between p53 and members of the HSP70 family correlates with increased transforming activity (Hinds *et al*. 1987; Finlay *et al*. 1988; Halevy, Michalovitz & Oren 1990; Hinds *et al*. 1990) and prolonged half-life of p53 (Finlay *et al*. 1988). Results concerning the relationship between HSP binding and subcellular localization

of p53 are ambiguous (Sturzbecher *et al.* 1987; Zerrahn *et al.* 1992; Merrick *et al.* 1996). Transfection with mortalin 2, a HSP70 family member, repressed p53-mediated transactivation of genes (Wadhwa *et al.* 1998) and sequestrated p53 to the cytoplasm (Wadhwa *et al.* 1999). Additionally, overexpression of mortalin 2 results in malignant transformation of NIH 3T3 cells (Kaul *et al.* 1998).

Mutant p53 also coprecipitates (or is copurified) with HSP90 (Blagosklonny *et al.* 1996; Selkirk *et al.* 1996; Sephernia *et al.* 1996; Dasgupta & Momand 1997; Whitesell *et al.* 1998) and other proteins of the cellular chaperone machinery (Whitesell *et al.* 1998). The coprecipitation of chaperone proteins with wild-type p53 was lost in conjunction with the restoration of its transcripional activity (Whitesell *et al.* 1998) and its ability to induce cell cycle arrest.

Disruption of HSP90-binding to mutant p53 by geldanamycin destabilizes p53 and prevents its nuclear translocation (Blagosklonny, Toretsky & Neckers 1995; Dasgupta & Momand 1997; Whitesell *et al.* 1997; Whitesell *et al.* 1998), stimulates ubiquitin-dependent and proteasome-mediated degradation (Whitesell *et al.* 1997) and leads to a loss of recognition by an antibody specific for the mutated conformation (Blagosklonny, Toretsky & Neckers 1995). *In vitro* translation experiments showed that HSP90 is required for acquisition of mutant p53 conformation (Blagosklonny, Toretsky & Neckers 1995). Geldanamycin does not appear to restore wild-type transcriptional activity to mutant p53 proteins (Whitesell *et al.* 1998), but there are reports that mutant p53 from geldanamycin-treated cells partially regained the ability to bind a wild-type specific p53 DNA consensus sequence (Blagosklonny, Toretsky & Neckers 1995). There are hints for a sequestration of p53 in the cytoplasm by complex formation with HSP90. Temperature-sensitive mutant p53 is associated with HSP90 in the cytoplasm at nonpermissive temperatures whereas at the permissive temperature p53 resides in the nucleus expressing a wild-type conformation devoid of HSP90-complexation (Sephernia *et al.* 1996). Additionally, geldanamycin induced the nuclear translocation of mutant p53 (Whitesell *et al.* 1998). However, other authors report that geldanamycin inhibits nuclear translocation of temperature-sensitive p53 that was induced by a temperature-shift (Dasgupta & Momand 1997).

p27/Kip1. HSC73 interacts with the Cdk2 inhibitor p27Kip1. The complex is formed during G1/S transition and correlates with p27/Kip2 degradation (Fig. 1). ATP dissociates this complex (Nakamura *et al.* 1999). Progression of G1/S transition is critically regulated by the degradation of p27/Kip1, which inhibits the kinase activity of the cyclin E/Cdk2 complex and/or cyclinD/Cdk4 complex (review: Peter 1997). Since p27/Kip1 is degraded via ubiquitination, it seems possible that HSC73 targets the protein for degradation.

Association with viral oncoproteins

Replication of many viruses requires that cells enter the cell cycle. Thus, these viruses have evolved the ability to stimulate cells to proliferate, leading in some cases to tumorigenesis. Viral oncoproteins alter the function of key cellular regulatory proteins, including members of the retinoblastoma protein family and p53.

Viral oncoproteins like SV40 super T antigen, SV40 large T, polyoma middle T antigen, SV40 small t antigen and polyoma virus large T antigen and EpsteinBarr virus nuclear antigen (EBNA)-LP were shown to interact with members of the HSP70 family (Walter *et al.* 1987; Sawai & Butel 1989; May *et al.* 1991; Kitay & Rowe 1996; Campbell *et al.* 1997; Sheng *et al.* 1997). Domains of EBNA-LP required for immortalization of B lymphocytes are responsible for interaction with HSP70 (Kitay & Rowe 1996). Khandjian & Türler (1983),

however, reported that they did not observe an association of SV40 T antigen with HPS70 and HSP90 by immunoprecipitation.

The aminoterminal sequences in the polyoma large T antigen and SV40 large T and small t antigens that govern the specific interaction with HSP70 are homologous to the J domain found in the DnaJ molecular chaperone, the bacterial homologue of HSP40 (Campbell *et al.* 1997; Sheng *et al.* 1997; Srinivasan *et al.* 1997). DnaJ proteins function as cofactors in regulating the activity of members of the HSP70 family. The J domain is proposed to be the interaction site for members of the HSP70 family (Hartl 1996). It was shown that this J domain-like sequence in viral proteins acts as a DnaJ-like chaperone since it stimulated ATPase activity of HSP70 and the release of the substrate from HSP70. Thus, it is hypothesized that the J domain of viral proteins directs the association of HSP70 to multiprotein complexes that are targets for viral oncoprotein action, e.g. the E2F-Rb complex (Srinivasan *et al.* 1997).

Viral T antigens use their J-domains to gain control over cell cycle progression. Mutations of the DnaJ-like sequence abolish polyoma large T antigen-induced transactivation of E2F-binding promoters and cell cycle progression by the polyoma large T antigen, a process that is dependent on the binding of the viral protein to pRb (Fig. 1). The same mutation affects binding to HSP70. Yet the mutants retain their ability to bind pRb. Cotransfection of a cellular DnaJ domain blocks polyoma large T antigen action (Sheng *et al.* 1997). Mutations of the J domain abrogates the ability to transform mammalian cells (Srinivasan *et al.* 1997). The J domain of SV40 large T antigen mediates a reduction in the abundance of phosphorylated pRb related proteins p130 and p107 and promotes degradation of p130 and p107 (Stubdal *et al.* 1997). Mutational analysis showed that the J domain of SV40 large T antigen is required to overcome the pRb, or p130-mediated, G1 arrest and repression of E2F-dependent transcriptional activity in cells overexpressing pRb or p130. Additionally only SV40 large T antigen with an intact J domain can disrupt p130-E2F DNA-binding complexes (Zalvide, Stubdal & Decaprio 1998).

Furthermore, the J-domain of the SV40 large T antigen is required for viral DNA replication *in vivo*. It is suggested that the viral J-domain supports SV40 DNA replication in a manner that is similar to the use of *E. coli* DnaJ by bacteriophage lambda in DNA replication (Campbell *et al.* 1997). Human papilloma virus replication initiator, the E1 helicase, is enhanced in its binding to the origin of DNA replication by purified human HSP70 and HSP40. This suggests an important role for chaperones in the assembly of the preinitiation complexes at the origin (Liu *et al.* 1998).

It was shown that HSP70 is needed for the nuclear import of SV40 large T antigen. Depletion of HSP70 from digitonin-permeabilized cells prevented nuclear import of SV40 large T antigen (Yang & DeFranco 1994). Swiss 3T3 cells transfected with a constitutively expressed human HSP70 can complement the nuclear translocation and transformation defect of mutant SV40 T antigen (Jeoung *et al.* 1991). Human cytomegalovirus (HCMV) induces the expression of HSP70, its nuclear translocation early in infection and its predominant cytoplasmic localization late in infection (Ohgitani *et al.* 1999).

Association with the centrosome

In a variety of mammalian cell lines members of the HSP70 family are associated with the centrosome (Rattner 1991; Brown *et al.* 1994; Perret *et al.* 1995). This suggested that HSP70 might be involved in microtubule nucleation or in centrosome assembly. The most prominent colocalization of HSP70 with the centrosome was observed during mitosis (Rattner *et al.* 1991; Brown *et al.* 1994). However, in adenovirus-transformed cells p53 and the adenovirus protein E1B were observed in the HSP70-containing structure that associates with the

centrosomes during interphase. Yet, upon entry into mitosis, the E1B/p53/HSP70 structure separated from the centrosome as the centrosome became engaged in mitotic spindle formation (Brown *et al*. 1994).

HSP70 during gametogenesis

Two members of the HSP70 family were found to be specifically expressed during spermatogenesis: Murine HSP70-2 is synthesized during the meiotic phase (Zakeri & Wolgemuth 1987; Zakeri, Wolgemuth & Hunt 1988; Dix *et al*. 1996b; Dix 1997a), and mouse testis-specific HSC70t is expressed in postmeiotic spermatids (Maekawa *et al*. 1989; Matsumoto & Fujimoto 1990). A human homologue of murine HSP70-2, HSPA2 has been observed in spermatocytes and spermatids (Son *et al*. 1999). While the function of HSP70t is still unknown, gene disruption of *hsp70–2* caused a defect in meiosis and germ cell apoptosis which lead to infertility (Dix *et al*. 1996a, 1997b; Mori *et al*. 1997).

HSP70–2 has been identified as a component of the synaptonemal complexes formed between paired homologous chromosomes during meiotic prophase in mouse and hamster spermatocytes (Allen *et al*. 1996). Synaptonemal complexes function in meiotic chromosome recombination and segregation. Desynapsis and disassembly of synaptonemal complexes is impaired in cells with a disrupted *hsp70–2* gene, and the cells are unable to progress to metaphase (Dix *et al*. 1996a, 1997b).

Disruption of *hsp70–2* in mice revealed another function of HSP70–2: An interaction between HSP70–2 and Cdc2 kinase (= Cdk1) was demonstrated (Fig. 1), which is required for Cdc2 complex formation with cyclin B1, kinase activation and progression of the meiotic cell cycle (Zhu, Dix & Eddy 1997). This may be the reason for the failure to complete meiosis in *hsp70–2*$^{-/-}$ mice. Spermatogenic cell development was arrested in prophase of meiosis I at the G2/M-phase transitions (Eddy 1999).

The small HSPs

HSP27 is an ubiquitous target of phosphorylation upon stimulation with a variety of growth factors and mitogens (Mehlen & Arrigo 1994; Satoh & Kim 1995; Guay *et al*. 1997), differentiation inducers (Spector *et al*. 1993; Chaufour, Mehlen & Arrigo 1996; Chiesa, Noguera & Sredy 1997; Horman *et al*. 1997), apoptosis inducing agents (Horman *et al*. 1997) and stressing treatments (Landry *et al*. 1992; Satoh & Kim 1995; Guay *et al*. 1997). Oligomeric structures of HSP27 are observed concommitant with phosphorylation (Mehlen & Arrigo 1994; Chaufour, Mehlen & Arrigo 1996). In addition, an accumulation of HSP27 is observed during stress (Landry *et al*. 1992; Lavoie *et al*. 1993; Wagner *et al*. 1999), after induction of differentiation (Shakoori *et al*. 1992; Kindas-Mügge & Trautinger 1994; García-Bermejo *et al*. 1995; Spector *et al*. 1995; Chaufour, Mehlen & Arrigo 1996; Chiesa, Noguera & Sredy 1997) and in actively growing tumour cells (Khalid *et al*. 1995; Morino *et al*. 1997 Takashi *et al*. 1997).

