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Recruitment of Hsp70 chaperones: a crucial part of viral survival strategies

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Abstract Virus proliferation depends on the successful recruitment of host cellular components for their own replication, protein synthesis, and virion assembly. In the course of virus particle production a large number of proteins are synthesized in a relatively short time, whereby protein folding can become a limiting step. Most viruses therefore need cellular chaperones during their life cycle. In addition to their own protein folding problems viruses need to interfere with cellular processes such as signal transduction, cell cycle regulation and induction of apoptosis in order to create a favorable environment for their proliferation and to avoid premature cell death. Chaperones are involved in the control of these cellular processes and some viruses reprogram their host cell by interacting with them. Hsp70 chaperones, as central components of the cellular chaperone network, are frequently recruited by viruses. This review focuses on the function of Hsp70 chaperones at the different stages of the viral life cycle emphasizing mechanistic aspects.

Introduction

The life cycle of a virus is a course with many obstacles that must be overcome in order to produce a sufficient number of progeny to guarantee evolutionary survival (Fig. 1). Viruses have to interact with cell surface receptors, induce endocytosis and/or membrane fusion and thereby achieve entry into the cell. Their capsid, which needs to be relatively stable outside of the cell to ensure sufficient protection of the viral genome against environmental impacts, must be disassembled or opened to allow the viral nucleic acid to gain access to the cytoplasm and nucleus where replication and transcription take place. The maturation process of viral proteins, which often consist of multiple domains or are produced as polyproteins, can be very complicated. New capsids have to assemble in an ordered way around the viral genome and release of new virions must be induced. In addition, cellular

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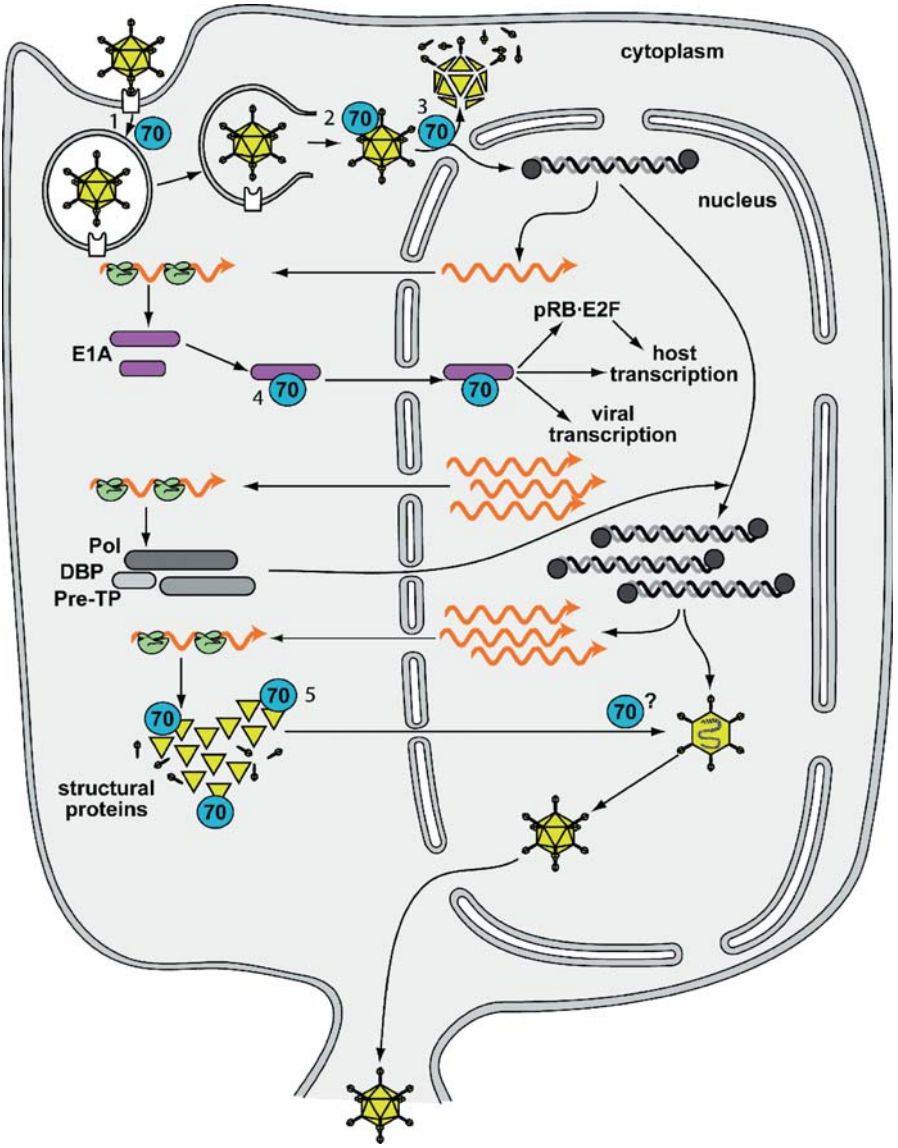

Fig. 1 Life cycle of adenovirus. Known and suspected interactions with Hsp70 are shown. *1*, Hsp70 is involved in the recycling of clathrin during the formation of clathrin coated pits and vesicles and afterwards in the uncoating of clathrin coated vesicles (Greene and Eisenberg 1990; Newmyer et al. 2003; Newmyer and Schmid 2001; Ungewickell 1985). *2*, Binding of Hsp70 to the hexon capsid protein. *3*, Hsp70-mediated release of the viral genome into the nucleus. *4*, Interaction of Hsp70 with the immediate early gene product E1A, dissociation of pRB·E2F complexes, regulation of viral and host transcription by E1A with likely participation of Hsp70. *5*, interaction of Hsp70 with newly synthesized coat proteins and possible role in the virion assembly

defense mechanisms must be overcome and sometimes cell differentiation prevented and start of the cell cycle induced.

Some of these obstacles involve protein folding processes and it is therefore not surprising that most viruses interact with cellular chaperones. In fact, two of the major chaperone systems in *Escherichia coli*, the Hsp70 (DnaK, DnaJ, GrpE) and the Hsp60 (GroEL, GroES) systems, were originally discovered as host factors essential for growth of bacterial viruses, the bacteriophages λ and T4 (Georgopoulos 1972, 1977; Sunshine et al. 1977). Early on it became clear that the folding tasks of the chaperones, in particular of the Hsp70s, involves not only the acceleration of the maturation of viral proteins but also the regulation of the viral life cycle and coordination of host and viral physiological states. In eukaryotic cells Hsp70 chaperones are involved in the regulation of fundamental cellular processes such as the cell cycle and apoptosis. The functional interaction of viruses with these chaperones therefore contributes to reprogramming the host cell, specifically to allow re-entry into the cell cycle and to avoid premature apoptosis. Hsp70 chaperones also seem to be involved in circumvention of cellular defense and sometimes even in avoidance of the host defense mechanisms.

This treatise deals mainly with the role of Hsp70 chaperones for virus proliferation. Before detailing the virus–Hsp70 interactions, the cellular functions and the molecular mechanism of Hsp70 chaperones and their various co-chaperones are introduced. The role of Hsp70 at different stages of the viral life cycle is discussed. Finally, an evolutionary facet of the virus–Hsp70 relationship will be considered.

Mechanism of Hsp70 chaperones

Members of the Hsp70 family of chaperones are involved in an astonishingly large variety of processes. Among these processes are the folding of newly synthesized polypeptides, the refolding of stress denatured proteins, the disaggregation of protein aggregates, the translocation of organellar and secretory proteins across membranes, the assembly and disassembly of oligomeric structures, and the control of the biological activity and stability of regulatory proteins (Bukau et al. 2000; Craig et al. 1999; Gething 1999; Hartl and Hayer-Hartl 2002; Neupert and Brunner 2002; Ryan and Pfanner 2002; Schlieker et al. 2002). Hsp70 chaperones not only continuously survey the folding status of proteins as part of their quality control function that is especially important under stress conditions, they are also involved in many cellular housekeeping functions including signal transduction and regulation of cell cycle and cell death (Beere and Green 2001; Helmbrecht and Rensing 1999). Among these housekeeping functions, it is especially noteworthy that in most organisms Hsp70s are involved in the regulation of the stress response (Arsene et al. 2000; Gabai et al. 1998; Morimoto 1999; Urano et al. 2000). In eukaryotic cells Hsp70 chaperones are found in virtually all compartments and even on the cell surface where specific receptors exist for the binding of Hsp70 proteins (Asea 2003).

The evolutionary adaptation to such a broad spectrum of functions was made possible by three basic properties of Hsp70s. First, they transiently interact with short hydrophobic peptide stretches within their target proteins and protein size is therefore not a limiting factor. Second, they are regulated in their activity by co-chaperones including the large family of modular J-domain proteins (JDPs) that target Hsp70s to their substrates. Third, for specific tasks they cooperate with other chaperone systems.

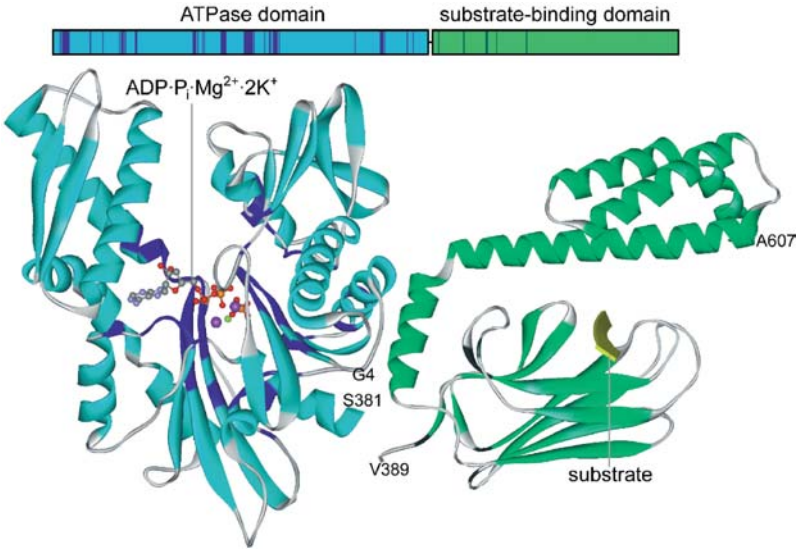

Fig. 2 Structure of Hsp70 chaperones. *Top*: domain structure of Hsp70s; the residues that are conserved in at least 11 out of the 12 known full-length sequences of viral Hsp70s are shown in *darker colors*. *Bottom*: secondary structure representation of the crystal structure of the ATPase domain of bovine Hsc70 (1BUP; *left*; Sousa and McKay 1998) and the substrate-binding domain of *E. coli* DnaK (1DKX; *right*; Zhu et al. 1996). In viral Hsp70s conserved residues are shown in *dark blue* (ATPase domain) and *dark green* (substrate-binding domain)

The ATPase cycle

Hsp70 homologs share the same overall structure, consisting of an N-terminal ATPase domain of 45 kDa and a C-terminal substrate binding domain of at least 25 kDa which is further subdivided into a β-sandwich subdomain of 15 kDa and a C-terminal α-helical subdomain (Fig. 2). ATP binding to the ATPase domain of Hsp70 proteins decreases the affinity of the substrate-binding domain for substrates by 5- to 85-fold (Mayer et al. 2000b; Palleros et al. 1993; Schmid et al. 1994). This decrease in affinity is due to an increase in the dissociation rate (k_{off}) of Hsp70-substrate complexes by two to three orders of magnitude and a concomitant increase of the association rate (k_{on}) for substrate binding by approximately 50-fold (Mayer et al. 2000b; Pierpaoli et al. 1997; Schmid et al. 1994; Theyssen et al. 1996). The ATPase cycle of Hsp70 thus consists of an alternation between the ATP state with low affinity and fast exchange rates for substrates, and the ADP state with high affinity and low exchange rates for substrates.