The role of small HSPs in the control of proliferation and differentiation, however, is not clear and may be different in different tissues (Welsh & Gaestel 1998): Transfection of *hsp27 to* a breast carcinoma cell line for example led to a lowered proliferation (Kindas-Mügge, Micksche & Trautinger 1998) whereas antisense inhibition of HSP27 in MCF-7 cells inhibited growth (Mairesse *et al*. 1996) and induced the acquisition of a secretory phenotype (Horman *et al*. 1999). HSP27 overexpression in rat colon carcinoma cell clones (Garrido *et al*. 1998) and in breast cancer cells (Lemieux *et al*. 1997) enhanced their tumorigenicity. Additionally, there are reports that antisense inhibition of HSP27 furthermore interfered with the differentiation process of HL-60 cells (Chaufour, Mehlen & Arrigo 1996) and reduced the

extent of cardiomyocyte differentiation (Davidson & Morange 2000). Overexpression of HSP27 induced resistance to apoptosis (Samali & Cotter 1996; Garrido *et al*. 1999).

The oligomer size of HSP27, its phosphorylation and function is regulated by the MAPKAP kinase 2/3, which is the only kinase known to phosphorylate HSP27. MAPKAP kinase 2/3 is phosphorylated and activated by the MAP kinase p38 and by the ERK-kinases (Landry *et al*. 1992; Stokol *et al*. 1992; Guay *et al*. 1997; Larsen *et al*. 1997) thus coupling the function of HSP27 to the mitogen-activated signal cascade (see below).

THE MAP KINASE CASCADE

The complex regulatory systems of the cell cycle are influenced by external signals, for example by growth factors via the mitogenic signal cascades, by inhibitory factors such as TGFβ, by viral infection and by stress. Stress may either act unspecifically upon gene expression and many other processes or act specifically, for example through changes in the activity of different signal cascades (see below).

MAP kinase cascade in cell cycle control

Converting extracellular stimuli into appropriate intracellular responses involves the coordinated action of specific intracellular pathways. One of the most prominent signal transduction pathways identified in all eukaryotic cells is the MAPK (mitogen activated protein kinase) cascade (Seger & Krebs 1995), which consists of hierarchically organized kinase modules that are sequentially activated and finally mediate the phosphorylation of specific cellular targets. Three distinct subgroups of MAPK cascades can be distinguished in mammalian cells (Davis 1994): the stress activated MAP kinases SAPK/JNK (stress activated protein kinase/c-jun terminal kinase) and the HOG-1 (high osmolarity glycerol) homologue p38, which are mainly activated upon environmental stress and the mitogen-stimulated MAP kinase ERK (extracellular signal-regulated kinase) predominantly activated by growth factors. Dependent on the cellular context, different cellular responses (proliferation, differentiation and apoptosis) are initiated through the preferential activation of a specific MAPK cascade. It is now well established that members of the mitogen-stimulated MAP kinases p44/ERK1 and p42/ERK2 play critical roles in the control of cell cycle progression during meiosis and mitosis, while the activation of SAPK by environmental stress leads to growth arrest or in some cell types to apoptosis (Pelech & Charest 1995).

A general activation scheme of ERK in response to external stimuli is shown in Fig. 2. Upon binding of growth factors to specific receptor tyrosine kinases (RTK) the receptor becomes autophosphorylated and associates with the adaptor protein Grb-2 (growth factor receptor bound protein 2) by its SH2 domain. Grb-2 binds the guanine nucleotide release protein (Gnrp or Sos) which causes a GDP-GTP exchange in the guanine nucleotide-binding protein Ras. Activated GTP-bound Ras in turn activates the serine/threonine kinase Raf by direct binding to the N-terminus of Raf and recruiting this kinase to the plasma membrane. Active Raf then acts as an upstream activator of the dual-specific MEK/MKK1 (MAP kinase/ERK kinase; mitogen activated kinase kinase). In addition MEK/MKK1 may be activated by other upstream kinases like Mos, a MAP kinase kinase kinase expressed during meiotic maturation of oocytes, and MEKK1 an upstream activator of the stress dependent protein kinase kinase SEK (Sagata 1997). Once activated, the dual specific MEK/MKK1 subsequently phosphorylates the MAP kinases ERK1,2 at threonine 183 and tyrosine 185 within a specific consensus sequence (TEY). The main cytoplasmic substrates of ERK are

PLA2 (phospholipase A2) and RSK (ribosomal S6 kinase) thereby connecting the MAP kinase cascade with other signalling pathways of the cellular communication network (Hunter 1995; Waskiewicz & Cooper 1995). ERKs as well as Cdks belong to the proline-directed kinases that phosphorylate threonine or serine residues surrounded by two proline residues in the −2 and +1 position (P-X-S/T-P, where X = any AA) (Alvarez, Nothwood & Gonzalez 1991; Treisman 1996).

A fraction of activated ERK translocates into the nucleus where it phosphorylates a set of transcription factors: c-Jun, c-Fos, Elk-1, SAP-1,2, ATF-2 and c-Myc, thereby modifying the expression of genes with TRE, SRE, or CRE-driven promoters (Treisman 1996). The TCFs (ternary complex factors): Elk-1 and SAP-1,2 form ternary complexes with SRF (serum response factor) and stimulate the transcription of genes which preclude the S phase of the cell cycle by binding to SRE (serum response element). Cell cycle entry of mammalian fibroblasts is accompanied by the sustained activation of p44 MAPK and p42 MAPK isoforms in the G1 phase and their inactivation at the G1/S transition (Meloche 1995). Pagés *et al*. (1993) showed that persistent activation of ERKs is required to pass the G1 restriction point in fibroblasts. One might speculate that MAP kinases are also involved in pRb phosphorylation in the G0/G1/S transition of the somatic cell cycle because it was recently shown that MAP kinases contribute to pRb phosphorylation during meiotic maturation of *Xenopus* oocytes (Taieb *et al*. 1998).

Lavoie *et al*. (1996) demonstrated that activation of ERK 1,2 is involved in mitogen-induced cyclin D1 expression, a regulatory subunit of Cdk 4/6. The upregulation of cyclin D1 expression is an essential event for the initation of a new cell cycle. G1 progression can be inhibited by dominant negative MEK/MKK1 or $p44^{MAPK}$ while constitutively active MEK/MKK1 dramatically increases the endogenous cyclin D1 expression. These results indicate a direct role of MAPK in the regulation of Cdk activity especially in the G1 phase of the cell cycle. Further studies reveal that activation of the MAP kinases ERK1,2 does not seem to be restricted to the G1 phase of cell cycle. These kinases are also active during the G2/M transition in CHO cells (Tamemoto *et al*. 1992) and are required for the progression of *Xenopus* oocytes from G2 into meiotic M phase. They are apparently not a limiting step for normal mitotic M phase entry in *Xenopus* (Cross & Smythe 1998).

In contrast to the proliferation-stimulating activity of ERKs they also play an important role in regulating cell cycle arrest and exit from the cell cycle during terminal differentiation. Cell cycle arrest at the spindle assembly checkpoint during mitosis of *Xenopus* cells depends on ERK2 activity and correlates with a reduced destruction of cyclin B (Minshull *et al*. 1994). During NGF-stimulated neuronal differentiation of PC12 cells, retinoic acid (RA)-induced differentiation of HL-60 myeloblastic leukaemia cells and PMA-induced megakaryocyte differentiation of K562 cells, ERKs play a critical role in transducing differentiation signals (Jaiswal *et al*. 1996; Herrera *et al*. 1998; Yen *et al*. 1998). Treating HL-60 cells with the MEK specific inhibitor PD98059 reveals that RA-induced differentiation depends on signalling through a pathway including Ras/Raf and MAPK and correlates with cell cycle arrest and pRb hypophosphorylation (Yen *et al*. 1998). In NGF stimulated differentiation of PC12 cells the capacity of B-Raf to activate the MAP kinase depends on its ability to form stable association with the molecular chaperone HSP90 (Jaiswal *et al*. 1996).

The observation that MAP/ERK transduced signals can initiate two differently directed cellular processes either toward proliferation or toward differentiation, raises the intriguing question of what enables the MAP/ERK kinase to either stimulate cell cycle progression or to stop growth? The most convincing hypothesis postulates that the decision between the two states depends on signal intensity and duration. This suggests that a short duration of ERK

activation favours the mitogenic response to growth factors and promotes proliferation while a long duration of ERK activation preferentially causes growth arrest and differentiation (Yen *et al*. 1998).

Association of chaperones with mitogenic signal transduction proteins

A variety of transcription factors and protein kinases involved in mitogenic signal transduction are recovered from cells in heterocomplexes with HSP90 (Pratt 1997, 1998). These heterocomplexes are formed by a multicomponent HSP90-based chaperone system consisting of at least HSP90, HSP70, p60 and p23, which represents an ubiquitous protein-folding system in the cytoplasm of eukaryotes (Pratt 1997, 1998; Chen & Smith 1998). It is suggested that the same cellular process that promotes the folding and assembly of nascent proteins plays a pivotal role in signal transduction by promoting the regulated folding or assembly and disassembly of mature signalling molecules between active and inactive states (Rutherford & Zuker 1994). In addition to binding several transcription factors, HSP90 forms stable complexes with a variety of protein kinases implicated in mitogenic signalling, cell cycle regulation or oncogenic transformation. Among these are tyrosine kinases v-Src (v-Fps, v-Yes, v-Fes, Lck), Wee1 kinase, and the serine/threonine kinases Raf/Gag-Mil (Pratt 1997).

Src. A complex between tyrosine kinase v-Src and HSP90 was observed frequently (Oppermann, Levinson & Bishop 1981). Newly synthesized Src molecules preferentially associate with HSP90 (Fig. 2; Xu & Lindquist 1993). Complexed with HSP90, v-Src remains inactive and hypophosphorylated during the transport to the membrane. When v-Scr is subsequently found attached to the plasma membrane, it is active and no longer associated with HSP90 (Courtneidge & Bishop 1982; Brugge,Yonemoto & Dallow 1983; Xu & Lindquist 1993).

The antibiotics geldanamycin and herbimycin A which interfere with HSP90-Src complex formation were found to revert transformation by v-Src (Whitesell *et al*. 1994; Uehara *et al*. 1986, 1988). Disruption of the complex by geldanamycin or herbimycin leads to destabilization of v-src (Uehara *et al*. 1989; Whitesell *et al*. 1994). In cells infected with temperature-sensitive *src* variants that are defective in cellular transformation, most Src molecules are recovered in stable heterocomplexes with HSP90 (Brugge, Yonemoto & Dallow 1983). In yeast in which *v-src* expression results in growth arrest mutants with a decreased HSP90 level are resistant to the cytotoxic effect of *v-src* transfection (Xu & Lindquist 1993). It is thus assumed that HSP90 functions in the maturation of v-Src, its transport to the membrane and kinase activation (Courtneidge & Bishop 1982; Brugge,Yonemoto & Dallow 1983; Xu & Lindquist 1993). Analysis of point mutations in the *hsp90* gene revealed that HSP90 function in the maturation of v-Src involves separable roles in protein accumulation and kinase activation (Nathan & Lindquist 1995). Even though native c-Src-HSP90 complexes have not been identified, they can be assembled in a reticulocyte lysate (Hutchison *et al*. 1992). Recently, it was shown that HSP90 is indeed necessary also for c-Src maturation in yeast, however, it is less sensitive to HSP90 perturbation than v-Src (Xu, Singer & Linquist 1999).

In addition, a series of Src-related transforming cellular and viral tyrosine kinases was found in complexes with HSP90: v-Fps, v-Yes, v-Fgr, c-Fgr, Lck, v-Fes, c-Fes (Adkins, Hunter & Sefton 1982; Lipsich, Cutt & Brugge 1982; Ziemiecki *et al*. 1986; Hartson & Matts 1994; Nair *et al*. 1996). The antibiotics geldanamycin and herbimycin A revert transformation by tyrosine kinase oncogenes, such as *lck*, *yes*, *ros*, *fps*, *abl* and *erbB* (Uehara *et al*. 1988; Hartson *et al*. 1996) presumably by disrupting the HSP90 kinase complex and by the

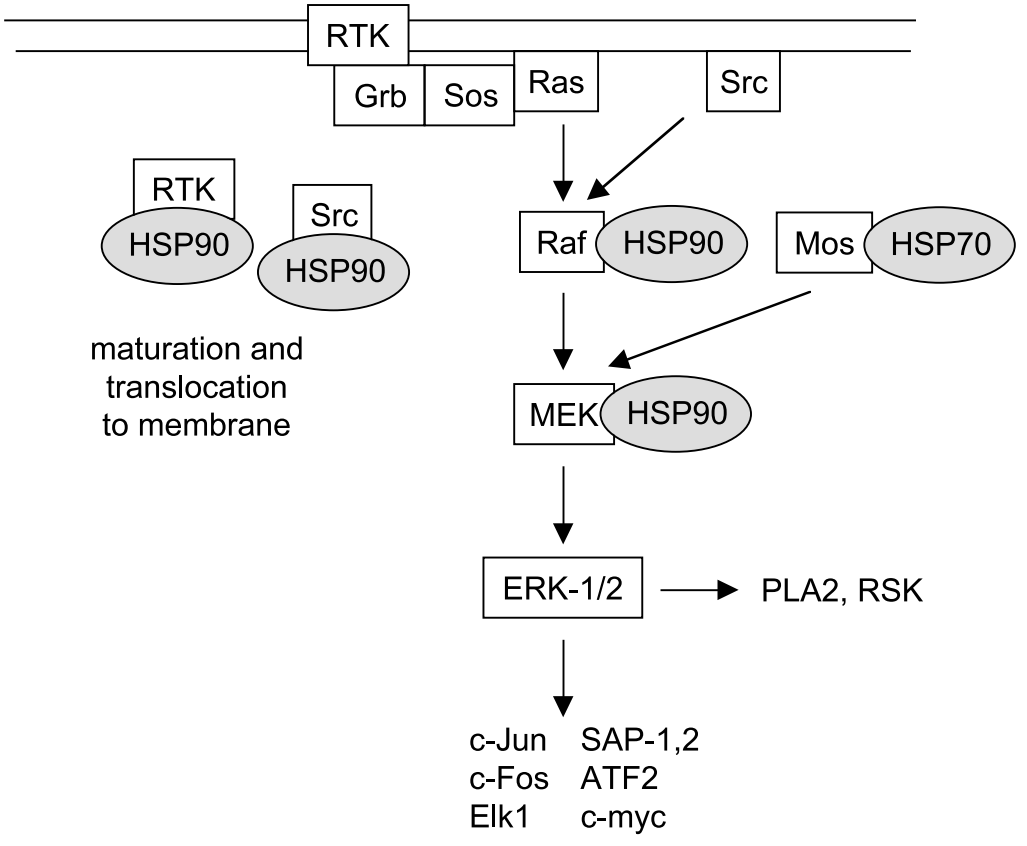

Figure 2. Association of chaperones (HSPs) with proteins of the mitogen-activated signal cascade. ERK, extracellular signal regulated kinases (also called mitogen-activated protein (MAP) kinases); Grb, growth factor receptor-bound protein, adaptor protein; MEK, ERK (MAPK) kinase; Mos, Raf, ERK (MAPK) kinase kinase; Ras, derived from rat sarcoma, activator of Raf; RTK-receptor tyrosine kinases; Sos 'son of sevenless', guanine nucleotide releasing protein; Src, derived from Rous sarcoma, non receptor tyrosine kinas;, c-Jun, c-Fos, Elk1, SAP1,2, ATF2, cMyc, transcription factors. PLA2, Phospholipase A2; RSK, ribosomal S6 kinase. Heat shock proteins, grey (for further explanation see text).

subsequent destabilization of the kinase (Hartson *et al*. 1996). A demonstration of the folding capacity of HSP90 was achieved with Lck (Hartson *et al*. 1996). It is proposed that the SH2 domain of Lck rapidly folds independently of HSP90 function followed by the slower HSP90-dependent folding of the catalytic domains (Hartson *et al*. 1998). Alternatively, the association of v-Src with HSP90 may represent a stress-like response of cells attempting to stabilize and/or sequester the abnormal kinase protein (Whitesell *et al*. 1994). Yeast Cdc37 shows *in vitro* chaperone activity similar to that of HSP90 and can compensate *in vivo* for HSP90 by maintaining the activity of v-Src (Kimura *et al*. 1997).

Tyrosine kinase receptors. Geldanamycin and herbimycin A treatment also destabilize other tyrosine kinases, such as the EGF receptor, EGF receptor precursor, insulin-like growth factor receptor and $p185^{cerbB2}$ receptor resulting in a loss of these receptors (Fig. 2; Miller *et al*. 1994; Murakami *et al*. 1994a, Murakami, Mizuno & Uehara 1994b; Sepp-Lorenzino *et al*. 1995; Chavany *et al*. 1996; Mimnaugh, Chavany & Neckers 1996). This loss is probably due

to ubiquitin-dependent degradation of the receptors in the proteasome (Sepp-Lorenzino *et al*. 1995; Mimnaugh, Chavany & Neckers 1996).

In the case of $p185^{cerbB2}$, the receptor-associated protein is the glucose-regulated and ER-located protein GRP94, which is a member of the HSP90 family and which also binds geldanamycin (Chavany *et al*. 1996; Mimnaugh, Chavany & Neckers 1996). Failure of newly synthesized $p185^{cerbB2}$ to associate with GRP94 prevents the translocation of the newly synthesized protein to the plasma membrane (Chavany *et al*. 1996).

Signalling by the sevenless receptor, required for differentiation of the R7 photoreceptor neurone in *Drosophila*, is reduced by mutations in the *hsp83* gene (a hsp90 family member) and in the gene that codes for Cdc37 (Cutforth & Rubin 1994). *Drosophila* p83 mutations suppress a gain-of-function mutation in the *torso* gene, coding for a receptor tyrosine kinase that is required for embryonic development (Doyle & Bishop 1993). Mutations in *Drosophila cdc37* impair signalling by the sevenless receptor and interact genetically with Cdc2 (= Cdk1 Cutforth & Rubin 1994).

Raf. Raf exists in part of a complex that includes HSP90 (Fig. 2; Stancato *et al*. 1993, 1997; Wartmann & Davis 1994; Schulte *et al*. 1995, 1996; Jaiswal *et al*. 1996; Pratt 1997; Van der Straten *et al*. 1997). The site of complex formation is the carboxyterminal catalytic domain of Raf (Stancato *et al*. 1993; Lovríc, Bischof & Moelling 1994). The assembly of Raf-HSP90-complexes does not depend upon receptor activation (Wartmann & Davis 1994; Jaiswal *et al*. 1996), but Raf activation caused by Ras is associated with translocation of the cytoplasmic Raf-HSP90 complex to the cell membrane (Wartmann & Davis 1994). Disruption of the Raf-HSP90 complex by geldanamycin leads to a decreased Raf stability, block of MAP kinase activation by Raf and prevention of trafficking of newly synthesized Raf to the membrane (Wartmann & Davis 1994; Schulte *et al*. 1995, 1996, Schulte, An & Necker 1997; Stancato *et al*. 1997). Mutations in the HSP90 equivalent in *Drosophila melanogaster* reduced Raf kinase activity (Van der Straten *et al*. 1997). Cdc37 (p50) is the primary determinant of HSP90 recruitment to Raf-1 in *Drosophila*: *cdc37* mutants inhibited Raf-1 and MAPK activation by growth factors (Grammatikakis *et al*. 1999). Coimmunoadsorption of insect HSP90 was observed also for MEK, the MAP kinase activating kinase (Stancato *et al*. 1997).

HSP90 complexes the majority of Gag-Mil protein kinase in MH2-transformed quail embryo fibroblasts at the carboxyterminal domain. The Mil portion of the virus encoded Gag-Mil fusion protein is homologous to the carboxyterminus of c-Mil, the avian counterpart of the mammalian c-Raf. During mitosis part of Gag-Mil is shifted to a form with reduced electrophoretic mobility and reduced association with HSP90 (Lovric, Bischof & Moelling 1994).

MOS, a germ cell-specific activator of MEK is associated with HSP70 in COS-1 cells. The MOS protein present in the immunoprecipitates is found to be an active MEK kinase/MAP kinase kinase kinase. Inclusion of ATP in cell extracts protected against loss of MOS kinase activity. In the absence of ATP, kinase activity of MOS was lost though MOS remained bound to HSP70. Mutations in the serine 3 residue of MOS that inhibits kinase activity and interaction between c-MOS catalytic domain and MEK also abolish association with HSP70 (Liu *et al*. 1999).

An interaction of HSP60 with Ras was observed by chemical crosslinking (de Gunzberg, Riehl & Weinberg 1989; Ikawa & Weinberg 1992). The portion of Ras bound to HSP60 is 5%, which increased upon serum stimulation (de Gunzberg, Riehl & Weinberg 1989). The association between Ras and HSP60 was not expected to be part of the chaperone activity of HSP60, since overexpression of Ras did not result in increased amounts of the complex. A puzzle derives from the fact that HSP60 localizes to the mitochondrial matrix, and Ras is associated with the plasma membrane. Yet there are hints for additional localizations of both

proteins (Ikawa & Weinberg 1992). Recently, a kinase suppressor of Ras (KSR) was detected that forms a multimolecular signalling complex with HSP90, HSP70, HSP68, p50(Cdc37), MEK1, MEK2, 14–3-3 and other, unidentified proteins (Stewart *et al.* 1999). These results suggest a role of chaperones in the scaffolding of the Ras-mitogen-activated protein kinase pathway.

CONCLUSIONS

Based on the evidence reviewed above one can conclude that chaperones/HSPs play important roles in cell cycle progression. The main reasons for this conclusion are the following.