ATP hydrolysis by Hsp70s is generally very slow (τ=5–15 min) but is stimulated by substrate association (two- to tenfold) and by a J-domain containing co-chaperones (in general $\leq$tenfold). The simultaneous interaction of Hsp70s with a substrate and a JDP synergistically stimulate the ATPase activity up to several thousand-fold (Barouch et al. 1997; Karzai and McMacken 1996; Laufen et al. 1999; Misselwitz et al. 1998). After ATP hydrolysis the substrate is tightly bound by the Hsp70 chaperone and for most Hsp70s nucleotide exchange is rate-limiting for substrate release, i.e., the rate with which ADP dissociates determines how long the substrate remains in complex with the Hsp70 proteins. For some prokaryotic, mitochondrial and plastidal Hsp70s nucleotide exchange is cat-

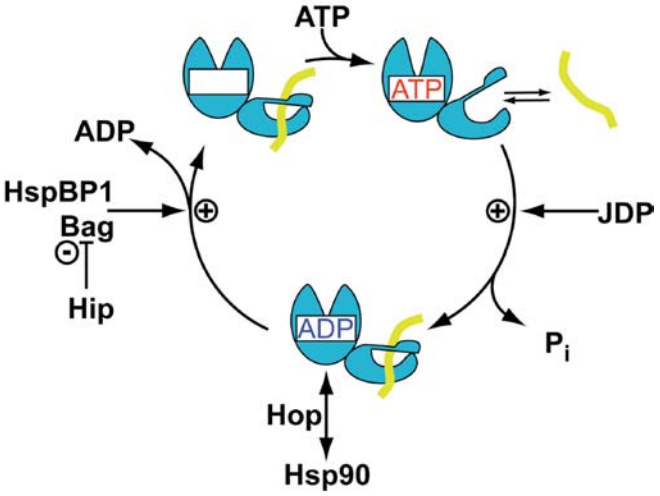

Fig. 3 ATPase cycle of Hsp70 chaperones and action of some co-chaperones on the ATPase cycle

alyzed by the nucleotide exchange factor GrpE (Harrison et al. 1997; Liberek et al. 1991; Packschies et al. 1997). For the cytosolic Hsc70-type Hsp70s the family of Bag proteins has been shown to accelerate ADP dissociation (Brehmer et al. 2001; Gässler et al. 2001; Höhfeld and Jentsch 1997; Sondermann et al. 2001) (Fig. 3).

Hsp70 substrate interactions

Hsp70 chaperones interact promiscuously with virtually all unfolded proteins but generally do not bind their native counterparts. Yet, they also recognize certain folded proteins with high specificity. An important question therefore is, how Hsp70 can combine within its substrate specificity both of these seemingly contradicting properties. Using a library of cellulose-bound peptides scanning the sequences of natural proteins the binding motif for the *E. coli* homolog DnaK was elucidated (Rüdiger et al. 1997). This motif consists of a core of five amino acids enriched in hydrophobic residues, flanked on both sides by a region where positively charged residues are preferred. Such motifs occur in virtually all proteins on average every 30–40 residues. In folded proteins they are mostly found in the hydrophobic core explaining the promiscuous binding to denatured proteins. In contrast, it is not completely clear how Hsp70s recognize specifically certain folded proteins, although the binding sites of Hsp70 proteins in a few native substrates has been determined (Hoff et al. 2002; Kim et al. 2002; M.P. Mayer and B. Bukau, unpublished results).

How Hsp70 systems refold denatured proteins is also still enigmatic. In analogy to the global unfolding hypothesis proposed for Hsp60 and Hsp100 chaperones (Shtilerman et al. 1999; Weber-Ban et al. 1999) it is proposed that Hsp70s induce local conformational changes in their substrate protein thereby giving them a new chance to fold productively (Mayer et al. 2000a; Pierpaoli et al. 1997; Slepenkov and Witt 2002).

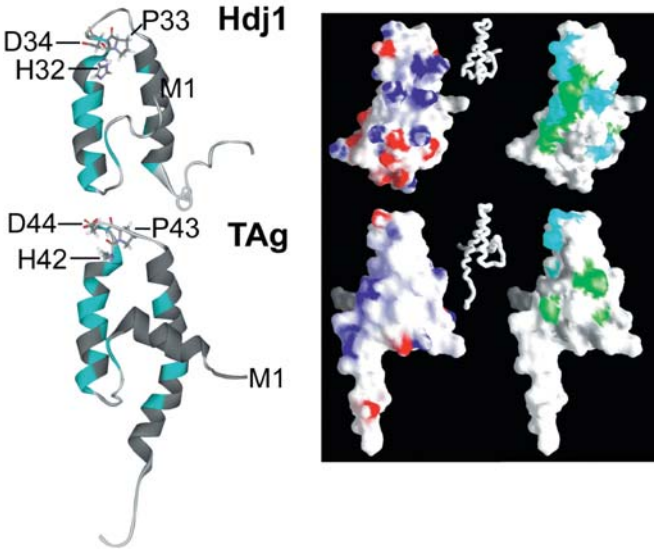

Fig. 4 Comparison of the J domains of Hdj1 and polyomavirus T antigen. *Left*: Secondary structure representation of one of the 20 energy minimized NMR structures of human Hdj1 (1HDJ; Qian et al. 1996) and mouse polyomavirus T antigen (1FAF; Berjanskii et al. 2000) with the highly conserved and essential His–Pro–Asp motif as stick model. The positions of the residues are shown in *cyan* for which in the corresponding residues in *E. coli* DnaJ J-domain line broadening and chemical shift perturbation is observed upon interaction with the *E. coli* Hsp70 homolog DnaK ATPase domain. *Right*: Surface representations of the two J-domains colored according to the surface potential (–10 to +10; *left*) and the residues that correspond to the DnaK interacting residues in DnaJ (*right*); *cyan*, identical residues as in DnaJ; *green*, conservative exchanges; *gray*, not conserved. The *inset* shows the orientation of the J-domains as worm representation. The surface representation and the electrochemical potentials were calculated using the GRASP program

The family of J-domain proteins: targeting of substrates to Hsp70s

The family of JDPs consists of modular multidomain proteins that are characterized by a conserved domain of 70–80 amino acids, the so-called J-domain (Cheetham and Caplan 1998; Kelley 1998; Laufen et al. 1998) (Fig. 4). The additional domains of JDPs serve as protein–protein interaction sites allowing JDPs to bind substrate proteins, to interact with other chaperones, or to target JDPs to specific cellular locations.

The J-domain is essential for the functional interaction of JDPs with Hsp70s, i.e., the stimulation of the ATPase activity, and mutations within this domain especially in the almost universally conserved tripeptide motif His-Pro-Asp (HPD-motif) abrogate the function of the JDPs as co-chaperones of Hsp70s. JDPs have been shown to promote the binding of Hsp70s to their substrates by simultaneous co-stimulation of the ATPase activity leading to the locking-in of the substrate into the substrate binding cavity of the Hsp70s (Karzai and McMacken 1996; Laufen et al. 1999; Misselwitz et al. 1998). This function of JDPs depends on close proximity of JDP and substrate, which for most JDPs is guaranteed by direct interaction with the Hsp70 substrate. How JDPs interact with substrates and mediate their transfer onto Hsp70 partner proteins is not clear.

Some JDPs have a broad substrate specificity such as *E. coli* DnaJ, yeast Ydj1 and human DjB1/Hdj1 and are able to prevent the aggregation of misfolded proteins, while others have more restricted substrate spectra, such as the clathrin-specific auxilin, or they

may not bind substrates themselves but rather be positioned in close proximity of substrates. The latter seems to be the case for Sec63 at the translocation pore in the endoplasmic reticulum (ER) (Corsi and Schekman 1996; Rapoport et al. 1996), Pam18 at the translocase of the inner mitochondrial membrane (D'Silva et al. 2003; Mokranjac et al. 2003; Truscott et al. 2003) and cysteine string proteins on the surface of neurosecretory vesicles (Buchner and Gundersen 1997; Evans et al. 2003).

GrpE and the Bag family of proteins: induction of substrate unloading

The 21-kDa nucleotide exchange factor GrpE has been found only in prokaryotic organisms and in some eukaryotic organelles (mitochondria and plastids). It forms an asymmetric homodimer when in complex with its Hsp70 partner protein DnaK and does not share any sequence or structural motifs with other known proteins. The family of Bag proteins are multidomain proteins that share a three-helix bundle domain of approximately 120 residues, the so-called Bag domain, which is essential for interaction with Hsp70 proteins (Briknarova et al. 2001; Sondermann et al. 2001; Takayama et al. 1999).

Bag proteins differ from GrpE proteins by their ability to associate with ligands other than Hsp70 proteins. These interactions link Bag proteins to a number of highly diverse cellular processes including signal transduction processes inducing apoptosis, proliferation, and differentiation. Bag proteins have been found in complexes with the anti-apoptotic Bcl-2, the protein kinase Raf, the transcription factor c-Jun, the receptors for vitamin D, androgen and glucocorticoids (Bardelli et al. 1996; Clevenger et al. 1997; Schneikert et al. 1999; Takayama et al. 1995; Wang et al. 1996; Zeiner et al. 1997; Zeiner and Gehring 1995), and the proteasome (Lüders et al. 2000). The known cellular processes in which Bag proteins are involved were recently reviewed comprehensively and are therefore not discussed in detail here (Takayama and Reed 2001).

Whatever the functions of Bag proteins are in the above cellular processes, it is clear from the work of several laboratories that Bag proteins are not strictly essential for the chaperone activity of Hsp70 proteins in folding of non-native proteins (Bimston et al. 1998; Gässler et al. 2001; Lüders et al. 1998; Nollen et al. 2000; Takayama et al. 1997, 1999; Zeiner et al. 1997). This is in contrast to the essential role for GrpE in the chaperone cycle of DnaK. In vivo and in vitro experiments demonstrated that Bag proteins, in particular Bag-1 M, can act as positive and negative regulatory factor for Hsp70 (Bimston et al. 1998; Gässler et al. 2001; Nollen et al. 2000; Takayama et al. 1997; Zeiner et al. 1997). Since the positive or negative regulatory function of Bag-1 depends on its concentration, which in most cell types is rather low (3% of the Hsc70 concentration; Kanelakis et al. 1999), it might be that the inhibitory effects of Bag-1 are restricted to special environmental and metabolic conditions of the cell. An attractive hypothesis is that in the cell, most Bag proteins are associated with their protein partners and exert their regulatory role only locally. Furthermore, it was shown that Bag-1, by accelerating nucleotide exchange, promotes substrate release thereby counteracting the activity of JDPs (Gässler et al. 2001). In the context of Bag-1 binding to the proteasome, Bag-1 could act as substrate discharging factor stimulating substrate release by Hsp70 at the location were the misfolded protein is to be delivered for degradation. Similarly, Bag-1 bound to receptors or kinases could stimulate the premature dissociation of Hsp70 thereby influencing the chaperone-mediated activation of these signaling molecules.

Additional Hsp70 cofactors: cooperation with other chaperones and the degradation machinery

The 35-kDa protein Chip interacts with Hsc70's substrate binding domain and thereby negatively influences the chaperone activity of Hsc70 (Ballinger et al. 1999). This cofactor recently turned out to be a ubiquitin E3 isopeptide ligase promoting the degradation of proteins that were also shown to be substrates of Hsc70 (Hatakeyama et al. 2001; Jiang et al. 2001). The hypothesis is therefore that Chip's interaction with the Hsp70 chaperone systems influences the decision whether a misfolded or regulatory protein is to be refolded into the active state or degraded by the proteasome (Connell et al. 2001; Meacham et al. 2001). The proteasome-interacting Bag-1 may be part of this decision-making system. In addition, Chip seems to have degradation-independent regulatory functions. The heat shock factor HSF1 was found to be activated by the interaction with Chip and translocated into the nucleus in complex with Chip and Hsp70 to activate transcription of heat shock genes (Dai et al. 2003).

The homodimeric 43-kDa Hsp70 interacting protein Hip was originally found in a two-hybrid screen using the Hsc70 ATPase domain as bait (Höhfeld et al. 1995; Velten et al. 2000). This Hsp70 cofactor was subsequently found to be part of the multi-chaperone folding machine that regulates steroid hormone receptors (Prapapanich et al. 1996). Although Hip was proposed to aid chaperone function of Hsp70s by slowing down their nucleotide exchange (Höhfeld and Jentsch 1997; Höhfeld et al. 1995; Lüders et al. 1998) more recent investigations could not provide evidence for such a function of Hip and its biochemical function is still unclear (M.P. Mayer, unpublished results). Rather then having a direct effect on the ATPase cycle of Hsc70 Hip seems to compete with Bag proteins for binding to the ATPase domain of Hsc70 and thereby counteracts the effect of Bag (Kanelakis et al. 2000) (see Fig. 3).

The homodimeric 60-kDa Hsp70–Hsp90 organizing protein Hop (yeast Sti1) was also found in the steroid hormone receptor chaperone complexes (Smith et al. 1993). Hop was shown to promote the assembly of the Hsp70–Hsp90–substrate complex with steroid hormone receptors (Fig. 3).

Chip, Hip, and Hop contain several tetratricopeptide repeat (TPR) domains that constitute protein–protein interaction motifs. The TPR motifs of Hop and Chip bind the EEVD motifs that are found at the C terminus of all known eukaryotic cytosolic Hsp70s and Hsp90s (Scheufler et al. 2000).

Viral control of production and localization of Hsp70s

Increase in Hsp70 chaperone levels following viral infection of cells has been widely observed (see Table 1) (Jindal and Malkovsky 1994). These observations prompted the questions how viruses influence the production of Hsp70 proteins, how specific this induction is and whether such an increase in Hsp70 chaperone levels is advantageous for viral proliferation. From the known regulatory circuits three alternative mechanisms can be inferred. First, the induction may occur indirectly through the production of a large number of proteins that are in an unfolded, aggregation-prone state. In the cytosol or nucleus these protein species could compete with the HSF for binding to Hsp70 thereby leading to an increase of free and active HSF that elicits the transcription of many heat shock genes in the course of the normal cellular stress response (Morimoto et al. 1994). In the ER misfolded

Table 1 Functional interactions of viruses with the Hsp70 system

Virus group	Infecting virus, viral protein	Component of the Hsp70 system	Functional interaction	References
dsDNA viruses				
Polyomaviridae	SV40, TAg	Hsp70	Induction of *hsp*70 transcription	Damania et al. 1998a, 1998b; Khandjian and Turler 1983; Kingston et al. 1986; Sainis et al. 1994; Simon et al. 1988
	SV40, TAg	Hsp70/ Hsc70	Translocation of T-Ag into nucleus	Yang and DeFranco 1994
	SV40, TAg	Hsp70/ Hsc70	Genome replication	Campbell et al. 1997; Li et al. 2001; Sullivan et al. 2000b; Weisshart et al. 1996
	SV40, VP1,2,3	Hsc70	Virion assembly	Chromy et al. 2003; Cripe et al. 1995
	SV40, TAg	Hsc70	Transformation	DeCaprio 1999; Kroll 2002; Sullivan et al. 2000a, 2000b
	JCV	Bag1, Hsc70	Viral transcription	Devireddy and Pater 2000
Papilomaviridae	HPV-11, E1, E2	Hsp70, Hdj1, Hdj2	Genome replication	Lin et al. 2002; Liu et al. 1998
Adenoviridae	AdV, E1A, E1B	Hsp70	Induction of *hsp*70/*hdj*1 transcription, mRNA export	Agoff and Wu 1994; Glotzer et al. 2000; Herrmann et al. 1987; Kao et al. 1985; Kao and Nevins 1983; Kraus et al. 1994; Lum et al. 1992; Milarski and Morimoto 1986; Moore et al. 1987; Nevins 1982; Phillips and Morimoto 1991; Simon et al. 1987; Theodorakis and Morimoto 1987; Williams et al. 1989; Wu et al. 1986; Yang et al. 1996
	AdV hexon	Hsp70, Hsc70	Virion disassembly?	Niewiarowska et al. 1992; Saphire et al. 2000
	AdV penton	Bag3	Virion disassembly?	Chroboczek et al. 2003
	AdV E1A	Hsc70	?	White et al. 1988
	AdV fiber protein	Hsp70	Virion assembly?	Macejak and Luftig 1991

Table 1 (continued)

Virus group	Infecting virus, viral protein	Component of the Hsp70 system	Functional interaction	References
Herpesviridae	HSV-1; VZV; HCMV, IE1, IE2; EBV	Hsp70, BiP	Induction of *hsp*70/*grp*78 transcription	Caswell et al. 1993; Cheung and Dosch 1993; Colberg-Poley and Santomenna 1988; Colberg-Poley et al. 1992; Hagemeier et al. 1992; Kobayashi et al. 1994; LaThangue et al. 1984; Mao et al. 2001; Phillips and Morimoto 1991; Santomenna and Colberg-Poley 1990
	HSV-2; VZV; HCMV	Hsp70	Nuclear localization	LaThangue and Latchman 1987; Ohgitani et al. 1998, 1999
	HSV-1, UL9	Hsp70, Hsp40, hTid1	Genome replication	Eom and Lehman 2002; Tanguy Le Gac and Boehmer 2002
	HCMV	Hsp70/Hsc70, Bag1	Viral transcription	Niyaz et al. 2003; Takahashi et al. 2001
	EBV, EBNA-LP	Hsp70, Hsc70	Transformation	Kitay and Rowe 1996
Poxviridae	Vaccinia virus	Hsp70	Induction of hsp70 transcription	Brum et al. 2003; Jindal and Young 1992; Sedger et al. 1996; Sedger and Ruby 1994
Siphoviridae	λ-phage	DnaK	Induction of dnaK transcription	Drahos and Hendrix 1982; Tilly et al. 1989
	λP	DnaK, DnaJ, GrpE	DNA replication	Alfano and McMacken 1989a, 1989b; Dodson et al. 1986, 1989; Hoffmann et al. 1992; LeBowitz and McMacken 1984; Liberek et al. 1988, 1990; Wyman et al. 1993; Yochem et al. 1978; Zylicz et al. 1983, 1988; 1989
Myoviridae	P1-phage	DnaK, DnaJ, GrpE	DNA replication	Dibbens et al. 1997; Tilly and Yarmolinsky 1989; Wickner 1990, Wickner et al. 1991a, 1991b, 1992
ssDNA viruses				
Parvoviridae	Parvovirus, H1	Hsp70	Induction of Hsp70 release	Moehler et al. 2003
dsRNA viruses				
Reoviridae	Rotavirus, VP4, VP7	Hsc70	Cell entry	Guerrero et al. 2002; Zarate et al. 2003
	Rotavirus, NSP4		Induction of grp78 transcription	Xu et al. 1998
	Rotavirus, VP7	BiP	Capsid protein folding	Mirazimi and Svensson 2000
	Reovirus	Hsp70	Capsid protein folding	Leone et al. 1996

Table 1 (continued)

Virus group	Infecting virus, viral protein	Component of the Hsp70 system	Functional interaction	References
(+)-strand ssRNA viruses				
Picornaviridae	HCV, E2	BiP	Induction of the UPR	Liberman et al. 1999
	HCV, E1, E2	BiP	Envelope protein maturation	Choukhi et al. 1998
	CAV-9	BiP	Cell entry	Triantafilou et al. 2002
	Poliovirus, CBV-1, P1	Hsp70	Virion assembly ?	Macejak and Sarnow 1992
Togaviridae	Sindbis virus E1, E2	BiP	Envelope protein maturation	Carleton and Brown 1996, 1997; Mulvey and Brown 1995
Closteroviridae	BYV	vHsp70	Cell-to-cell movement	Agranovsky et al. 1998; Alzhanova et al. 2000, 2001; Peremyslov and Dolja 2002; Peremyslov et al. 1999
	BYV, CTV	vHsp70	Virion assembly	Napuli et al. 2000; Satyanarayana et al. 2000, 2004
Tobamoviridae	TMV, CP	Hsp70	Induction of heat shock response	Jockusch et al. 2001
(−)-strand ssRNA viruses				
Bunyaviridae	Topsovirus NSm	JDP	Cell-to-cell movement	Soellick et al. 2000
Rhabdoviridae	Rabies virus; VSV	Hsc70	Present in virion particle	Sagara and Kawai 1992
	VSV-G	BiP	Envelope protein folding/maturation	de Silva et al. 1990, 1993; Hammond and Helenius 1994; Machamer et al. 1990
Paramyxoviridae	NDV; SV5, HN; measles virus; sendai virus	BiP	Induction of UPR	Bolt 2001; Collins and Hightower 1982; Ng et al. 1989; Peluso et al. 1978; Watowich et al. 1991
	SV5, HN, SV5, F	BiP	Envelope protein folding/maturation	Ng et al. 1989; Parks and Lamb 1990; Roux 1990; Tomita et al. 1999; Watowich et al. 1991
	CDV	Hsp70, Hsc70	Capsid protein folding	Oglesbee and Krakowka 1993; Oglesbee et al. 1990; Zhang et al. 2002
	CDV, measles virus	Hsp70, Hsc70	Genome replication	Oglesbee et al. 1996; Zhang et al. 2002
Orthomyxoviridae	Influenza A, H	BiP	Envelope protein folding/maturation	Hogue and Nayak 1992; Singh et al. 1990
	Influenza A	Hsc70	Present in virion particle	Sagara and Kawai 1992
	Influenza A	$p58^{IPK}$, Hsc70	General regulation of translation	Melville et al. 1997, 1999; Yan et al. 2002

Table 1 (continued)

Virus group	Infecting virus, viral protein	Component of the Hsp70 system	Functional interaction	References
Retroidviruses				
Retroviridae	RSV	BiP	Induction grp78 transcription	Peluso et al. 1978; Stoeckle et al. 1988
	HTLV-1	Hsc70	Cell-to-cell movment	Fang et al. 1999; Sagara et al. 1998
	HTLV-1, Tax	Hsp70, hTid1	Transformation	Cheng et al. 2001
	HIV, gp120	Hsp70	Induction of stress response	Furlini et al. 1994; Wainberg et al. 1997
	HIV	Hsp70	Nuclear import of pre-integration complex	Agostini et al. 2000
	HIV, Tat	Hsp70	Viral transcription	O'Keeffe et al. 2000
	HIV, gp160	BiP	Envelope protein maturation	Earl et al. 1991; Otteken et al. 1996
	HIV	Hsp70, Hsc70	Present in virion particle	Gurer et al. 2002
Hepadnaviridae	HBV, Pol	Hsp70, Hsp40	Reverse transcription	Beck and Nassal 2001, 2003; Gyoo Park et al. 2002; Hu and Anselmo 2000; Hu and Seeger 1996; Hu et al. 2002; Hu et al. 1997
	HBV, L	Hsp70	Envelope protein maturation	Cho et al. 2003; Lambert and Prange 2003; Löffler-Mary et al. 1997; Prange et al. 1999

Abbreviations. Viruses: AdV, adenovirus; BYV, beet yellow virus; CAV-9, coxsackievirus A9; CDV, canine distemper virus; CTV, citrus tristeza virus; EBV, Epstein–Barr virus; HBV, hepatitis B virus; HCMV, human cytomegalovirus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papilomavirus; HSV, herpes simplex virus; HTLV-1, human T cell leukemia virus 1; JCV, JC virus; NDV, Newcastle disease virus; RSV, Rous sarcoma virus; SV5, simian virus 5; SV40, simian virus 40; TMV, tobacco mosaic virus; VSV, vesicular stomatitis virus; VZV, varicella zoster virus. Proteins: HN, haemagglutinin-neuraminidase; TAg, large T antigen; vHsp70, virus encoded Hsp70; BiP/Grp78, immunglobulin binding protein/glucose regulated protein 78 kDa (ER Hsp70); all other abbreviation name viral proteins

proteins would bind to the ER Hsp70 homolog BiP/Grp78 and thereby elicit the unfolded protein response that increases the transcription of genes encoding for ER resident chaperones (Urano et al. 2000). Second, the viruses could interfere with stress signal transduction pathways upstream or independent of HSF thereby using pathways that are independent of unfolded proteins inside the cell. Third, specialized viral proteins may induce directly the increased production of Hsp70 proteins, either by binding to the promoters of specific Hsp70-encoding genes or by stabilizing certain mRNAs and activating their translation. As detailed in the following parts, all three mechanisms have been observed. While the changes in the levels of Hsp70 proteins were investigated in many systems, changes in the levels of co-chaperones of Hsp70 may be inferred but are generally not mentioned.