- increased amounts of HSPs in proliferating (and transformed) cells
- serum/growth factor-induced synthesis
- protooncogene/oncogene-dependent induction
- virus-stimulated expression
- cell cycle phase-dependent synthesis and translocation
- stimulation of the cell cycle by overexpression, inhibition by suppression of HSP synthesis

The exact details of this role of chaperones in the cell cycle are, however, not yet known. Several possibilities are being discussed:

- Essential function in the initiation of DNA replication by binding to parts of the preinitiation complex, similar to what is known for bacteriophages. An essential function is also proposed for transformation
- Auxiliary function in stabilizing the conformation of active/inactive forms of essential cell cycle or signal cascade proteins such as Cdk4, pRb, p27, Wee-1, Src, Raf
- Auxiliary function in the nuclear transport of DNA inititation factors and transcription factors such as E2F or in the transport of cell cycle inhibitors to the degradation machinery, as assumed in the case of p27.
- Auxiliary function in the maintenance of the general functioning of the cell, i.e. gene expression, protein transport and degradation, stability of the cytoskeleton, etc., which are prerequisites for DNA synthesis and mitosis.

The involvement of chaperones/HSPs in numerous auxiliary processes makes it difficult to determine putative essential functions. In addition, the different chaperones/HSPs have partly similar, partly different functions, which may apply also to their role in cell cycle progression.

When discussing the role of chaperones/HSPs in the mitogenic signal cascade, it was suggested that the same ancient cellular processes that promote the folding and assembly of nascent proteins play a pivotal role in signal transduction by promoting the regulated folding or assembly and disassembly of mature signalling molecules between active and inactive states. Members of the protein folding machinery mediate the activity of various kinases, receptors, and transcription factors (Ruther & Zuker 1994).

HSP90 heterocomplexes with receptor tyrosine kinases (TRKs) as well as with nonreceptor tyrosine kinases of the Src-type were frequently observed and also contain other proteins such as HSP70, p60 and p23. One major function of this complex may be to fold the kinase and to keep it hypophosphorylated (inactive) until it reaches its ultimate location. This

translocation function of HSP90/HSP70 may also be realized in the case of the Raf/HSP90 complex. The significance of complex formation between Ras and HSP60 ist not clear.

Molecules of the MAP kinase cascade, as well as other signal transduction pathways, thus form multicomponent complexes through tight association with various anchoring proteins (Jaiswal *et al*. 1996; Zanke *et al*. 1996; Stewart *et al*. 1999). These large signalling complexes often contain molecular chaperones like HSP70 and/or HSP90. The physical association between individual components of a signalling cascade provides an efficient tool to segregate distinct signalling cascades via specific protein protein interactions and promotes specificity by preferential activation of the associated components. The immobilized signal cascades might increase the speed and specificity of signal transduction (Pelech & Chares 1995). The role of molecular chaperones in these intramolecular associations seems to reside in a support of aggregation and stabilization of specific multiprotein signalling complexes and in preventing association with other signal transduction cascades (Zanke *et al*. 1996).

Stress, such as heat shock or oxidative stress, upsets many cellular processes including the cell cycle, which is arrested mainly at the G1/S and the G2/M transitions (Kühl & Rensing, 2000). After cells have responded to stress by an increased synthesis of HSPs they reach a state of acquired thermotolerance, which is less sensitive to further stress exposures. This is also true for heat shock effects on the cell cycle (Kühl, Kunz & Rensing 2000), a result that supports the idea of a stabilizing role of HSPs/chaperones for cell cycle processes. Increased amounts of HSPs apparently counteract the effects of stress on regulatory cell cycle proteins as well as on basic cell cycle-sustaining processes.

ACKNOWLEDGEMENTS

We thank M.G. Vicker for helpful discussion and for critically reading the manuscript.

REFERENCES

Adkins B, Hunter T, Sefton BM (1982) The transforming proteins of PRCII virus and Rous sarcoma virus form a complex with the same two cellular phosphoproteins. *J. Virol.* **43,** 448.

Agoff SN, Hou J, Linzer DI, Wu B (1993) Regulation of the human hsp70 promoter by p53. *Science* **259,** 84.

Alfano C, McMacken R (1989a) Ordered assembly of nucleoprotein structures at the bacteriophage λ replication origin during the initiation of DNA replication. *J. Biol. Chem.* **264,** 10699.

Alfano C, McMacken R (1989b) Heat shock protein-mediated disassembly of nucleoprotein structures is required for the initiation of bacteriophage λ DNA replication. *J. Biol. Chem.* **264,** 10709.

Algue R, Akhaven-Niak H, Russell P (1994) A. role for HSP90 in cell cycle control: Wee 1 tyrosine kinase activity requires interaction with HSP90. *EMBO J.* **13,** 6099.

Allen JW, Dix DJ, Collins BW *et al*. (1996) HSP70-2 is part of the synaptonemal complex in mouse and hamster spermatocytes. *Chromosoma* **104,** 414.

Alvarez E, Northwood IC, Gonzalez FA *et al*. (1991) Pro-Leu-Ser/Thr-Pro is a consensus primary sequence for substrate protein phosphorylation. Characterization of the phosphorylation of c-myc and c-jun proteins by an epidermal growth factor receptor threonine 669 protein kinase. *J. Biol. Chem.* **266,** 15277.

Becker J, Craig EA (1994) Heat-shock proteins as molecular chaperones. *Eur. J. Biochem.* **219,** 11.

Beissinger M, Buchner J (1998) How chaperones fold proteins. *Biol. Chem.* **379,** 245.

Black AR, Azizkhan-Clifford J (1999) Regulation of E2F. a family of transcription factors involved in proliferation control. *Gene* **273,** 281.

Blagosklonny MV, Toretsky J, Neckers L (1995) Geldanamycin selectively destabilizes conformationally altered mutated p53. *Oncogene* **11,** 933.

Blagosklonny MV, Toretsky J, Bohen S, Neckers L (1996) Mutant conformation of 53 translated *in vitro* or *in vivo* requires functional HSP90. *Proc. Natl. Acad. Sci. USA* **93,** 8379.

Brown CR, Doxsey SJ, White E, Welch WJ (1994) Both viral (adenovirus E1B) and cellular (Hsp 70, p53) components interact with centrosomes. *J. Cell. Physiol.* **160,** 47.

Brugge J, Yonemoto W, Darrow D (1983) Interaction between the Rous sarcoma virus transforming protein and two cellular phosphoproteins. Analysis of the turnover and distribution of this complex. *Mol. Cell. Biol.* **3,** 9.

Campbell KS, Mullane KP, Aksoy IA *et al.* (1997) DnaJ/HSP 40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. *Genes Dev.* **11,** 1098.

Chaufour S, Mehlen P, Arrigo AP (1996) Transient accumulation, phosphorylation and changes in the oligomerization of HSP27 during retinoic acid-induced differentiation of HL 60 cells: possible role in the control of cellular growth and differentiation. *Cell Stress Chaperones* **1,** 225.

Chavany C, Mimnaugh E, Miller P *et al.* (1996) $P185^{cerbB2}$ binds to GRP94 *in vivo*. *J. Biol. Chem.* **271,** 4974.

Chen CF, Chen Y, Dai D, Chen PL, Riley DJ, Lee WH (1996) A new member of the HSP90 family of molecular chaperones interacts with the retinoblastoma protein during mitosis and after heat shock. *Mol. Cell. Biol.* **16,** 4691.

Chen S, Smith DF (1998) Hop as an adaptor in the heat shock protein 70 (HSP70) and HSP90 chaperone machinery. *J. Biol. Chem.* **273,** 35194.

Cheung RK, Dosch HM (1993) The growth transformation of human B cells involves superinduction of *hsp70* and *hsp90*. *Virology* **193,** 700.

Chiesa R, Noguera I, Sredy J (1997) Phosphorylation of HSP25 during lens cell differentiation. *Exp. Eye Res.* **65,** 223.

Courtneidge SA, Bishop JM (1982) Transit of pp 60v-src to the plasma membrane. *Proc. Natl. Acad. Sci. USA* **79,** 7117.

Cross DAE, Smythe C (1998) PD98059 prevents establishment of the spindle assembly checkpoint and inhibits the G2-M transition in meiotic but not mitotic cell cycles in *Xenopus*. *Exp. Cell Res.* **241,** 12.

Csermely P, Schnaider T, Szántó I (1995) Signaling and transport through the nuclear membrane. *Biochim. Biophys. Acta* **1241,** 425.

Cutforth T, Rubin GM (1994) Mutations in *hsp83* and *cdc37* impair signaling by the sevenless receptor tyrosine kinase in *Drosophila*. *Cell* **77,** 1027.

D'Onofrio C, Puglianiello A, Amici C, Faraoni I, Lanzilli G, Bonmassar E (1995) HSP70 production and inhibition of cell proliferation in Molt-4 T-cells after cell-to-cell transmission of HTLV-I: Effect of PGA_1. *Leuk. Res.* **19,** 345.

Dai K, Kobayashi R, Beach D (1996) Physical interaction of mammalian CDC37 with CDK4. *J. Biol. Chem.* **271,** 22030.

Dasgupta G, Momand J (1997) Geldanamycin prevents nuclear translocation of mutant p53. *Exp. Cell Res.* **237,** 29.

Davidoff AM, Iglehart JD, Marks JR (1992) Immune response to p53 is dependent upon p53/HSP70 complexes in breast cancers. *Proc. Natl. Acad. Sci. USA* **89,** 3439.

Davidson SM, Morange M (2000) HSP25 and the p38 MAPK pathway are involved in differentiation of cardiomyocytes. *Dev. Biol.* **218,** 146.

Davis RJ (1994) MAPKs: New JNK Expands the Group. *TIBS* **19,** 470.

de Gunzberg J, Riehl R, Weinberg RA (1989) Identification of a protein associated with p21Ras by chemical cross linking. *Proc. Natl. Acad. Sci. USA* **86,** 4007.

De Maio A (1999) Heat shock proteins. *Facts, Thoughts Dreams. Shock* **11,** 1.

Dey B, Lightbody JJ, Boschelli F (1996) CDC37 is required for p60v-src activity in yeast. *Mol. Biol. Cell* **7,** 1405.

Dix DJ (1997a) HSP 70 expression and function during gametogenesis. *Cell Stress Chaperones* **2,** 73.

Dix DJ, Allen JW, Collins BW *et al.* (1996a) Targeted gene disruption of *HSP70–2* results in failed meiosis, germ cell apoptosis, and male infertility. *Proc. Natl. Acad. Sci. USA* **93,** 3264.

Dix DJ, Allen JW, Collins BW *et al.* (1997b) HSP70-2 is required for desynapsis of synaptonemal complexes during meiotic prophase in juvenile and adult mouse spermatocytes. *Development* **124,** 4595.

Dix DJ, Rosario-Herrle M, Gotoh H *et al.* (1996b) Developmentally regulated expression of HSP70–2 and a HSP70–2/lacZ transgene during spermatogenesis. *Dev. Biol.* **174,** 310.