Induction of the stress response

The stress response to unfolded proteins is apparently the reason for the induction of heat shock gene transcription during infection with the varicella zoster virus (VZV, *Herpesviridae*). Inclusion bodies, which are formed in the nucleus of the host cells in the course of the virus infection, sequester Hsp70 and presumably liberate HSF for heat shock gene transcription (Ohgitani et al. 1998). More direct evidence for this mode of viral heat shock response induction was provided by Rajes and coworkers (Jockusch et al. 2001). Analyzing Hsp70 and Hsp18 mRNA and protein levels in tobacco leaves after infection with wild-type tobacco mosaic virus (TMV, *Tobamoviridae*) or a mutant virus, which encodes a temperature sensitive coat protein, they found that degree and time of induction paralleled the amount of insoluble viral coat protein suggesting that the amount of unfolded protein is the inducing agent.

Such an induction mechanism was also found for the ER resident Hsp70 and Hsp90 homologs, BiP/Grp78 and Grp94, which are overproduced in the course of a hepatitis C virus (HCV, *Picornaviridae*) infection. The HCV envelope protein E2 is retained in a pre-Golgi compartment in an at least partially misfolded state and bound by BiP (Liberman et al. 1999). Since BiP is part of the sensing system that detects misfolding in the ER, the interaction of E2 with BiP activates the feedback mechanism of the unfolded protein response leading to the overproduction of a number of ER resident chaperones including BiP and Grp94 (Kozutsumi et al. 1988). Similarly, the BiP encoding gene is induced during the infection with the paramyxoviruses measles virus, Sendai virus and simian virus 5 (SV5) (Bolt 2001; Peluso et al. 1978). However, the inducing viral component, the hemagglutinin–neuraminidase glycoprotein, is not retained in the ER in an unfolded state. The high flux of this protein through the ER seems to suffice for the induction of the unfolded protein response (Ng et al. 1989; Watowich et al. 1991). The case of the rotavirus (*Reoviridae*) nonstructural glycoprotein NSP4, which also induces the production of BiP and Grp94, seems to be slightly different, since an interaction with BiP or Grp94 could not be detected. The mechanism of this induction is not clear (Xu et al. 1998).

Interference with stress signaling pathways is described for the human immune deficiency virus (HIV). HIV induces the synthesis of *hsp70* and *hsp27* mRNA through the interaction of its envelope glycoprotein gp120 with cell surface receptors (Furlini et al. 1994; Wainberg et al. 1997). Isolated gp120 is also able to induce the heat shock genes in the absence of intact virus particles. The Epstein–Barr virus also seems to induce the *hsp70* and *hsp90* genes through the interference with signal transduction pathways since

the induction is clearly dependent on virus attachment but independent of viral protein synthesis and involves an influx of Ca^{2+} (Cheung and Dosch 1993).

Selective *hsp* gene induction

A specific *hsp* induction is realized by a number of viruses, e.g., adenovirus (AdV), herpes simplex virus (HSV), and simian virus 40 (SV40) and is characterized by the selective induction of specific chaperone-encoding genes while the expression of other heat shock genes is not influenced. The transactivating viral proteins thereby recognize the specific promoters directly by binding to *cis*-acting DNA elements or by interacting with promoter-specific transcription factors. The SV40 large T-antigen (TAg), for example, induces the *hsp70* gene expression (Kingston et al. 1986; Sainis et al. 1994; Simon et al. 1988) by binding to the general transcription initiation factors, the TATA-binding protein (TBP) and TFIIA, and stabilizing the transcription pre-initiation complex at the *hsp70* promoter TATA-element, but not at two other promoters tested (Damania et al. 1998a; Gilinger and Alwine 1993). The adenoviral early gene products E1A and E1B synergistically stimulate *hsp70* gene expression (Herrmann et al. 1987; Kao et al. 1985; Phillips et al. 1991; Wu et al. 1986) in a cell cycle specific manner (Milarski and Morimoto 1986; Simon et al. 1987). This stimulation is mediated through an interaction of E1A with the CCAAT-box binding factor (Agoff and Wu 1994; Lum et al. 1992; Williams et al. 1989) and the disruption of the inhibitory complex of DR1 with TBP (Kraus et al. 1994). Furthermore, AdV specifically promotes the nuclear export of the *hsp70* mRNA by interaction of the mRNA with E1B, thereby circumventing the general virus-induced export block for cellular mRNAs (Moore et al. 1987; Yang et al. 1996). Late during AdV infections the *hsp70* mRNA levels decline precipitously while transcription continues demonstrating the complexity of the viral regulation of *hsp70* expression (Theodorakis and Morimoto 1987).

In addition to the induction of production of Hsp70, viruses are able to influence the localization of the Hsp70 systems. The avian AdV CELO gene product Gam1 for example leads to a strong accumulation of Hsp70 and its co-chaperone Hdj1 in the nucleus (Glotzer et al. 2000). Similarly, the human cytomegalovirus (HCMV) directs Hsp70 to the nucleus in the early phase of infection but redirects it to the cytoplasm in the late phase (Ohgitani et al. 1999).

What is the significance of the virus induced *hsp70* expression? In view of the fact that transcription of most cellular genes is shut down and mRNA processing, transport and translation is regulated by many viruses, the virus-induced increase of Hsp70 levels in the cell seems to be important for the viral life cycle. Two clear examples where the essentiality of the Hsp70 system for virus proliferation was demonstrated beyond doubt are the bacteriophage λ and the avian AdV CELO. The *E. coli* Hsp70 homolog DnaK (GroPC) was originally identified as host factor essential for bacteriophage λ proliferation and loss of function mutations in *dnaK*, *dnaJ*, and *grpE* can only be complemented by transduction of the respective gene (Georgopoulos 1977; Saito and Uchida 1977). The CELO inducer of *hsp70* and *hdj1* transcription, Gam1 is also essential for virus proliferation. However, Gam1 can be replaced by a heat shock, being most efficient immediately before infection, or the transduction of the *hdj1* gene (Glotzer et al. 2000).

Functions of the Hsp70 system in the viral life cycle

Hsp70 systems are potentially involved in all phases of the viral life cycle including cell entry, virion disassembly, the transfer of viral genome into the nucleus, replication of the viral genome, morphogenesis of the virion particles, and transformation of the cell. The following discussion is ordered according to these stages of the life cycle rather than to virus systematics and the same viruses are discussed in several sections in different contexts. For a systematic summary see Table 1.

Cell entry: interaction of viruses with cell-surface exposed Hsp70s

Before animal viruses can transfer their virion particles into host cells by fusion of their envelope with the plasma membrane or by receptor-mediated endocytosis, they must attach to specific surface receptors. This surface attachment is, at least in some cases, a multistep process that can involve surface-exposed Hsp70 proteins. Although Hsp70 and Hsc70 do not contain export signal sequences and more importantly depend in their chaperone function on repetitive cycles of ATP hydrolysis, they are found on the cell surface of a number of different cell types including tumor cells (Hantschel et al. 2000; Kaur et al. 1998; Multhoff et al. 1998; Multhoff and Hightower 1996; Shin et al. 2003), virus infected cells (Brenner and Wainberg 1999; Chouchane et al. 1994), spermatogenic cells (Boulanger et al. 1995; Miller et al. 1992), epidermal cells (Rocchi et al. 1993), arterial smooth muscle cells (Johnson and Tytell 1993), monocytes and B cells (Manara et al. 1993; VanBuskirk et al. 1989, 1991). It is not clear in each case how Hsc70 and Hsp70 locate to the cell surface, however, for antigen-presenting cells it was shown that specific receptors exist that bind Hsp70 and Hsc70 for uptake out of the extracellular space (Arnold-Schild et al. 1999; Asea et al. 2002; Becker et al. 2002; Binder et al. 2000; Lipsker et al. 2002; Sondermann et al. 2000). This uptake is proposed to signal necrotic and lytic processes and to induce an inflammatory response (Dybdahl et al. 2002; Milani et al. 2002; Vabulas et al. 2002). Furthermore, it was proposed that Hsp70-bound peptides are transferred in the endosomal pathway onto MHC II molecules for antigen presentation (Becker et al. 2002; Domanico et al. 1993; Kishi et al. 2001; Nicchitta 2000; Panjwani et al. 1999; Pierce 1994; Roth et al. 2002; Suzue et al. 1997; VanBuskirk et al. 1989).

The nonenveloped rotavirus, a double-stranded RNA virus, needs cell surface-exposed Hsc70 at a postattachment step for the successful infection of epithelial cells (Arias et al. 2002). Treatment of the epithelial cells with monoclonal antibodies against Hsc70 prevented internalization and infection by rotavirus but did not obliterate its attachment to the cells, while infection with poliovirus and reovirus was not affected by the antibody treatment (Guerrero et al. 2002). Hsc70 was shown to bind to free rotavirus particles and preincubation of the virus particles with Hsc70 significantly decreased their infectivity (Guerrero et al. 2002). The C-terminal half of the viral coat protein VP5 was demonstrated to be sufficient for interaction with Hsc70 and the interaction of VP5 as well as whole virus particles with Hsc70 could be competed with a 17-mer peptide containing a sequence stretch of the VP5 C-terminal domain (Zarate et al. 2003). These observations pose interesting questions regarding the mechanism of this interaction: What is the role of Hsc70 in the internalization of rotavirus? Are substrate-binding by Hsc70 and its chaperone activity involved?

In view of the fact that Hsc70 proteins loaded with substrate peptides are internalized through receptor-mediated endocytosis, it is tempting to speculate that the internalization process may depend on, or even may be triggered by, peptide binding to the substrate-binding domain of Hsc70. Rotavirus could use such a mechanism for efficient cell entry by providing the substrate signal to Hsc70. Since there is no ATP available for cell surface exposed Hsc70, the interaction of the substrate-binding domain of Hsc70 with a substrate polypeptide would be a very slow reaction ($\tau \approx 100$–$1{,}000$ s; Pierpaoli et al. 1997; Takeda and McKay 1996). These limitations, however, do not preclude such a mechanism for the interaction since attachment steps prior to the interaction with Hsc70 are necessary for rotavirus internalization providing the time window and the high local concentration necessary for an efficient binding. Since substrate dissociation rates are very low in the ADP or nucleotide-free state, the rotavirus would be firmly attached to the cell once associated to Hsc70. Such a mechanism could be tested by competing rotavirus infection with high concentrations of peptides, which are known to have a high affinity for Hsc70 and are unrelated to the VP5 sequence, or by replacing surface exposed wild-type Hsc70 by mutant proteins, which are defective in their affinity for substrates (compare Mayer et al. 2000b). Alternatively, rotavirus could bind to any other part of the Hsc70 molecule without involving the chaperone mechanism of Hsc70. For example a co-chaperone binding site could serve as interaction site, as is the case for the Hsc70 receptor CD40, which competes with the co-chaperone Hip for binding to the ATPase domain of Hsc70 (Becker et al. 2002). In favor of this mode of binding is the fact that the peptide, which was able to compete with rotavirus infection, does not seem to be a high-affinity substrate for Hsc70 as judged from the sequence of the peptide using the DnaK algorithm and the BiP scoring system (Blond-Elguindi et al. 1993; Rüdiger et al. 1997). Rotavirus would then be internalized as a hitchhiker on Hsc70. A more extensive function of the cell-surface exposed Hsc70, for example by chaperoning a viral coat protein to promote translocation through the membrane or uncoating of the virion, seems unlikely because Hsc70 depends in its chaperone function on ATP and the assistance of a JDP both of which have not yet been found on the cell surface. At later stages, however, in endosomal vesicles, such a chaperone action may be possible, because ATP as well as co-chaperones are present in the ER and could be transferred by vesicular flow to endocytotic vesicles.

Similar to rotavirus, the coxsackievirus A9 (CAV-9), a nonenveloped RNA virus of the *Picornaviridae* family, also interacts with a cell-surface exposed Hsp70 homolog. Its target Hsp70, the primarily ER resident BiP/Grp78, was shown by fluorescence energy transfer experiments to be associated to major histocompatibility complex (MHC) I molecules (Triantafilou et al. 2002). BiP is known to interact with MHC molecules already in the ER supporting their folding and assembly (Paulsson and Wang 2003). It therefore may occasionally remain in complex with the MHC proteins and migrate with them to the plasma membrane. Monoclonal BiP-specific antibodies prevent attachment and cell entry of CAV-9 (Triantafilou et al. 2002).