Dodson M, McMacken, Echols H (1989) Specialized nucleoprotein structures at the origin of replication of bacteriophage λ. *J. Biol. Chem.* **264,** 10719.

Doyle HJ, Bishop JM (1993) Torso, a receptor tyrosine kinase required for embryonic pattern formation, shares substrates with the sevenless and EGF-R pathways in *Drosophila*. *Genes Dev.* **7,** 633.

Eddy MA (1999) Role of heat shock protein HSP70–2 in spermatogenesis. *Rev. Reprod.* **4,** 23.

Endicott JA, Noble ME, Tucker JA (1999) Cyclin-dependent kinases: inhibition and substrate recognition. *Curr. Opinion Struct. Biol.* **9,** 738.

Ezaki B, Ogura T, Mori H, Niki H, Hiraga S (1989) Involvement of DnaK protein in mini-F plasmid replication: temperature-sensitive *seg* mutations are located in the DnaK gene. *Mol. Gen. Genet.* **218,** 183.

Feldherr CM, Akin D (1990) The permeability of the nuclear envelope in dividing and nondividing cell cultures. *J. Cell Biol.* **111,** 1.

Feldherr CM, Akin D (1993) Regulation of nuclear transport in proliferating and quiescent cells. *Exp. Cell Res.* **205,** 179.

Ferrarini M, Heltai S, Zocchi MR, Rugarli C (1992) Unusual expression and localization of heat-shock proteins in human tumor cells. *Int. J. Cancer* **51,** 613.

Ferris DK, Harel-Bellan A, Morimoto RI, Welch WJ, Farrar WL (1988) Mitogen and lymphokine stimulation of heat shock proteins in T lymphocytes. *Proc. Natl. Acad. Sci. USA* **85,** 3850.

Finlay CA, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ (1988) Activating mutations for transformation by p53 produce a gene product that forms an HSC70-p53 complex with an altered halfe-life. *Mol. Cell. Biol.* **8,** 531.

Foos G, Natour S, Klempnauer KH (1993) TATA box dependent trans-activation of the human HSP70 promoter by Myb proteins. *Oncogene* **8,** 1775.

Galea-Lauri J, Latchman DS, Katz DR (1996) The role of the 90-kDa heat shock protein in cell cycle control and differentiation of the monoblastoid cell line U937. *Exp. Cell Res.* **226,** 243.

Gannon JV, Greaves R, Iggo R, Lane DP (1990) Activating mutations in p53 produce a common conformational effect. A monoclonal antibody specific for the mutant form. *EMBO J.* **9,** 1595.

García-Bermejo L, Vilaboa NE, Perez C, de Blas E, Calle C, Aller P (1995) Modulation of HSP70 and HSP27 gene expression by the differentiation inducer sodium butyrate in U-937 human promonocytic leukemia cells. *Leu. Res.* **19,** 713.

Garrido C, Bruey JM, Fromentin A, Hammann A, Arrigo AP, Solary E (1999) HSP 27 inhibits cytochrome c-dependent activation of procaspase-9. *FASEB J.* **13,** 2061.

Garrido C, Fromentin A, Bonnotte B *et al.* (1998) Heat shock protein 27 enhances the tumorigenicity of immunogenic rat colon carcinoma cell clones. *Cancer Res.* **58,** 5495.

Grammatikakis N, Lin JH, Grammatikakis A, Tsichlis PN, Cochran BH (1999) p50 (cdc37) acting in concert with HSP90 is required for Raf-1 function. *Mol. Cell Biol.* **19,** 1661.

Guay J, Lambert H, Gingras-Breton G, Lavoie JN, Huot J, Landry J (1997) Regulation of actin filament dynamics by p38 map kinase-mediated phosphorylation of the heat shock protein 27. *J. Cell Sci.* **110,** 357.

Hainaut P, Milner J (1992) Interaction of heat-shock protein 70 with p53 translated *in vitro*: evidence for interaction with dimeric p53 and for a role in the regulation of p53 conformation. *EMBO J.* **11,** 3513.

Haire RN, Peterson MS, O'Leary JJ (1988) Mitogen activation induces the enhanced synthesis of two heat-shock proteins in human lymphocytes. *J. Cell Biol.* **106,** 883.

Halevy O, Michalovitz D, Oren M (1990) Different tumor-derived p53 mutants exhibit distinct biological activities. *Science* **250,** 113.

Hang H, Fox MH (1995) Expression of hsp70 induced in CHO cells by 45.0° C hyperthermia is cell cycle associated and DNA synthesis dependent. *Cytometry* **19,** 119.

Hansen LK, Houchins JP, O'Leary JJ (1991) Differential regulation of HSC70, HSP70, HSP90, and HSP90ß mRNA expression by mitogen activation and heat shock in human lymphocytes. *Exp. Cell Res.* **192,** 587.

Hartl FU (1996) Molecular chaperones in cellular protein folding. *Nature* **381,** 571.

Hartson SD, Barrett DJ, Burn P, Matts RL (1996) HSP90-mediated folding of the lymphoid cell kinase $p56^{lck}$. *Biochemistry* **35,** 13451.

Hartson SD, Matts RL (1994) Association of HSP90 with cellular *Src*-family kinases in a cell-free system correlates with altered kinase structure and function. *Biochemistry* **33,** 8912.

Hartson SD, Ottinger EA, Huang W, Barany G, Burn P, Matts RL (1998) Modular folding and evidence for phosphorylation-induced stabilization of a HSP90-dependent kinase. *J. Biol. Chem.* **273,** 8475.

Helmbrecht K, Rensing L (1999) Differential constitutive heat shock protein 70 expression during proliferation and differentiation of rat C6 glioma cells. *Neurochem. Res.* **24,** 1293.

Henriksson M, Classon M, Axelson H, Klein G, Thyberg J (1992) Nuclear colocalization of *c-myc* protein and HSP70 in cells transfected with human wild-type and mutant *c-myc* genes. *Exp. Cell Res.* **203,** 383.

Hensold JO, Houseman DE (1988) Decreased expression of the stress protein HSP70 is an early event in murine erytholeukemic cell differentiation. *Mol. Cell. Biol.* **8,** 2219.

Herrera R, Hubbel S, Decker S, Petruzzelli L (1998) A role for the MEK/MAPK pathway in PNA-induced cell cycle arrest: modulation of megakaryocytic differentiation of K562 cells. *Exp. Cell Res.* **238,** 407.

Herrmann CH, Dery CV, Mathews MB (1987) Transactivation of host and viral genes by the adenovirus E1B 19K tumor antigen. *Oncogene* **2,** 25.

Hickey E, Randon SE, Smale G, Lloyd D, Weber LA (1989) Sequence and regulation of a gene encoding a human 89-kilodalton heat shock protein. *Mol. Cell. Biol.* **9,** 2615.

Hinds PW, Finlay CA, Frey AB, Levine AJ (1987) Immunological evidence for the association of p53 with a heat shock protein, HSC70, in p53-plus-*ras*-transformed cell lines. *Mol. Cell. Biol.* **7,** 2863.

Hinds PW, Finlay CA, Quartin RS *et al.* (1990) Mutant p53 DNA clones from human colon carcinomas cooperate with ras in transforming primary rat cells: a comparison of the 'hot spot' mutant phenotypes. *Cell Growth Differ.* **1,** 571.

Hoffmann HJ, Lyman SK, Lu C, Petit MA, Echols H (1992) Activity of the Hsp70 chaperone complex – DnaK, DnaJ, and GrpE in initiating phage λ DNA replication by sequestering and releasing P protein. *Proc. Nat. Acad. Sci. USA* **89,** 12108.

Horman S, Fokan D, Mosselmans R, Mairesse N, Galand P (1999) Anti-sense inhibition of small-heat-shock-protein (HSP27) expression in MCF-7 mammary-carcinoma cells induces their spontaneous acquisition of a secretory phenotype. *Int. J. Cancer* **12,** 574.

Horman S, Galand P, Mosselmans R, Legros N, Leclercq G, Mairesse N (1997) Changes in the phosphorylation status of the 27 kDa heat shock protein (HSP27) associated with the modulation of growth and/or differentiation in MCF-7 cells. *Cell Prolif.* **30,** 21.

Hunter T (1995) Protein kinases and phosphatases: The ying and yang of protein phosphorylation and signaling. *Cell* **89,** 225.

Hutchison KA, De Brott BK, Leon JH, Perdew GH, Jove R, Pratt WB (1992) Reconstitution of the multiprotein complex of pp 60src, HSP90 and p50 in a cell-free system. *J. Biol. Chem.* **267,** 2902.

Ikawa S, Weinberg RA (1992) An interaction between $p21^{ras}$ and heat shock protein HSP60. *Proc. Nat. Acad. Sci. USA* **89,** 2012.

Imamoto N, Matsuoka Y, Kurihara T *et al.* (1992) Antibodies against 70-kDa heat shock cognate protein inhibit mediated nuclear import of karyophilic proteins. *J. Cell Biol.* **119,** 1047.

Inoue A, Torigoe T, Sogahata K *et al.* (1995) 70-kDa heat shock cognate protein interacts directly with the N-terminal region of the retinoblastoma gene product pRb. *J. Biol. Chem.* **270,** 22571.

Jäättelä M (1995) Over-expression of hsp70 confers tumorigenicity to mouse fibrosarcoma cells. *Int. J. Cancer.* **60,** 689.

Jaiswal RK, Weissinger E, Kolch W, Landreh GE (1996) Nerve growth factor-mediated activation of the mitogen-activated protein (MAP) kinase cascade involves a signaling complex containing B-Raf and HSP90. *J. Biol. Chem.* **271,** 23626.

Jeoung DI, Chen S, Windsor J, Pollack RE (1991) Human major HSP70 protein complements the localization and functional defects of cytoplasmic mutant SV40 T antigen in Swiss 3T3 mouse fibroblast cells. *Genes Dev.* **5,** 2235.

Jérôme Y, Leger J, Devin J, Baulieu EE, Catelli MG (1991) Growth factors acting via tyrosine kinase receptors induce HSP90 α gene expression. *Growth Factors* **4,** 317.

Jérôme Y, Vourc'h C, Baulieu EE, Catelli MG (1993) Cell cycle regulation of the chicken hsp90a expression. *Exp. Cell Res.* **205,** 44.

Johnson DG, Schneider-Broussard R (1998) Role of E2F in cell cycle control and cancer. *Frontiers Bioscience* **3,** 447.

Johnson DG, Walker CL (1999) Cyclins and cell cycle checkpoints. *Ann. Rev. Pharmacol. Toxicol.* **39,** 295.

Kao HT, Nevins JR (1983) Transcriptional activation and subsequent control of the human heat shock gene during adenovirus infection. *Mol. Cell. Biol.* **3,** 2058.

Karlseder J, Wissing D, Holzer G *et al.* (1996) HSP70 overexpression mediates the escape of a doxorubicin-induced G2 cell cycle arrest. *Biochem. Biophys. Res. Comm.* **220,** 153.

Kaul SC, Duncan EL, Englezou A *et al.* (1998) Malignant transformation of NIH3T3 cells by overexpression of mot-2 protein. *Oncogene* **17,** 907.