Another example of a virus interacting with Hsp70 proteins on the cell surface is the retrovirus human T lymphotropic virus type 1 (HTLV-1). The cell-free infectivity of this virus is very low (Clapham et al. 1983) but as in many other retrovirus infections, close cell-to-cell interactions between HTLV-1 harboring cells and target cells leads to syncytium formation allowing direct cell-to-cell transfer of the virus (Hoshino et al. 1983). Cell-surface exposed Hsc70 is essential for this process as shown by blocking syncytium formation with Hsc70-specific monoclonal antibodies or a peptide derived from the sequence

of the HTLV-1 glycoprotein gp46, which binds to Hsc70 (Fang et al. 1999; Sagara et al. 1998). This peptide seems to be a good Hsc70 substrate as judged by the DnaK-algorithm (Rüdiger et al. 1997) and syncytium formation could therefore involve the substrate binding properties of Hsc70.

Uncoating and genome release

The viral genomes are packaged in nucleocapsid structures which allow condensation of the viral nucleic acid in a very small space and serve as protective coat against a hostile environment. For successful infection the viral genome has to be released from the virion particle either by disassembly of the coat or by opening of a pore. The coats, however, must be stable to prevent genome release outside of a host cell and uncoating of the virion particle is most likely a thermodynamically unfavorable process. The differences between extra- and intracellular environment, oxidizing versus reducing conditions and the different ionic milieu, which are experienced by the capsid of nonenveloped viruses, may not be sufficient for the destabilization of the virion particle. If the virion particle would be stable in the extracellular but unstable in the intracellular milieu, thermodynamics would require that the assembly reaction in the cytoplasm should be unfavorable. Therefore, viruses for which the uncoating does not occur in endocytic vesicles aided by acidification may involve cellular components in the genome release process. Since Hsp70 chaperones are known to be involved in the disassembly of oligomeric protein structures, the best example of which is the uncoating of clathrin coated vesicles (Greene and Eisenberg 1990; Ungewickell 1985), it is conceivable that the Hsp70 chaperone machinery assists the uncoating of virion particles.

Such a process was described for an AdV. Soon after the release of the virion particle from endocytic vesicles into the cytoplasm, Hsp70 and Hsc70 can be found attached to the hexon protein, the major AdV coat proteins (Niewiarowska et al. 1992). In addition, Hsp70 and its co-chaperone Bag3 interact with the penton protein, the base and fiber-forming virion component (Chroboczek et al. 2003). The intact nucleocapsid is transported to the nuclear pore complex using the normal nuclear localization signal (NLS)-dependent nuclear import machinery as demonstrated by competition with classical NLS-containing proteins and inhibition with nuclear import inhibitors like GTPγS (Saphire et al. 2000). The nucleocapsid docks with the nuclear pore by interaction of its hexon protein with components of the pore complex without transiting into the nucleus. The viral DNA is subsequently transferred into the nucleus in an Hsp70-dependent manner. Since purified hexon can enter the nucleus in an Hsp70-independent manner while the viral DNA cannot (Saphire et al. 2000), it is plausible that the nucleocapsid, which is too large to pass through the nuclear pore complex, is disassembled in an Hsp70-dependent manner allowing the viral DNA to enter the nucleus. The final proof for such a mechanism is, however, still lacking because in a reconstituted import assay containing the necessary import factors for hexon import supplemented with Hsp70 the viral DNA was not transferred into the nucleus to any significant extent. The inability of the reconstituted system used to uncoat the virion particle was most likely due to the absence of Hsp70 co-chaperones such as JDPs or Bag-domain proteins. Similar to the uncoating of clathrin coated vesicles where the clathrin associated JDP auxilin targets Hsc70 to clathrin for the multiple ATPase cycles requiring uncoating reaction, JDPs may be necessary for targeting Hsp70 proteins to the AdV coat and disassembly may require multiple J-domain stimulated rounds of ATP

hydrolysis. Furthermore, proteolytic processes including the proteasome may also be involved since three ubiquitin-protein isopeptide ligases were found associated with the virion (Chroboczek et al. 2003).

Replication and reverse transcription

The involvement of Hsp70 systems in viral DNA replication was first demonstrated by genetic and biochemical means for the *E. coli* bacteriophage λ (Alfano and McMacken 1989b; Georgopoulos 1977; Georgopoulos and Herskowitz 1971; Mensa-Wilmot et al. 1989; Saito and Uchida 1977; Zylicz et al. 1989; for review see Zylicz et al. 1999). The bacteriophage protein λP sequesters the *E. coli* DNA helicase DnaB and recruits it to the origin of replication oriλ of the bacteriophage genome, where it interacts with the four dimers of λO assembled at oriλ to form a multimeric complex (Dodson et al. 1985; Liberek et al. 1988; Roberts and McMacken 1983). In the absence of the Hsp70 system of *E. coli*, DnaK, DnaJ and GrpE, the λO·λP·DnaB complex is stalled at the oriλ and DNA unwinding and therefore replication cannot start. The most likely reason for this block is that the affinity of λP to DnaB is significantly higher (at least fivefold) than the affinity between the *E. coli* replication initiation factor DnaC and DnaB (Mallory et al. 1990). λP therefore outcompetes DnaC for binding to DnaB with the consequence that the λP·DnaB complex cannot spontaneously disassemble. The thermodynamically stable complex of λO, λP, and DnaB at oriλ is disassembled by the chaperone action of DnaJ and DnaK, which interact with and sequester λP in an ATP-dependent process thereby liberating DnaB for the unwinding of the DNA and the initiation of replication (Alfano and McMacken 1989a; Dodson et al. 1989; Hoffmann et al. 1992; Liberek et al. 1988; Zylicz et al. 1989). If DnaK is in large excess over λP the nucleotide exchange factor GrpE is not essential. However, if DnaK is at more stoichiometric concentrations GrpE enhances the efficiency of the process greatly, demonstrating that nucleotide exchange and therefore multiple rounds of the ATPase cycle with substrate binding and release is central to the efficiency of the disassembly reaction (Alfano and McMacken 1989a; Alfano and McMacken 1989b; Zylicz et al. 1988, 1989).

For the replication of the P1 phage genome, as well as for the replication of the F-plasmid, the DnaK/DnaJ/GrpE-system plays a different role (Kawasaki et al. 1990; Tilly and Yarmolinsky 1989; Wickner et al. 1991a; , 1991b, 1992; Wickner 1990). The initiator proteins RepA for P1 phage and RepE for the F-plasmid form stable homodimers in vivo and in vitro (Ishiai et al. 1994; Swack et al. 1987; Wickner et al. 1991b). RepA does not bind DNA in its dimeric form, while RepE binds to an inverted repeat sequence motif, the operator of the *repE* gene repressing its transcription (Ishiai et al. 1994). Both proteins can only bind in their monomeric form to the direct repeat sequence motifs, the so-called iterons, within their respective origin of replication to initiate DNA replication. The conversion into monomers requires the chaperone action of DnaK, DnaJ, and GrpE, as demonstrated in vitro and by mutant analysis in vivo (Dibbens et al. 1997; Ishiai et al. 1994; Wickner et al. 1991b, 1992). A constitutively monomeric mutant protein of RepE is able to initiate replication of the P1 genome independent of the DnaK system (Matsunaga et al. 1997). The crystal structure of the constitutively monomeric mutant protein of RepE in complex with its iteron DNA suggested that large conformational changes are necessary for the dimer–monomer interconvertion explaining the necessity of Hsp70 action (Komori et al. 1999; Sharma et al. 2004).

The involvement of the Hsp70 system in genome replication has also been demonstrated for eukaryotic viruses. The binding of the human papillomavirus-11 (HPV) DNA helicase E1 to DNA is enhanced by Hsp70, Hdj1, and Hdj2, whereby the action of Hsp70 is ATP dependent (Liu et al. 1998). Hdj2 stabilizes E1 in a dihexameric state on supercoiled and relaxed DNA. In the presence of topoisomerase I and single-stranded DNA-binding protein E1 unwinds supercoiled DNA. This unwinding reaction, however, was independent of an origin of replication. The HPV origin binding protein E2, which is essential for the formation of the pre-initiation complex at the origin of replication but dispensable for the elongation reaction, binds with high affinity to E1 and inhibits the unwinding reaction. This inhibition is abrogated by the Hsp70 chaperone system (Lin et al. 2002). Together these data suggest the following model. The E2 protein recognizes the origin of replication of the HPV genome and tethers the E1 helicase to the origin DNA. The Hsp70 chaperone machinery enhances this assembly process significantly, maybe by remodeling the hexameric, ring-shaped E1 to allow efficient DNA threading. The Hdj-proteins seem to stabilize a dihexameric form of E1 probably in preparation for bidirectional unwinding. Finally, Hsp70 and Hdj-proteins remove the E2 protein in a reaction similar to the *E. coli* DnaK machinery removing the λP protein from the pre-initiation complex. Consequently, the block on the stalled complex is lifted allowing the start of DNA unwinding by E1 and the action of DNA primase (Lin et al. 2002).

The Hsp70 chaperone machinery also seems to be essential for replication of the SV40 genome. The SV40 TAg is a multifunctional protein that among other functions acts as replication initiator protein and DNA helicase. It contains at its N terminus the signature motif for JDPs, the J-domain (Fig. 4). Many point mutations or deletions within this domain lead to a reduction or even loss of helicase function (Campbell et al. 1997; Li et al. 2001; Weisshart et al. 1996). SV40 proteins with a deletion of the N-terminal J-domain does not assemble into the correct hexameric structure and exhibits a lower affinity for the SV40 origin of replication (Weisshart et al. 1996). The J-domain also seems to contribute to the interaction with DNA polymerase α-primase (Dornreiter et al. 1990).

The DNA-replication of HSV is also affected by Hsp70 chaperones. The affinity of the dimeric replication initiator protein UL9 to oriS, one of the viral origins of replication, is greatly stimulated by Hsp70 and Hdj1 (Tanguy Le Gac and Boehmer 2002). This effect is largely due to an increase in association rate whereas the dissociation rate is seemingly unaltered. In contrast, the Hsp70 chaperone team does not affect the affinity of UL9 to single-stranded DNA as well as the helicase activity. Interestingly, the monomeric C-terminal origin-binding domain of UL9 binds independent of Hsp70 or Hdj1 to oriS with even higher affinity (Tanguy Le Gac and Boehmer 2002). One possible interpretation of these results is that Hsp70 monomerizes UL9 similar to the effect of the *E. coli* DnaK/DnaJ/GrpE system on the replication initiator proteins of P1 phage, RepA, and of the F-plasmid, RepE, thereby enhancing the affinity and specificity of UL9 for oriS. Alternatively, Hsp70 could assist a conformational transition of UL9 that would increase the accessibility of the origin-binding site of UL9 in its C-terminal domain and thereby increase the affinity of dimeric UL9 for oriS. Since the strand-opening reaction is strictly dependent on the amount of UL9 bound to its high and low-affinity sites flanking the central A/T-rich region of oriS, Hsp70 increases with the UL9 occupancy on oriS the efficiency of DNA replication in HSV.

In canine distemper virus (CDV, *Paramyxoviridae*) the replication of the negative strand RNA occurs in a ribonucleocapsid particle containing the virus-encoded proteins N

and P, the major core protein and the RNA-dependent RNA polymerase. The observation that the induction of the stress response promoted cytopathic effects of CDV infection and an association of Hsp70 proteins with the nucleocapsid particles suggested a possible contribution of Hsp70 proteins to viral replication (Oglesbee and Krakowka 1993; Oglesbee et al. 1990). Isolation of nucleocapsid particles from stressed and unstressed cells under conditions of ATP depletion demonstrated that Hsp70 and Hsc70 are associated under normal as well as under stress conditions. Furthermore, antibodies against Hsp70 reduced the RNA polymerase activity associated with the nucleocapsid particles, while addition of purified Hsp70/Hsc70 proteins increased the polymerase activity (Oglesbee et al. 1996). Isolation of nucleocapsid particles in the presence of ATP, which led to a depletion of Hsp70 proteins, yielded particle devoid of polymerase activity. These data clearly demonstrate the importance of Hsp70 association with the nucleocapsid particles for viral genome replication and transcription. The mechanism of this interaction is still unknown. Two possible modes of action could be that the Hsp70 proteins interact and remodel the N protein to make the RNA accessible to the polymerase, or they could directly chaperone the polymerase and thereby enhance its activity.

It has also been reported that the reverse transcriptase of the retroid hepadnavirus depends on the Hsp70 folding machinery for activity (Beck and Nassal 2001; Hu et al. 1997). In this case cellular folding processes are used that are normally involved in the control of stability and activity of regulatory proteins such as receptors and protein kinases involved in signal transduction, cell cycle regulation, and apoptosis. In addition to JDP and Hsp70 proteins, these processes involve the Hsp90 chaperones and an increasing number of co-chaperones including Hop, Hip, p23, $p50^{cdc37}$, and immunophilines (Pearl and Prodromou 2002; Pratt and Toft 2003; Richter and Buchner 2001; Young et al. 2001). The current model of the action of this chaperone machine is derived from the assembly reaction of steroid hormone receptors. The reaction cycle starts with the interaction of Hdj1 and Hsp70 with the so-called client protein cotranslationally or directly after de novo synthesis. The TPR-containing protein Hop assembles Hsp70, Hsp90 and the client in an early complex. p23 and immunophilins replace Hop and Hsp70 to yield the mature complex which dissociates with a half-life of approximately 5 min (Smith 2000). Hdj1 and Hsp70 can rebind the released client to restart the cycle. It is believed that the chaperone activity of Hsp70 brings the client into a certain conformation in which it is captured by the clamp mechanism of Hsp90. In this conformation the client is inactive but rapidly activatable by protein modifications such as phosphorylation or by binding to a ligand or a partner protein. After the initial observation that Hsp90 is associated with the reverse transcriptase of the duck hepatitis B virus (HBV) (Hu and Seeger 1996), a number of in vitro reconstitution experiments demonstrated that the activation of the reverse transcriptase follows the general scheme of the activation of cellular regulatory proteins (Beck and Nassal 2001, 2003; Gyoo Park et al. 2002; Hu and Anselmo 2000; Hu et al. 1997, 2002). Only after interaction with Hdj1, Hsp70, Hop, and Hsp90 in an ATP dependent reaction, is the reverse transcriptase able to assemble with the ϵRNA located near the 5′ end of the pregenome as a template and with the core protein into a nucleocapsid, where DNA synthesis commences with the protein priming reaction and the template switch from the 5′ to the 3′ end (Bartenschlager et al. 1990; Bartenschlager and Schaller 1992; Pollack and Ganem 1994; Tavis et al. 1994; Wang and Seeger 1992, 1993). The co-chaperone p23 increases the efficiency of the process. A more recent study demonstrating that Hdj1 and Hsp70 alone are able to activate the reverse transcriptase supports the notion that Hsp70 transforms the re-

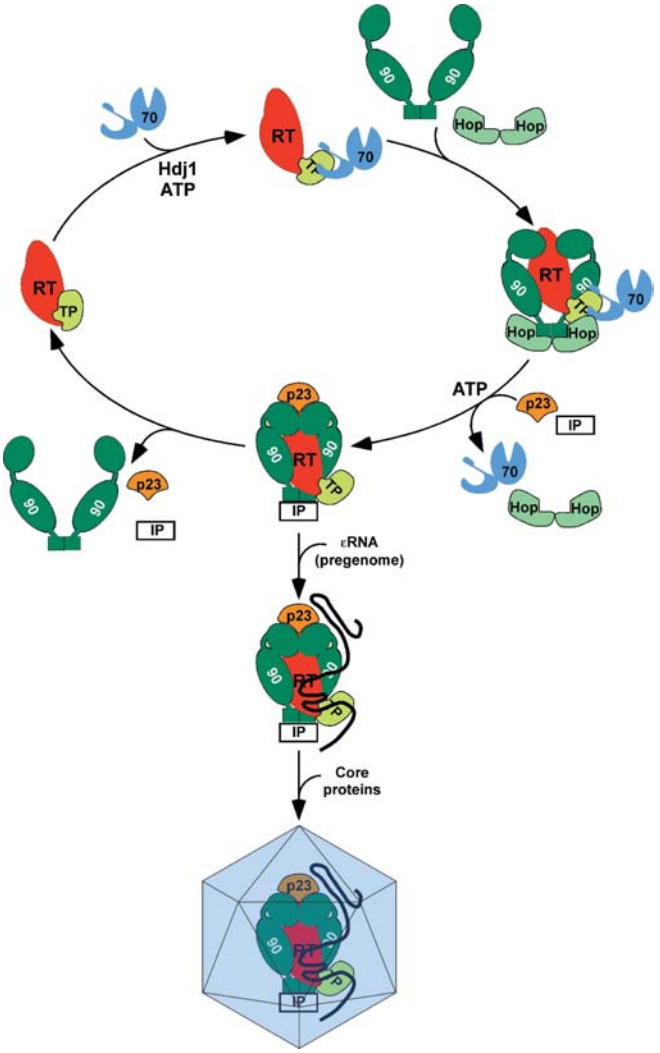

Fig. 5 Activation of the reverse transcriptase/DNA polymerase of hepadnavirus. The activation combines the model for the activation of steroid hormone receptor by Hsp90, the Hsp90 ATPase cycle with proposed conformational changes in Hsp90 and the activation model from Hu et al. (Hu et al. 1997; Mayer et al. 2002; Pearl and Prodromou 2002; Pratt 1997; Richter and Buchner 2001; Smith 2000; Young et al. 2001). *RT*, Hepadnavirus P protein reverse transcriptase/DNA polymerase; *TP*, N-terminal domain of the P protein; *70*, Hsp70; 90, Hsp90 with N-terminal ATPase domain, middle domain and C-terminal domain; *IP*, immunophilins. The interaction sites of the Hsp70 and Hsp90 are not known and therefore arbitrary

verse transcriptase by a modulation of its conformation into the active state in which it is stabilized by Hsp90 and co-chaperones and which allows association with the RNA and the core proteins to form the reverse transcription-competent nucleocapsid particle (Beck and Nassal 2003) (summarized in Fig. 5). Finally, Hsp70 and Hsp90 are found incorporated in the released virus particles supporting the idea that the two chaperones stabilize the reverse transcriptase during transmission in the extracellular space and allow immediate

activation after reintroduction into a suitable host cell. Since Hsp70 is also found in other viruses including the retrovirus human immune deficiency virus (HIV) and non-retroviruses of the negative-strand RNA group (rabies virus, vesicular stomatitis virus, influenza A virus, etc.) in similar amounts as the polymerase it is possible that the chaperones assist reverse transcription and RNA-dependent RNA polymerase reactions in other viruses as well (Gurer et al. 2002; Sagara and Kawai 1992).

In summary, Hsp70s are involved in viral genome replication by monomerization of initiator proteins, assisting assembly and disassembly of preinitiation complexes, and by stabilizing and activating helicases and polymerases.

Viral gene expression

Most viruses exploit the cellular transcription and translation machineries for the expression of their genes and therefore recruit initiation and elongation factors. Since some of the involved host factors interact with components of the Hsp70 system, the chaperone system is also important for this stage of the viral life cycle. Several transcription initiation factors interact physically with the Hsp70 co-chaperone Bag1 in vitro and Bag1 stimulates general transcription activity in vitro and when overexpressed in vivo in an Hsp70-dependent manner (Niyaz et al. 2003; Niyaz et al. 2001; Zeiner et al. 1999). Such a stimulation of transcription was also observed when viral promoters were used in reporter gene constructs with promoters of the human polyomavirus JCV and HCMV (Devireddy et al. 2000; Takahashi et al. 2001). The molecular mechanism of the general transcriptional activation, however, is still unclear.

As discussed above, Bag1 stimulates ADP dissociation from Hsp70 proteins. In the presence of physiological ATP concentrations, ATP rebinds rapidly inducing the conformational change in Hsp70 and Hsc70 that leads to substrate release (Gässler et al. 2001; Höhfeld and Jentsch 1997; Sondermann et al. 2001). Taking these properties into account it is possible that Bag1, tethered to DNA by its own unspecific DNA binding activity and/or by interaction with a transcription initiation factor, assists the assembly of transcription initiation complexes by triggering the release of Hsc70-bound transcription factors at the site of the promoter. Alternatively or in addition, Bag1–Hsc70 interaction could remodel initiation complexes thereby stimulating the promoter clearance of the RNA polymerase.

A different way of stimulating virus specific transcription is used by HIV-1. The Tat protein specifically binds to the TAR stem–loop structure at the 5’ end of the nascent viral transcript and activates HIV-1 transcription by enhancing the processivity of RNA polymerase (Cullen 1998; Jones 1997). This activation is mediated by the human transcription elongation factor P-TEFb, which directly interacts with Tat and phosphorylates the C-terminal tail of RNA polymerase II (Chen et al. 1999). The protein kinase subunit of P-TEFb is the cyclin-dependent protein kinase Cdk9 which is a Hsp70 and Hsp90 client. Interference with the Hsp70–Hsp90 chaperones, for example using the Hsp90 inhibitor geldanamycin, prevents the formation of the functional Cdk9·cyclin T1 complex and the transcriptional stimulation by Tat (O’Keeffe et al. 2000).

A common problem for gene expression of RNA viruses is that double-stranded RNA intermediates of their replication and gene expression induce the protein kinase PKR (protein kinase-RNA-activated) which shuts down translation by phosphorylating the translation initiation factor eIF2α (Galabru and Hovanessian 1987; Gale et al. 1998). PKR is also induced by interferon and as such it is part of the host defense strategy against viral infec-

tions. To circumvent the PKR-mediated block to viral proliferation influenza A virus induces the cellular TPR-domains containing JDP $p58^{IPK}$, which down-regulate PKR in an Hsp70-dependent manner (Lee et al. 1990, 1992, 1994; Tang et al. 1996; Melville et al. 1997, 1999). In uninfected, unstressed cells $p58^{IPK}$ forms a complex with Hdj1 which is proposed to be the inactive form of $p58^{IPK}$ (Melville et al. 1997, 1999). During influenza A virus infection the amount of Hdj1 that co-precipitates with $p58^{IPK}$ first increases about twofold and then decreases to zero. The Hsp70-mediated dissociation of the $p58^{IPK}$-Hdj1 complex is suggested to lead to an activation of $p58^{IPK}$ allowing the interaction, monomerization and consequently inhibition of PKR (Melville et al. 1999). The exact mechanism of this process, in particular why two J-domain containing proteins are involved is not clear.

In summary, Hsp70 can be involved in viral gene expression at the level of transcription initiation and transcription elongation. In addition, Hsp70 is instumentalized by viruses to circumvent the general translation block induced by double-stranded RNA and interferon.

Morphogenesis

There is ample circumstantial evidence, based on "guilt by association", that Hsp70 systems may also be involved in viral morphogenesis assisting folding of capsid monomers, assembly of nucleocapsids, and facilitating folding of cytoplasmic of luminal domains of envelope proteins (Choukhi et al. 1998; Liberman et al. 1999; Macejak and Luftig 1991). Howerver, conclusive evidence that these interactions lead to higher yields of properly folded capsids or envelope proteins and more efficient virion assembly is still missing for most of the investigated viral model systems. A few more conclusive examples are detailed here. Hsp70 was shown to interact with the capsid proteins VP1, VP2, and VP3 of polyomavirus. Expression of these proteins in a variety of systems including A31 mouse fibroblasts, reticulocyte lysate, Sf9 insect cells, and *E. coli* leads to the formation of an ATP-sensitive complex with Hsp70 proteins. During infection the capsid protein–Hsp70 complex is first detected in the cytoplasm and subsequently imported into the nucleus. These observations prompted the speculation that Hsp70 assists folding of the capsid proteins to an assembly competent state but prevents premature virion assembly until translocation into the nucleus and genome replication have been completed (Cripe et al. 1995). Chromy et al. demonstrated that purified VP1 and VP3 assembles in vitro into polymorphic higher oligomeric structures upon addition of unphysiological concentrations of Ca^{2+} (0.5 mM), while the addition of the prokaryotic DnaK or mammalian Hsc70, which bound to the C terminus of VP1, inhibited the Ca^{2+} induced assembly. In contrast, the addition of the complete prokaryotic DnaK, DnaJ, GrpE chaperone team assembled VP1 and VP3 into virion-like structures in an ATP-dependent but Ca^{2+}-independent process. The mammalian Hsc70 could also assemble correct icosahedral virion particles in an ATP-dependent process when the SV40 large T antigen with a functional J-domain was present as its JDP partner (Chromy et al. 2003).