Kawasaki Y, Wada C, Yura T (1990) Roles of *Escherichia coli* heat shock proteins DnaK, DnaJ and GrpE in mini-F plasmid replication. *Mol. Gen. Genet.* **220,** 277.

Khalid H, Tsutsumi K, Yamashita H, Kishikawa M, Yasunaga A, Shibata S (1995) Expression of the small heat shock protein (HSP) 27 in human astrocytomas correlates with histologic grades and tumor growth fractions. *Cell. Mol. Neuroiol.* **15,** 257.

Khandjian EW, Türler H (1983) Simian virus 40 and polyoma virus induce synthesis of heat shock proteins in permissive cells. *Mol. Cell. Biol.* **3,** 1.

Kimura Y, Rutherford SL, Miyata Y *et al.* (1997) Cdc37 is a molecular chaperone with specific functions in signal transduction. Genes Dev. 11 1775.

Kindas-Mügge I, Micksche M, Trautinger F (1998) Modification of growth in small heat shock (HSP27) gene transfected breast carcinoma. *Anticancer Res.* **18,** 413.

Kindas-Mügge I, Trautinger F (1994) Increased expression of the M (r) 27,000 heat shock protein (HSP27) in in vitro differentiated normal human keratinocytes. *Cell Growth Diff.* **5,** 777.

Kingston RE, Baldwin AS Jr, Sharp PA (1984) Regulation of heat shock protein 70 gene expression by c-myc. *Nature* **312,** 280.

Kitay MK, Rowe DT (1996) Proteinprotein interactions between Epstein-Barr virus nuclear antigen-LP and cellular gene products. Binding of 70-kilodalton heat shock protein. *Virology* **220,** 91.

Koskinen PJ, Sistonen L, Evan G, Morimoto R, Alitalo K (1991) Nuclear colocalization of cellular and viral *myc* proteins with HSP70 in *myc*-overexpressing cells. *J. Virol.* **65,** 842.

Kühl NM, Kunz J, Rensing L (2000) Heat shock-induced arrests in different cell cycle phases of rat C6-glioma cells are attennuated in heat shock-primed thermotolerant cells. *Cell Prolif.* **33,** 147.

Kühl NM, Rensing L (2000) Heat shock effects on cell cycle progression. *CMLS* **57,** 450.

Kuroita T, Tachibana H, Ohashi H, Shirahata S, Murakami H (1992) Growth stimulating activity of heat shock protein 90 a to lymphoid cell lines in serum-free medium. *Cytotechnology* **8,** 109.

Landry J, Lambert H, Zhou M *et al.* (1992) Human HSP27 is phosphorylated at serines 78 and 82 by heat shock and mitogen-activated kinases that recognize the same amino acid motif as S6 kinase II. *J. Biol. Chem.* **267,** 794.

Larsen JK, Yamboliev IA, Weber LA, Gerthoffer WT (1997) Phosphorylation of the 27-kDa heat shock protein via p38 MAP kinase and MAPKAP kinase in smooth muscle. *Am. J. Physiol.* **273,** L930.

Laufen T, Mayer MP, Beisel C, Klostermeier D, Mogk A, Reinstein J, Bukau B. (1999) Mechanism of regulation of HSP70 chaperones by DnaJ cochaperones. *Proc. Natl. Acad. Sci.* **96,** 5452.

Lavia P, Jansen-Durr P (1999) E2F target genes and cell cycle checkpoint control. *Bioassays* **21,** 221.

Lavoie JN, Gingras-Breton G, Tanguay RM, Landry J (1993) Induction of Chinese hamster HSP27 gene expression in mouse cells confers resistance to heat shock. HSP27 stabilization of the microfilament organization. *J. Biol. Chem.* **268,** 3420.

Lavoie JN, L'Allemain G, Brunet A, Müller R, Pouysségur J (1996) Cyclin D1 expression is regulated positively by the p42/p44^{MAPK} and negatively by the p38/HOPG^{MAPK} pathway. *J. Biol. Chem.* **271,** 20608.

Lemieux P, Österreich S, Lawrence JA *et al.* (1997) The small heat shock protein HSP27 increases invasiveness but decreases motility of breast cancer cells. *Invasion Metastasis* **17,** 113.

Liberek K, Georgopoulos C, Zylicz M (1988) Role of the *Escherichia coli* DnaK and DnaJ heat shock proteins in the initiation of bacteriophage λ DNA replication. *Proc. Nat. Acad. Sci. USA* **85,** 6632.

Lipinski MM, Jacks T (1999) The retinoblastoma gene family in differentiation and development. *Oncogene* **18,** 7873.

Lipsich LA, Cutt JR, Brugge JS (1982) Association of the transforming proteins of Rous, Fujinami, and Y73 avian sarcoma viruses with the same two cellular proteins. *Mol. Cell. Biol.* **2,** 875.

Liu JS, Kuo SR, Makhov AM *et al.* (1998) Human HSP70 and HSP40 chaperone proteins facilitate human papillomavirus-11 E1 protein binding to the origin and stimulate cell-free DNA replication. *J. Biol. Chem.* **273,** 30704.

Liu H, Vuyyura VB, Pham CH, Yang Y, Singh B (1999) Evidence of an interaction between Mos and HSP70. a role of the Mos residue serine 3 in mediating HSP70 association. *Oncogene* **18,** 3461.

Lovríc J, Bischof O, Moelling K (1994) Cell cycle-dependent association of Gag-Mil and HSP90. *FEBS L.* **343,** 15.

Maekawa M, O'Brien DA, Allen RL, Eddy EM (1989) Heat-shock cognate protein (HSC71) and related proteins in mouse spermatogenic cells. *Biol. Reprod.* **40,** 843.

Mairesse N, Horman S, Mosselmans R, Galand P (1996) Antisense inhibition of the 27 kDa heat shock protein production affects growth rate and cytoskeletal organization in MCF-7 cells. *Cell Biol. Int.* **20,** 205.

Matsumoto M, Fujimoto H (1990) Cloning of a *hsp*70-related gene expressed in mouse spermatids. *Biochem. Biophys. Res. Comm.* **166,** 43.

May E, Breugnot C, Duthu A, May P (1991) Immunological evidence for the association between simian virus 40, 115-kDa super T antigen and HSP70 proteins in rat, monkey, and human cells. *Virology* **180,** 285.

Mehlen P, Arrigo AP (1994) The serum-induced phosphorylation of mammalian HSP27 correlates with changes in its intracellular localization and levels of oligomerization. *Eur. J. Biochem.* **221,** 327.

Meloche S (1995) Cell cycle entry of mammalian fibroblasts is accompanied by the sustained activation of p44 MAPK and p42 MAPK isoforms in the G1 phase and their inactivation at the G1/S transition. *J. Cell. Physiol.* **163,** 577.

Merrick BA, He C, Witcher LL *et al.* (1996) HSP70 binding and mitochondrial localization of p53 protein in human HT1080 and mouse C3H10T1/2 cell lines. *Biochim. Biophys. Acta* **1297,** 57.

Milarski KL, Morimoto RI (1986) Expression of human HSP70 during the synthetic phase of the cell cycle. *Proc. Natl. Acad. Sci. USA* **83,** 9517.

Milarski KL, Welch WJ, Morimoto RI (1989) Cell cycle-dependent association of HSP70 with specific cellular proteins. *J. Cell Biol.* **108,** 413.

Miller P, Dirio C, Moyer M *et al.* (1994) Depletion of the *erbB*-2 gene product p185 by benzoquinoid ansamycins. *Cancer Res.* **54,** 2724.

Milner J (1995) Flexibility: the key to p53 function. *TIBS* **20,** 49.

Milner J, Cook A, Mason J (1990) p53 is associated with $p34^{cdc2}$ in transformed cells. *EMBO J.* **9,** 2885.

Mimnaugh EG, Chavany C, Neckers L (1996) Polyubiquitination and proteasomal degradation of the $p185^{cerbB2}$ receptor protein-tyrosine kinase induced by geldanamycin. *J. Biol. Chem.* **271,** 22796.

Minshull J, Sun H, Tonks NK, Murray AV (1994) A MAP kinase dependent spindle assembly checkpoint in *Xenopus* egg extracts. *Cell* **79,** 475.

Morgan DO (1997) Cyclin-dependent kinases. Engines, Clocks Microprocessors. *Ann. Rev. Cell Dev. Biol.* **13,** 261.

Mori C, Nakamura N, Dix DJ *et al.* (1997) Morphological analysis of germ cell apoptosis during postnatal testis development in normal and HSP70-2 knockout mice. *Dev. Dyn.* **208,** 125.

Morino M, Tsuzuki T, Ishikawa Y *et al.* (1997) Specific expression of HSP27 in human tumor cell lines in vitro. *In Vivo* **11,** 179.

Murakami Y, Fukazawa H, Mizuno S, Uehara Y (1994a) Conversion of epidermal growth factor (EGF) into a stimulatory ligand for A431-cell growth by herbimycin A by decreasing the level of expression of EGF receptor. *Biochem. J.* **301,** 57.

Murakami Y, Mizuno S, Uehara Y (1994b) Accelerated degradation of 160 kDa epidermal growth factor (EGF) receptor precursor by the tyrosine kinase inhibitor herbimycin A in the endoplasmatic reticulum of A431 human epidermoid carcinoma cells. *Biochem. J.* **301,** 63.

Murakami T, Ohmori H, Gotoh S *et al.* (1991) Down modulation of N-myc, heat-shock protein 70, and nucleolin during the differentiation of human neuroblastoma cells. *J. Biochem.* **110,** 146.

Nair SC, Toran EJ, Rimerman RA, Hjermstad S, Smithgall TE, Smith DF (1996) A. pathway of multichaperone interactions common to diverse regulatory proteins: estrogen receptor, Fes tyrosine kinase, heat shock transcription factor Hsf1, and the aryl hydrocarbon receptor. *Cell Stress Chaperones* **1,** 237.

Nakamura S, Tatuno I, Noguchi Y *et al.* (1999) 73-kDa heat shock cognate protein interacts directly with p27Kip1, a cyclin-dependent kinase inhibitor, during G1/S transition. *Biochem. Biophys. Res. Comm* **257,** 340.

Nathan DF, Lindquist S (1995) Mutational analysis of HSP90 function. Interactions with a steroid receptor and a protein kinase. *Mol. Cell. Biol.* **15,** 3917.

Nevins JR (1982) Induction of the synthesis of a 70, 000 dalton mammalian heat shock protein by the adenovirus E1A product. *Cell* **29,** 913.

Nihei T, Sato N, Takahashi S *et al.* (1993a) Demonstration of selective protein complexes of p53 with 73 kDa heat shock cognate protein, but not with 72 kDa heat shock protein in human tumor cells. *Cancer L.* **73,** 181.

Nihei T, Takahashi S, Sagae S, Sato N, Kikuchi K (1993b) Protein interaction of retinoblastoma gene product pRb110 with Mr 73,000 heat shock cognate protein. *Cancer Res.* **53,** 1702.

Ohgitani E, Kobayashi K, Takeshita K, Imanishi J (1999) Biphasic translocation of a 70 kDa heat shock protein in human cytomegalovirus-infected cells. *J. Gen. Virol.* **80,** 63.

Ohnishi T, Matsumoto H, Takahashi A, Shimura M, Majima HJ (1995) Accumulation of mutant p53 and HSP72 by heat treatment, and their association in a human glioblastoma cell line. *Int. J. Hyperthermia.* **11,** 663.

Okuno Y, Imamoto N, Yoneda Y (1993) 70-kDa Heat-shock cognate protein colocalizes with karyophilic proteins into the nucleus during their transport in vitro. *Exp. Cell Res.* **206,** 134.

Oppermann H, Levinson W, Bishop JM (1981) A cellular protein that associates with the transforming potein of Rous sarcoma virus is also a heat-shock protein. *Proc. Nat. Acad. Sci. USA* **78,** 1067.

Pagés G, Lenormand P, L'Allemain G, Chambard JC, Meloche S, Pouysségur J (1993) Mitogen-activated protein kinases p42MAPK and p44MAPK are required for fibroblast proliferation. *Proc. Natl. Acad. Sci. USA* **90,** 8319.

Parsell DA, Lindquist S (1993) The function of heat-shock proteins in stress tolerance. Degradation and reactivation of damaged proteins. *Annu. Rev. Genetics* **27,** 437.

Pelech SL, Charest DL (1995) MAP kinase-dependent pathways in cell cycle control. *Prog. Cell Cycle Research* **1,** 33.

Perdew GH, Wiegand H, Vanden Heuver JP, Mitchell C, Singh SS (1997) A 50 kilodalton protein associated with Raf and pp 60 (v-Src) protein kinases is a mammalian homolog of the cell cycle control protein Cdc37. *Biochemistry* **36,** 3600.

Perret E, Moudjou M, Geraud ML, Derancourt J, Soyer-Gobillard MO, Bornens M (1995) Identification of an HSP70-related protein associated with the centrosome from dinoflagellates to human cells. *J. Cell Sci.* **108,** 711.

Peter M (1997) The regulation of cyclin-dependent kinase inhibitors (Ckis). *Prog. Cell Cycle Res.* **3,** 99.

Phillips B, Abravaya K, Morimoto RI (1991) Analysis of the specificity and mechanism of transcriptional activation of the human *hsp*70 gene through infection by DNA viruses. *J. Virol.* **65,** 5680.

Pimhasi-Kimhi O, Michalovitz D, Ben-Zeew A, Oren M (1986) Specific interaction between the p53 cellular tumor antigen and major heat shock proteins. *Nature* **320,** 182.

Pratt WB (1997) The role of the HSP90-based chaperone system in signal transduction by nuclear receptors and receptors signaling via MAP kinase. *Annu. Rev. Pharmacol. Toxicol.* **37,** 297.

Pratt WB (1998) The HSP90-based chaperone system: involvement in signal transduction from a variety of hormone and growth factor receptors. *Proc. Soc. Exp. Biol. Med.* **217,** 420.

Rattner JB (1991) HSP70 is localized to the centrosome of dividing HeLa cells. *Exp. Cell Res.* **195,** 110.

Rutherford SL, Zuker CS (1994) Protein folding and the regulation of signaling pathways. *Cell* **79,** 1129.

Ryan JJ, Clarke MF (1994) Alteration of 53 conformation and induction of apoptosis in a murine erythroleukemia cell line by dimethylsulfoxide. *Leuk. Res.* **18,** 617.

Sagata N (1997) What does Mos do in oocytes and somatic cells? *Bioessays* **19,** 13.

Sainis I, Angelidis C, Pagoulatos G, Lazaridis I (1994) The hsc70 gene which is slightly induced by heat is the main virus inducible member of the hsp70 gene family. *FEBS L.* **355,** 282.

Samali A, Cotter TG (1996) Heat shock proteins increase resistance to apoptosis. *Exp. Cell Res.* **223,** 163.

Santomenna LD, Colberg-Poley AM (1990) Induction of cellular *hsp70* expression by human cytomegalovirus. *J. Virol.* **64,** 2033.

Sato N, Torigoe T (1998) The molecular chaperones in cell cycle control in 'Stress of Life. From Molecules to Man'. *Ann. NY Acad. Sci.* **851,** 61.

Satoh J, Kim SU (1995) Cytokines and growth factors induce HSP27 phosphorylation in human astrocytes. *J. Neuropathol. Exp. Neurol.* **54,** 504.

Sawai ET, Butel JS (1989) Association of a cellular heat shock protein with simian virus 40 large T antigen in transformed cells. *J. Virol.* **63,** 3961.

Scheibel T, Buchner J (1997) The HSP90 family – an overview. M.J. Gething ed. Oxford. Oxford University Press, pp. 147151.

Schulte TW, An WG, Neckers LM (1997) Geldanamycin-induced destabilization of Raf-1 involves the proteasome. *Biochem. Biophys. Res. Comm.* **239,** 655.

Schulte TW, Blagosklonny MV, Ingui C, Neckers L (1995) Disruption of the Raf-1-Hsp90 molecular complex results in detabilization of Raf-1 and loss of Raf-1Ras association. *J. Biol. Chem.* **270,** 24585.

Schulte TW, Blagosklonny MV, Romanova L *et al.* (1996) Destabilization of Raf-1 by geldanamycin leads to disruptions of the Raf-1-MEK-mitogen-activated protein kinase signaling pathway. *Mol. Cell. Biol.* **16,** 5839.

Seger R, Krebs E (1995) The MAPK signaling cascade. *FASEB J.* **9,** 726.

Selkirk JK, He C, Patterson RM, Merrick BA (1996) Tumor suppressor p53 gene forms multiple isoforms. Evidence for single locus origin and cytoplasmic complex formation with heat shock proteins. *Electrophoresis* **17,** 1764.

Sephernia B, Paz IB, Dasgupta G, Momand J (1996) Heat shock protein 84 forms a complex with mutant p53 protein predominantly within a cytoplasmic compartment of the cell. *J. Biol. Chem.* **271,** 15084.

Sepp-Lorenzino L, Ma Z, Lebwohl DE, Vinitsky A, Rosen N (1995) Herbimycin A induces the 20 S proteasome- and ubiquitin-dependent degradation of receptor tyrosine kinases. *J. Biol. Chem.* **270,** 16580.

Shakoori AR, Oberdorf A, Owen TA *et al.* (1992) Expresssion of heat shock genes during differentiation of mammalian osteoblasts and promyelocytic leukemia cells. *J. Cell. Biochem.* **48,** 277.

Sheng Q, Denis D, Ratnofsky M, Roberts TM, Decaprio JA, Schaffhausen B (1997) The DnaJ domain of polyomavirus large T antigen is required to regulate Rb family tumor suppressor function. *J. Virol.* **71,** 9410.

Shi Y, Thomas JO (1992) The transport of proteins into the nucleus requires the 70-kilodalton heat shock protein or its cytosolic cognate. *Mol. Cell. Biol.* **12,** 2186.

Shulga N, Roberts P, Gu ZY *et al.* (1996) In vivo nuclear transport kinetics in *Saccharomyces cerevisiae*: a role for heat shock protein 70 during targeting and translocation. *J. Cell Biol.* **135,** 329.

Silverstein AM, Grammatikakis N, Cochran BH, Chinkers M, Pratt WB (1998) p50 (cdc37) binds directly to the catalytic domain of Raf as well as to a site on HSP90 that is topologically adjacent to the tetratricopeptide repeat binding site. *J. Biol. Chem.* **273,** 20090.

Simon MC, Kitchener K, Kao HT *et al.* (1987) Selective induction of human heat shock gene transcription by the adenovirus E1A gene products, including the 12S E1A product. *Mol. Cell. Biol.* 7**,** 2884.

Sionor RV, Haupt Y (1999) The cellular response to p53: the decision between life and death. *Oncogene* **18,** 6145.

Son WY, Hwang SH, Han CT, Lee JH, Kim S, Kim YC (1999) Specific expression of heat shock protein HSPA2 in human male germ cells. *Mol. Hum. Reprod.* **5,** 1122.

Spector NL, Hardy L, Ryan C *et al.* (1995) 28-kDa mammalian heat shock protein, a novel substrate of a growth regulatory protease involved in differentiation of human leukemia cells. *J. Biol. Chem.* **270,** 1003.

Spector NL, Ryan C, Samson W, Levine H, Nadler LM, Arrigo AP (1993) Heat shock protein 28 is a unique marker of growth arrest during macrophage differentiation of HL-60 cells. *J. Cell Physiol.* **156,** 619.

Srinivasan A, McClellan AJ, Vartikar J *et al.* (1997) The amino-terminal transforming region of simian virus 40 large T and small t antigens functions as a J domain. *Mol. Cell. Biol.* **17,** 4761.

Stancato LF, Chow YH, Hutchison KA, Perdew GH, Jove R, Pratt WB (1993) Raf exists in a native heterocomplex with HSP90 and p50 that can be reconstituted in a cell-free system. *J. Biol. Chem.* **268,** 21711.

Stancato LF, Silverstein AM, Owens-Grillo JK, Chow YH, Jove R, Pratt WB (1997) The HSP90-binding antibiotic geldanamycin decreases Raf levels and epidermal growth factor signaling without disrupting formation of signaling complexes or reducing the specific enzymatic activity of Raf kinase. *J. Biol. Chem.* **272,** 4013.

Stepanova L, Leng X, Barker SB, Harper JW (1996) Mammalian $p50^{Cdc37}$ is a protein kinase-targeting subunit of Hsp90 that binds and stabilizes Cdk4. *Genes Dev.* **10,** 1491.

Stewart S, Sundaram M, Zhang Y, Lee J, Han M, Guan KL (1999) Kinase suppression of Ras forms a multiprotein signaling complex and modulates MEK localization. *Mol. Cell. Biol.* **19,** 5523.

Stock J (1999) Gyrating protein kinases. *Curr. Biol.* **9,** R364.

Stokol D, Engel K, Campbell DG, Cohen P, Gaestel M (1992) Identification of MAPKAP kinase 2 as a major enzyme responsible for the phosphorylation of the small mammalian heat shock proteins. *FEBS L.* **313,** 307.

Stubdal H, Zalvide J, Campbell KS, Schweitzer C, Roberts TM, Decaprio JA (1997) Inactivation of pRB-related proteins p130 and p107 mediated by the J domain of simian virus 40 large T antigen. *Mol. Cell. Biol.* **17,** 4979.

Sturzbecher HW, Chumakov P, Welch WJ, Jenkins JR (1987) Mutant p53 proteins bind HSP72/73 cellular heat shock-related proteins in SV40-transformed monkey cells. *Oncogene* **1,** 201.

Sugito K, Yamame M, Hattori H *et al.* (1995) Interaction between HSP70 and HSP40, eukaryotic homologues of DnaK and DnaJ, in human cells expressing mutant-type p53. *FEBS L.* **358,** 161.

Suzuki K, Watanabe M (1992) Augmented expression of HSP72 protein in normal human fibroblasts irradiated with ultraviolet light. *Biochem. Biophys. Res. Comm.* **186,** 1257.