In the positive-stranded RNA closteroviruses Hsp70, which in this case is virus encoded as discussed in detail below, plays a different role in the assembly of the helical symmetric capsid. Genetic analysis demonstrated that deletion of the viral Hsp70, or mutations that abrogated its ATPase activity, dramatically reduced the formation of full-length virions (Satyanarayana et al. 2000). In a biochemical analysis of the filamentous virion

particles the Hsp70 protein was found to be a component of the virion together with the major and the minor coat proteins (CP, CPm) and a fourth protein called p61 (Citrus tristeza virus, CTV) or p64 (Beet yellow virus) (Napuli et al. 2000, 2003; Satyanarayana et al. 2000). A more detailed analysis using a minimal CTV replicon, which contained only the gene encoding CPm with or without the genes encoding Hsp70 and p61, revealed that CPm starts incapsidation of the RNA at a 5′ nontranslated region, which previously was shown to be essential for virus replication (Gowda et al. 2003), and covers the RNA to different extents. When Hsp70 and p61 are present, incapsidation by CPm is restricted to about 630 nucleotides of the 5′ end consistent with the observation that in wild-type virions only a short tail is covered with CPm while the majority of the 20 kb RNA genome is covered with CP (Satyanarayana et al. 2004). Hsp70 therefore seems to be important for a coordinated incapsidation of the RNA. The mechanism of this process and whether Hsp70 has additional functions in the coat assembly reaction is unknown. It is also unclear why in contrast to other helical viruses like TMV two different coat proteins are necessary for the formation of this filamentous helical structure. One hypothesis is that CP forms a more stable coat around most of the RNA to protect the genome during the transition outside the plant cells, while encapsidation by CPm is less stable to allow efficient disassembly after reentry into a host cell. Thereby the origin of replication becomes accessible for translation and replication, two processes which could aid complete uncoating. The incorporation of Hsp70 into the CPm coated part of the capsid could stabilize CPm especially during extracellular transition where ATP is absent and Hsp70 release is slow.

During morphogenesis of the double-stranded RNA reovirus cellular Hsp70 assists the assembly of the trimeric lollipop-shaped sigma 1 protein that is responsible for the interaction with the host cell receptor. While the N-terminal filamentous part of the sigma 1 protein folds and trimerizes cotranslationally in a Hsp70-independent manner, the C-terminal globular domain folds post-translationally Hsp70-dependently. In this process Hsp70 binds already cotranslationally to a protein segment located downstream of the N-terminal triple α helical coiled-coil presumably inhibiting unwanted interactions and misfolding. After release from the ribosome trimerization of the C-terminal domain is coupled to ATP-mediated release of Hsp70 (Leone et al. 1996).

For the envelope proteins of a number of viruses including Sindbis virus, VSV, influenza A virus and HIV a transient interaction with the ER resident Hsp70 chaperone BiP/Grp78 was demonstrated (Carleton and Brown 1996; de Silva et al. 1990, 1993; Earl et al. 1991; Hammond and Helenius 1994; Hogue and Nayak 1992; Machamer et al. 1990; Mulvey and Brown 1995; Otteken et al. 1996; Singh et al. 1990). This interaction was prolonged when the folding of the protein or its assembly into an oligomeric structure was delayed by ATP depletion, prevention of disulfide bond formation, inhibition of glycosylation, temperature sensitive mutations at nonpermissive temperatures, and missing or misfolded oligomerization partners. These data strongly suggest that BiP plays an integral part of the folding of viral envelope proteins.

The cytoplasmic Hsp70 chaperone, however, can also be involved as demonstrated for the HBV glycoprotein L (Lambert and Prange 2003; Löffler-Mary et al. 1997; Prange et al. 1999). Hsc70, in conjunction with its J-domain co-chaperone Hdj1 and regulated in an antagonistic fashion by the co-chaperones Hip and Bag1, plays a functional role in the topogenesis of the L protein. The L protein is a polytopic membrane protein with initially three transmembrane helices which are inserted cotranslationally into the ER membrane in a topology where the N terminus is cytoplasmic and the C terminus is luminal. Post-trans-

lationally the N terminus is translocated into the ER lumen in approximately 50% of the molecules resulting in a mixed topology that is preserved during virus maturation allowing the N terminus to perform dual functions as nucleocapsid matrix protein and in receptor recognition (Bruss and Vieluf 1995; Le Seyec et al. 1999). Hsc70 binds to the N terminus and assists and regulates its translocation as evidenced by the following observations. Deletion of the Hsc70 binding site leads to a cotranslational translocation of the N terminus and a uniform topology (Löffler-Mary et al. 1997). Increasing intracellular Bag1 levels significantly enhance post-translational translocation of the N terminus consistent with its role as substrate release factor (see above; Gässler et al. 2001). In contrast, overproduction of Hip reduced post-translational translocation consistent with its proposed function as antagonist of Bag1 (Kanelakis et al. 2000). Together these data clearly show that the Hsp70 chaperone system regulates the mixed topology of the HBV envelope protein (Lambert and Prange 2003).

Taken together, Hsp70 assists folding and maturation of capsid and envelope proteins as well as the multimeric assembly reactions of subunits or entire virions.

Transformation

Viruses which do not provide their own polymerase are dependent on the host replication machinery. In order to proliferate in quiescent cells the virus has to reinitiate the cell cycle thereby transforming the cell. A number of mechanisms have evolved enabling viruses to overcome the restriction points of the cell cycle. The best investigated example demonstrating the involvement of Hsp70 systems is the DNA tumor virus SV40, the prototype of *Polyomaviridae* family, which also include the human BK and JC tumor viruses. Central to the transforming ability of SV40 virus are the large and small T antigen (TAg), both of which contain the signature motif of an Hsp70 co-chaperone, the J-domain, at their N terminus. Mutations within the J-domain that affect the functional interaction of the TAg with Hsp70s have been demonsrated to obliterate the ability of TAg to transform mammalian cells (Srinivasan et al. 1997) reviewed recently in full depth by Sullivan and Pipas (Sullivan and Pipas 2002). Among other functions the SV40 TAg sequesters the retinoblastoma family gene products pRb, p107 and p130 and liberates members of the E2F family of transcription factors in an Hsc70 and ATP hydrolysis-dependent manner (Sullivan et al. 2000a, 2001). The free E2F proteins subsequently trigger the expression of the S-phase genes leading to DNA replication (Dyson 1998). The most likely mechanism involves the following steps. First, the large TAg binds to the pRB–E2F complex. Second, Hsc70 in its ATP-bound form with high substrate association rates associates with the pRB–E2F–TAg complex. Third, the J-domain of TAg stimulates ATP hydrolysis by Hsc70 triggering the transition to the high-affinity conformation of Hsc70's substrate-binding domain thereby trapping the substrate protein, either pRB or E2F. Forth, Hsc70 induces a conformational change in the substrate protein leading to the dissociation of pRB and E2F, whereby pRB is still in complex with TAg and Hsc70 is bound to either pRB or E2F. ADP dissociation and rebinding of ATP to Hsc70 leads to the dissociation of the Hsc70–substrate complex resulting in free E2F and the pRB–TAg complex. The pRB–TAg complex may decay with a certain half-life spontaneously or induced through an ATPase driven interaction cycle with Hsc70 liberating TAg for other tasks. The fact that Hsc70 binds to TAg itself in an ATP-dependent manner is not surprising and also observed by other JDPs (e.g. Laufen et al. 1999; Mayer et al. 1999; Wittung-Stafshede et al.

2003). The J-domain signals to Hsp70 proteins the close proximity of a substrate. For lack of a substrate bound to the JDP Hsp70 binds to the JDP itself (compare Schirmbeck et al. 2002).

A second task of the TAg, the transactivation of E2F promoters independent of the disruption of the pRB–E2F complex also involves the J-domain and consequently a Hsp70 protein (Chao et al. 2000; Harris et al. 1998; Sheng et al. 1997; Sheng et al. 2000; Zalvide et al. 1998). A possible mechanism could involve the Hsc70 assisted assembly of a transcription initiation complex on the respective promoters similar to the role of Hsc70 in the Bag1 stimulated transcription discussed above. Alternatively, TAg could induce Hsc70 to disassemble an inhibitory silencer complex or to assist in remodeling the chromatin.

Although no clear indication was found so far for a participation of the J-domain of TAg in the interaction with p53 and ensuing inactivation of p53 which contributes to cell transformation and prevention of apoptosis, it should be mentioned that p53 interacts with the Hsp70–Hsp90 chaperone machinery (King et al. 2001; Zylicz et al. 2001) and an Hsp70-mediated transfer of p53 into a complex with TAg is possible. A JDP involved in this process may be TPR2 instead of TAg because it was suggested that TPR2 mediates the retrograde transfer of an Hsp90 bound client onto Hsp70 (Brychzy et al. 2003).

HPV and AdVs have similar transforming activities using the proteins E7 (HPV) and E1A (AdV) to disrupt the complexes between pRB family proteins and E2F family proteins. Although neither E7 nor E1A contains a J-domain both proteins could transform cells in a way similar to that described for SV40 TAg. E7 interacts with the JDP hTid-1, the homolog of the *Drosophila* protein Tid56, which acts as a tumor suppressor (Schilling et al. 1998). The C terminus of E7, which mediates the interaction with hTid-1, has also been shown to be essential for the physical disruption of the pRB–E2F complex albeit it is not necessary for direct interaction with pRB (Patrick et al. 1994; Wu et al. 1993). These observations suggest that the interaction with hTid-1 is involved in the disruption of the pRB–E2F complex providing E7 with the J-domain necessary to recruit Hsc70 for the complex dissociation in analogy to the function of SV40 TAg. Alternatively, the binding of E7 to hTid-1 could transform cells through an inhibition of the assumed tumor suppressor function of hTid-1. E1A, on the other side, directly interacts with Hsc70 (White et al. 1988) and could use Hsc70 in this way to disrupt the pRB–E2F complex. However, in addition to Hsc70 a JDP would be necessary for efficient stimulation of Hsc70's ATPase activity.

In conclusion it can be said that most double-stranded DNA viruses depend on Hsp70 chaperones for reprogramming of the host cells to reenter into the cell cycle. The dependency of cell transformation on Hsp70 chaperones is also observed in many tumor cells.

Cell survival and apoptosis

Hsp70 systems are essential for the survival under stress conditions. Therefore, the induction of Hsp70 protein production may serve an additional purpose for the virus, namely, the prevention or delay of host cell death until the progeny is ready to leave. It is therefore not surprising that interference with apoptotic signal transduction pathways in both directions–inhibiting and activating–is a quite common phenomenon accompanying viral infections (reviewed in Benedict et al. 2002; Hardwick 2001; Roulston et al. 1999). The induction of cell death during viral infection can have different reasons. First, many DNA viruses replicating in quiescent cells need to induce reentry into the cell cycle by providing

proliferation signals. Such untimely proliferation signals very often induce apoptosis as first line of cellular defense against viruses as well as against tumor growth (Debbas and White 1993; Fromm et al. 1994; Lowe and Ruley 1993; Symonds et al. 1994). Second, many different kinds of stress induce apoptosis and viral infection may impose such a lethal stress. Third, viral infection of a cell can induce the release of interferons triggering apoptosis (Castelli et al. 1997; Diaz-Guerra et al. 1997; Rivas et al. 1998). It is self-evident that viruses have evolved means to interfere with the cell death response because apoptosis is detrimental for viral proliferation during the early phase of viral life cycle. In contrast, when the viral particles are ready assembled apoptosis can be advantageous for virus spreading (Anderson 2001; Best et al. 2002; Goh et al. 2001; Henke et al. 2000, 2001; Muthumani et al. 2002a, 2002b; Schultz-Cherry et al. 2001). If virions are incorporated into apoptotic bodies which are engulfed by neighboring phagocytic cells, viruses can infect new cells without the danger of being exposed to the host's immune system (Fazakerley 2001; Fazakerley and Allsopp 2001).

It has been shown that Hsp70 plays an important role in apoptosis. Hsp70 interferes with the signal transduction pathways leading to programmed cell death at several levels including inhibition of (1) the apoptosis inducing Jun N-terminal kinase, (2) cytocrome C release out of mitochondria, (3) apoptosome assembly by interaction with Apaf-1, and (4) execution of apoptosis acting even downstream of caspase 3 (Beere and Green 2001; Beere et al. 2000; Gabai et al. 1997, 1998; Jäättelä et al. 1998; Jolly and Morimoto 2000; Li et al. 2000; Mosser et al. 1997, 2000; Ravagnan et al. 2001; Saleh et al. 2000; Yaglom et al. 1999). In addition, Jäättelä and coworkers demonstrated that depletion of Hsp70 in *hsp70* overexpressing tumor cells leads to an induction of apoptosis (Nylandsted et al. 2000). It is therefore conceivable that upregulation of *hsp70* expression may also serve as a tool to prevent premature apoptotic cell death, and the precipitous decrease of *hsp70* mRNA levels at the end of AdV infections may lead to the timed induction of apoptosis.

In this context it is also interesting that a decrease of the BiP concentration in tumor cell lines inhibits tumor progression and eliminates resistance to T-cell mediated cytotoxicity (Jamora et al. 1996; Sugawara et al. 1993). In analogy, viruses may evade T-cell mediated induction of apoptosis by upregulating the concentration of the ER resident Hsp70 homolog.

Virus-encoded components of the Hsp70 system

Not all viruses seem to be content with inducing the expression of the genes encoding the components of the Hsp70 system and then compete with cellular substrates for their chaperone power. There are examples of viruses that bring with them their own components of the Hsp70 system.

Viral J-domain containing proteins

The multifunctional large and small TAg of the simian and human DNA tumor viruses SV40, JC and BK virus, contain an N-terminal J-domain that functionally interacts with Hsp70 chaperones acting like a bona fide J-domain (Campbell et al. 1997; Genevaux et al. 2003; Kelley and Georgopoulos 1997; Kelley and Landry 1994). This J-domain induces Hsc70 binding to the TAg in an ATP-dependent manner (Sullivan et al. 2001). The TAg

are involved in almost all viral activities, including genome replication, regulation of transcription of viral and cellular genes, virion assembly, and cell transformation and many of these functions are abrogated by mutations in the J-domain (DeCaprio 1999; Kelley 1999). It is therefore assumed that its mode of action is based on the targeting of the Hsp70 chaperone machine to certain protein complexes inducing remodeling and change of activity of these complexes (for review see Sullivan and Pipas 2002).

The MC013L protein of the DNA poxvirus causing molluscum contagiosum also contains a J-domain (Moratilla et al. 1997). This protein interacts with the glucocorticoid and vitamin D receptors and downregulates their transcriptional activity, possibly by interfering with the Hsp70–Hsp90 chaperone machine (Chen et al. 2000).

The need for a specialized chaperone to assist folding and assembly of viral and host proteins is reminiscent of the T4-like phages that encode an Hsp60 co-chaperone in order to ensure the proper folding of their capsid protein (for a review see Ang et al. 2000).

The question arises why the polyoma- and poxviruses bring their own JDP along. Two alternative hypotheses are possible. First, the viral J-domain has a unique property that is not present in cellular JDPs. Second, the fusion of a J-domain to a viral protein could be more beneficial for the protein's function than the recruitment of a host JDP would be. Comparing the NMR structures of the J-domain of murin TAg and human Hdj1 reveals clear differences, in particular, when the electrostatic potential at the surface is analyzed (Fig. 4). In addition, many of the residues corresponding to *E. coli* DnaJ residues which are important for interaction with DnaJ's Hsp70 partner DnaK are conserved in Hdj1 but not in TAg (Fig. 4) (Genevaux et al. 2002; Greene et al. 1998). Nevertheless, the J-domains of SV40, JC and BK TAg can substitute for the J-domain of DnaJ in supporting Hsp70 function in *E. coli* (Genevaux et al. 2003; Kelley and Georgopoulos 1997). To address the question of specific effects of the TAg J-domain in more detail, domain-swap experiments were carried out and TAg function was investigated. The J-domain of SV40 TAg was replaced by two different J-domains of cellular JDPs without loss of functionality as measured by the ability to promote viral DNA replication (Campbell et al. 1997), interaction with pRB family proteins, and in vivo viral proliferation (Stubdal et al. 1997). These results clearly suggest that the viral J-domain functions as a normal J-domain without any specific properties. On the other hand, the J-domain of polyomaviruses contains in helix 1 a highly conserved ^{13}LXXLLXL^{19} motif that is not conserved in other J-domains. Mutational alteration of this sequence reduced viral DNA replication 100-fold while the effects on pRB, including release and activation of E2F, were unimpaired (Li et al. 2001). Magnusson and coworkers suggest from these data that the sequence of the viral J-domain may have, in some contexts, a specific function. However, of the three substitutions tested (L13M, L13I, L13V) only L13V had a phenotype. Given the fact that the consensus residue in other JDPs is the large hydrophobic amino acid tyrosine it is likely that a minimum size is necessary to stabilize the hydrophobic core between helix 1, 2, and 3 and valine is just too small, leading to a destabilization of helix 1 or the entire J-domain. This interpretation is supported by the fact that the small TAg with this mutation is unstable.

Taken together, all available evidence indicates that the evolutionary fusion of a J-domain to the viral protein increased the functionality of this protein by the more efficient recruitment of the Hsp70 chaperones as compared to other viral proteins that interact with host JDPs.

Virus-encoded Hsp70 proteins

The plant pathogenic closteroviruses, including beet yellow virus, lettuce infectious yellow virus, and CTV, went one step further and encode for their own proper Hsp70 chaperone. This Hsp70 protein is essential for cell-to-cell transmission (Agranovsky et al. 1998; Alzhanova et al. 2000, 2001; Peremyslov et al. 1999) and has been implicated in virion assembly as discussed above (Napuli et al. 2003; Napuli et al. 2000; Satyanarayana et al. 2000, 2004). In addition to its function in virion assembly, the viral Hsp70 also binds to microtubules and is found associated with the plasmodesmata, the open cytoplasmic cell-to-cell contacts, in closterovirus infected plant cells (Karasev et al. 1992; Medina et al. 1999) and interacts with a long-distance transport factor (Prokhnevsky et al. 2002). It is therefore possible that it has supplementary functions in viral transmission.

Similar to the situation with the virus-encoded JDPs, there are two possible explanations why closteroviruses encode for their own Hsp70 protein. First, coding for and regulating the production of a proper Hsp70 chaperone could supplement the viral need for chaperone power and even make the virus independent of the host Hsp70s. Second, the virus-encoded Hsp70 could have specific properties of the ATPase cycle and substrate specificity/affinity which are necessary for the folding of specific viral and host substrates but not supplied by the cellular Hsp70 proteins. Since it has not yet been attempted to complement the loss of function of the viral Hsp70 by the overproduction of a host Hsp70 protein, and the viral Hsp70s are not yet biochemically characterized, it is not possible to distinguish the two alternative hypotheses at present. However, in the unrelated tomato spotted wilt virus, a negative-stranded RNA virus of the *Bunyaviridae* family, the viral movement protein binds to the viral nucleocapsid and a plant JDP indicating the recruitment of a host Hsp70 for the cell-to-cell movement process (Soellick et al. 2000). This observation demonstrates that the plant virus specific process of cell-to-cell movement as such does not require a specialized Hsp70 chaperone. Furthermore, the fact that the Hsp70 proteins of the different members of the *Closteroviridae* family are not highly conserved (7.6% identity, 25% similarity in a multiple sequence alignment; see Fig. 2) and only slightly closer related among each other than to human Hsc70 (87% of the residues completely conserved within the *Closteroviridae* Hsp70s are also found in human Hsc70) does not support the hypothesis of an adaptation to a specific folding task. However, the co-evolution of the viral Hsp70 with a specific viral protein such as the small capsid protein would not necessarily be apparent in sequence identity among the different *Closteroviridae* Hsp70s. Furthermore, all viral-encoded Hsp70s are on average 82 residues shorter than the eukaryotic cytosolic Hsp70 proteins. Since the viral Hsp70s remain incorporated in the nucleocapsid (about 10 molecules per capsid; Napuli et al. 2000), the size of the protein may be an issue.

Taken together, although the currently available evidence provides no indication for an adaptation to a specific folding task, co-evolution with a specific substrate (capsid protein) and the requirement to fit into the nucleocapsid cannot be excluded as a driving force for the evolution of a virus-encoded Hsp70 protein.

Evolutionary aspects of the virus–Hsp70 interaction

Many mutational alterations lead to a destabilization of proteins and increase their tendency to misfold and aggregate. Since chaperones recognize misfolded proteins, prevent their aggregation and promote their refolding into the native state, chaperones can keep a mutationally altered protein functional and thereby buffer detrimental mutations. Such a function as evolutionary capacitor was shown for the Hsp90 chaperone (Queitsch et al. 2002; Rutherford and Lindquist 1998; Sollars et al. 2003). Rutherford and Lindquist demonstrated that the reduction of Hsp90 activity in *Drosophila melanogaster* by genetic or pharmacological means leads to the appearance of strain specific morphological alterations suggesting that Hsp90 preserves the natural function of certain morphogenetic factors that in its absence would not be active or would have a different activity. Continuous selection for a given trait under low Hsp90 activity conditions (mutant Hsp90, presence of an Hsp90 inhibitor or stress) could make the trait independent of Hsp90 activity. Similarly, Queitsch and Lindquist showed that plasticity in plants is also Hsp90 dependent. In principle, a similar function could also be performed by Hsp70 chaperones. Like Hsp90, Hsp70s are involved in chaperoning signaling molecules such as transcription factors and protein kinases, which constitute some of the morphogenetic factors. Since Hsp70, but not Hsp90, can refold denatured proteins Hsp70s could buffer many destabilizing alterations in structual proteins.

RNA replication is a very error-prone process with a rate of 1 misincorporation per 10^4 replicated bases. In the absence of repair mechanisms this means that the progeny viruses differ from each other and from the parent, a fact that is expressed in the term ‘quasi-species’ for RNA viruses because they are defined by a sequence space and not by a unique sequence found in the majority of the species’ members. The virus-encoded Hsp70 proteins are an impressive example for the accumulation of mutational alterations. While Hsp70s belong to the most conserved proteins with about 50% sequence identity between *E. coli* and man, the sequence variations found within the virus-encoded Hsp70s is so high that only 7.6% of the residues remained unchanged within the entire family (see Fig. 2). It therefore is very likely that destabilizing mutations occur frequently in viral genes increasing the likelihood for chaperone dependency of the encoded proteins. The minimal number of fully functional progeny required to give a sufficiently high chance for a successful new infection defines the mutational window for the virus species which is widened by the action of Hsp70 chaperones. Under certain circumstances Hsp70 chaperones may even be the prerequisite for an evolutionary survival of RNA viruses.

Concluding remarks

The ample evidence demonstrating the participation of Hsp70 chaperones at distinct steps in the life cycles of a variety of viruses suggests that Hsp70 involvement in virus proliferation is a widespread phenomenon. Since Hsp70 function is essential for eukaryotic cells and complete knockouts of the major *hsp70* genes are therefore not available, it is difficult to show in how many viral processes Hsp70s are involved and to rigorously demonstrate that the Hsp70 contribution to these processes is essential. The reduction of the Hsp70 levels through the use of gene knockdown technologies (siRNA) may give valuable indications in future studies. In addition, as more and more in vitro reconstitution systems for

the different steps of the viral life cycle become available, the mechanism of the Hsp70-mediated viral folding and assembly processes will be elucidated.

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