Suzuki K, Watanabe M (1994) Modulation of cell growth and mutation induction by introduction of the expression vector of human HSP70 gene. *Exp. Cell Res.* **215,** 75.

Taieb F, Karaiskou A, Rime H, Jessus C (1998) Human retinoblastoma protein (RB) is phosphorylated by Cdc2 kinase and MAP kinase in *Xenopus* maturing oocytes. *FEBS L.* **425,** 465.

Taira T, Negishi Y, Kihara F, Iguchi-Ariga SM, Ariga H (1992) c-myc protein complex binds to two sites in human *hsp*70 promoter region. *Biochim. Biophys. Acta* **1130,** 166.

Takashi M, Sakata T, Ohmura M, Kato K (1997) Elevated concentrations of the small stress protein HSP27 in rat renal tumors. *Urol. Res.* **25,** 173.

Tamemoto H, Kadowaki T, Tobe K *et al.* (1992) Biphasic activation of two mitogen-activated protein kinases during the cell cycle in mammalian cells. *J. Biol. Chem.* **267** (29)**,** 20293.

Tilly K, Yarmolinsky M (1989) Participation of *Eschericha coli* heat shock proteins DnaJ, DnaK, and GrpE in P1 plasmid replication. *J. Bacteriol.* **171,** 6025.

Treisman R (1996) Regulation of transcription by MAP kinase cascades. *Curr. Opin. Cell Biol.* **8,** 205.

Uehara Y, Hori M, Takeuchi T, Umezawa H (1986) Phenotypic change from transformed to normal induced by benzoquinonoid ansamycins accompanies inactivation of p60src in rat kidney cells infected with Rous sarcoma virus. *Mol. Cell. Biol.* **6,** 2198.

Uehara Y, Murakami Y, Mizuno S, Kawai S (1988) Inhibition of transforming activity of tyrosine kinase oncogenes by herbimycin A. *Virology* **164,** 294.

Uehara Y, Murakami Y, Sugimoto Y, Mizuno S (1989) Mechanism of reversion of Rous sarcoma virus transformation by herbimycin A: reduction of total phosphotyrosine levels due to reduced kinase activity and increased turnover of p60v-src1. *Cancer Res.* **49,** 780.

Van der Straten A, Rommel C, Dickson B, Hafen E (1997) The heat shock protein 83 (HSP83) is required for Raf-mediated signaling in *Drosophila*. *EMBO J.* **16,** 1961.

Volloch VZ, Sherman MY (1999) Oncogenic potential of Hsp72. *Oncogene* **18,** 3648.

Wadhwa R, Takano S, Mitsui Y, Kaul SC (1999) NIH 3T. 3 cells malignantly transformed by mot-2 show inactivation and cytoplasmic sequestration of the p53 protein. *Cell Res.* **9,** 261.

Wadhwa R, Takano S, Robert M *et al.* (1998) Inactivation of tumor suppressor p53 by Mot-2, a HSP70 family member. *J. Biol. Chem.* **273,** 29586.

Wagner M, Hermanns I, Bittinger F, Kirkpatrick CJ (1999) Induction of stress proteins in human endothelial cells by heavy metal ions and heat shock. *Am. J. Physiol.* 2**77,** L1026.

Wainberg Z, Oliveira M, Lerner S, Tao Y, Brenner BG (1997) Modulation of stress protein (HSP27 and HSP70) expression in CD4+ lymphocytic cells following acute infection with human immunodeficiency virus type-1. *Virology* **233,** 364.

Walter G, Carbone A, Welch WJ (1987) Medium tumor antigen of polyomavirus transformation-defective mutant NG59 is associated with 73-kilodalton heat shock protein. *J. Virol.* **61,** 405.

Wartmann M, Davis RJ (1994) The native structure of the activated Raf protein kinase is a membrane-bound multi-subunit complex. *J. Biol. Chem.* **269,** 6695.

Waskiewicz AJ, Cooper JA (1995) Mitogen and stress response pathways. MAP kinase cascades and phosphatase regulation in mammals and yeast. *Curr. Opin. Cell Biol.* **7,** 798.

Wei Y, Zhao X, Kariya Y, Teshigawara K, Uchida A (1995) Inhibition of proliferation and induction of apoptosis by abrogation of heat shock protein (HSP) 70 expression in tumor cells. *Cancer Immunol. Immunother.* **40,** 73.

Welsh MJ, Gaestel M (1998) Small heat shock protein family: Function Health Disease. *Ann. NY Acad. Sci.* **851,** 28.

Whitelaw ML, Hutchison K, Perdew GH (1991) A 50-kDa cytosolic protein complexed with the 90-kDa heat shock protein (HSP90) is the same protein complexed with pp 60v-src HSP90 in cells transformed by the Rous sarcoma virus. *J. Biol. Chem.* **266,** 16436.

Whitesell L, Mimnaugh EG, De Costa B, Myers CE, Neckers LM (1994) Inhibition of heat shock protein HSP90-pp. 60^{vsrc} heteroprotein complex formation by benzoquinone ansamycins. Essential role for stress proteins in oncogenic transformation. *Proc. Nat. Acad. Sci. USA* **91,** 8324.

Whitesell L, Sutphin P, An WG, Schulte T, Blagosklonny MV, Neckers L (1997) Geldanamycin-stimulated destabilization of mutated p53 is mediated by the proteasome *in vivo*. *Oncogene* **14,** 2809.

Whitesell L, Sutphin PD, Pulcini EJ, Martinez JD, Cook PH (1998) The physical association of multiple molecular chaperone proteins with mutant p53 is altered by geldanamycin, a HSP90-binding agent. *Mol. Cell. Biol.* **18,** 1517.

Wickner SH, Chattoraj DK (1987) Replication of mini-P1 plasmid DNA in vitro requires two initiation proteins, encoded by the repA gene of phage P1 and the *dnaA* gene of *Escherichia coli*. *Proc. Nat. Acad. Sci. USA* **84,** 3668.

Wickner S, Hoskins J, McKenney K (1991a) Function of DnaJ and DnaK as chaperones in origin-specific DNA binding by RepA. *Nature* **350,** 165.

Wickner S, Hoskins J, McKenney K (1991b) Monomerization of RepA dimers by heat shock proteins activates binding to DNA replication origin. *Proc. Nat. Acad. Sci. USA* **88,** 7903.

Wickner S, Skowyra D, Hoskins J, McKenney K (1992) DnaJ, DnaK and GrpE heat shock proteins are required in *ori*P1 DNA replication solely at the RepA monomerization step. *Proc. Nat. Acad. Sci. USA* **89,** 10345.

Wu BJ, Hurst HC, Jones NC, Morimoto RI (1986) The E1A 13S product of adenovirus 5 activates transcription of the cellular human HSP70 gene. *Mol. Cell. Biol.* **6,** 2994.

Wu BJ, Morimoto RI (1985) Transcription of the human *hsp70* genes is induced by serum stimulation. *Proc. Nat. Acad. Sci. USA* **82,** 6070.

Wyman C, Vasilikiotis C, Ang D, Georgopoulos C, Echols H (1993) Function of the GrpE heat shock protein in bidirectional unwinding and replication from the origin of phage λ. *J. Biol. Chem.* **268,** 25192.

Xu Y, Lindquist S (1993) Heat-shock protein HSP90 governs the activity of pp 60^{vsrc} kinase. *Proc. Natl. Acad. Sci. USA* **90,** 7074.

Xu Y, Singer MA, Lindquist S (1999) Maturation of the tyrosine kinase c-Src as a kinase and as a substrate depends on the molecular chaperone HSP90. *Proc. Natl. Acad. Sci. USA* **96,** 109.

Yang J, Defranco DB (1994) Differential roles of heat shock protein 70 in the in vitro nuclear import of glucocorticoid receptor and simian virus 40 large tumor antigen. *Mol. Cell. Biol.* **14,** 5088.

Yano M, Naito Z, Tanaka S, Asano G (1996) Expression and roles of heat shock proteins in human breast cancer. *Jpn. J. Cancer Res.* **87,** 908.

Yano M, Naito Z, Yokoyama M *et al.* (1999) Expression of HSP90 and cyclin D1 in human breast cancer. *Cancer L.* **137,** 45.

Yehiely F, Oren M (1992) The gene for the rat heat-shock cognate, *hsc70*, can suppress oncogene-mediated transformation. *Cell Growth Differ.* **3,** 803.

Yen A, Roberson MR, Varvayanis S, Lee AT (1998) Retinoic acid induced mitogen-activated protein (MAP) extracellular signal-regulated kinase (ERK) kinase-dependent MAP kinase activation needed to elicit HL-60 cell differentiation and growth arrest. *Cancer Res.* **58,** 3163.

Yufu Y, Nishimura J, Nawata H (1992) High constitutive expression of heat shock protein 90 a in human acute leukemia cells. *Leuk. Res.* **16,** 597.

Zakeri ZF, Wolgemuth DJ (1987) Developmental-stage-specific expression of the *hsp70* gene family during differentiation of the mammalian male germ line. *Mol. Cell. Biol.* **7,** 1791.

Zakeri ZF, Wolgemuth DJ, Hunt CR (1988) Identification and sequence analysis of a new member of the mouse *hsp70* gene family and characterization of its unique cellular and developmental pattern of expression in the male germ line. *Mol. Cell. Biol.* **8,** 2925.

Zalvide J, Stubdal H, Decaprio JA (1998) The J domain of simian virus 40 large T antigen is required to functionally inactivate RB family proteins. *Mol. Cell. Biol.* **18,** 1408.

Zanke B, Rubie EA, Winnett E *et al.* (1996) Mammalian mitogen-activated kinase pathways are regulated through formation of specific kinase-activator complexes. *J. Cell Biol.* **271,** 29876.

Zeise E, Kühl N, Kunz J, Rensing L (1998) Nuclear translocation of stress protein HSC70 during S phase in rat C6-glioma cells. *Cell Stress Chaperones* **3,** 94.

Zerrahn J, Deppert W, Weidemann D, Patschinsky T, Richards F, Milner J (1992) Correlation between the conformational phenotype of p53 and its subcellular location. *Oncogene* **7,** 1371.

Zhang W, Hu G, Estey E, Hester J, Deisseroth A (1992) Altered conformation of the p53 protein in myeloid leukemia cells and mitogen-stimulated normal blood cells. *Oncogene* **7,** 1645.

Zhu D, Dix DJ, Eddy EM (1997) HSP702 is required for CDC2 kinase activity in meiosis I of mouse spermatocytes. *Development* **124,** 3007.

Ziemiecki A, Catelli MG, Joab I, Moncharmont B (1986) Association of the heat shock protein HSP90 with steroid hormone receptors and tyrosine kinase oncogene products. *Biochem. Biophys. Res. Comm.* **138,** 1298.

Zylicz M, Ang D, Liberek K, Georgopoulos C (1989) Initiation of λ DNA replication with purified host- and bacteriophage-encoded proteins: the dnaK, dnaJ and grpE heat shock proteins. *EMBO J.* **8,** 1601.