Biological activities of HAP46/BAG-1

The HAP46/BAG-1 protein: regulator of HSP70 chaperones, DNA-binding protein and stimulator of transcription

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HAP46/BAG-1M and its isoforms affect the protein-folding activities of mammalian HSP70 chaperones. They interact with the ATP-binding domain of HSP70 or HSC70, leaving the substrate-binding site available for further interactions. Trimeric complexes can therefore form with, for example, transcription factors. Moreover, HAP46/BAG-1M and the larger isoform HAP50/BAG-1L bind to DNA non-specifically and enhance transcription *in vitro* and upon overexpression in intact cells. These factors are linked to positive effects on cell proliferation and survival. This review focuses on DNA-binding activity and transcriptional stimulation by HAP46/BAG-1M, and presents a molecular model for the underlying mechanism. It is proposed that transcription factors are recruited into complexes with HAP46/BAG-1M or HAP50/BAG-1L through HSP70/HSC70 and that response-element-bound complexes that contain HAP46/BAG-1M and/or HAP50/BAG-1L along with HSP70s target and affect the basal transcription machinery.

Keywords: Bcl-2-associated athanogene BAG-1; DNA binding; HSP70/HSC70-associating protein HAP46; HSP70/HSC70 molecular chaperones; transcriptional stimulation

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Introduction

HAP46/BAG-1M was discovered independently by three groups who used expression screening to search for partners of the respective proteins that they were studying. Takayama *et al* (1995) screened a mouse embryo cDNA library with Bcl-2 and cloned a cDNA that encodes a 219-residue protein, which they called Bcl-2-associated athanogene 1 (BAG-1). The name 'athanogene' (from the Greek word *athánatos*, meaning 'against death') was chosen because transfected cells responded less drastically to apoptotic stimuli. Similar sequences were cloned by Bardelli *et al* (1996),

who screened the same library with the plasma-membrane receptor for hepatocyte growth factor. The third group used a human expression library and glucocorticoid receptors, and so discovered the 274-residue human protein (Zeiner & Gehring, 1995). This has an apparent molecular weight of 46 kDa and was first named receptor-associating protein RAP46, then later renamed HSP70/HSC70-associating protein of apparent molecular size 46 kDa (HAP46) (Zeiner *et al*, 1997) for the reasons discussed below. The gene is located on region p12 of human chromosome 9 (Takayama *et al*, 1996). It gives rise to a single transcript of ~1.4 kb (Zeiner & Gehring, 1995) and the promoter contains binding sites for several transcription factors that are important for expression (Yang *et al*, 1999).

The three sequences (Takayama *et al*, 1995; Zeiner & Gehring, 1995; Takayama *et al*, 1996) are homologous, with the exception of 55 additional amino acids that are present in the human protein. The mouse cDNAs have subsequently been extended so that their similarity covers almost the entire sequence (Packham *et al*, 1997; Takayama *et al*, 1998). Such homology was surprising considering that different target proteins had been used for the interaction screening. This became even more puzzling when additional proteins were found to interact with the HAP46/BAG-1 protein, such as other members of the nuclear-receptor superfamily (Zeiner & Gehring, 1995; Froesch *et al*, 1998; Liu *et al*, 1998), several unrelated transcription factors (Zeiner *et al*, 1997), the platelet-derived growth-factor receptor (Bardelli *et al*, 1996), the protein kinase RAF-1 (Song *et al*, 2001), the human homologue SIAH-1A of *Drosophila* Seven in absentia (Matsuzawa *et al*, 1998) and proteasomes (Lüders *et al*, 2000a).

A breakthrough came with the discovery that the heat-shock protein HSP70 and the constitutive HSC70 are important interacting partners of the protein (Gebauer *et al*, 1997; Höhfeld & Jentsch, 1997; Takayama *et al*, 1997; Zeiner *et al*, 1997). This interaction occurs with mammalian and insect HSP70s, but not with endoplasmic BIP or HSP70s that are of plant, yeast or bacterial origin (Zeiner *et al*, 1997). This is noteworthy because the bait proteins that were used for cloning were produced in the baculovirus system, which contains insect HSP70s, and therefore explains the large range of interacting partners of HAP46/BAG-1.

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HAP46/BAG-1 affects HSP70 activities

HSP70s are a prominent and highly conserved family of chaperones that are involved in many biological processes. They support the folding of newly synthesized and misfolded polypeptides, prevent protein aggregation, aid protein translocation across membranes (see, for example, Mayer *et al*, 2001) and also have a role in the assembly of multimeric proteins. A well-known example is the generation of heteromeric states of steroid hormone receptors (Mayer *et al*, 2001), but there are other instances of similar functions (Niyaz *et al*, 2003). HSP70s characteristically consist of two different parts: the ATP-binding domain and the substrate-binding domain. They cooperate closely and their three-dimensional structures are known (Mayer *et al*, 2001). The ATP domain consists of two roughly symmetrical subdomains, I and II, with a central cleft that harbours the nucleotide-binding site. Bound ATP is slowly hydrolysed and the resulting ADP is exchanged for new ATP. The substrate-binding domain has the ability to interact with an almost unlimited range of proteins (Mayer *et al*, 2001).

Importantly, the interaction of HAP46/BAG-1 with HSP70/HSC70 makes use of the ATP-binding domain rather than the substrate-binding domain (Gebauer *et al*, 1997; Höhfeld & Jentsch, 1997; Takayama *et al*, 1997; Zeiner *et al*, 1997), and trimeric complexes can be formed with HSP70s as mediators between HAP46/BAG-1 and various proteins attached to the substrate domain (Takayama *et al*, 1997; Zeiner *et al*, 1997; Niyaz *et al*, 2003). Deletion of the last 47 residues from HAP46/BAG-1 showed that the carboxy-terminal portion is responsible for binding HSP70s (Takayama *et al*, 1997). As this interaction is such a prominent feature of HAP46/BAG-1 and its isoforms, this domain was named the BAG domain (Takayama *et al*, 1999). The interaction of this domain with HSP70s has been analysed in detail by X-ray analysis and nuclear magnetic resonance spectroscopy (NMR), complemented by mutagenesis. The BAG domain consists of a bundle of three α -helices that contact subdomain II of the HSP70s, mostly through electrostatic interactions (Briknarová *et al*, 2001; Sondermann *et al*, 2001). Of particular importance is Arg 237 of HAP46/BAG-1M (human sequence), as the conversion of this residue to Ala greatly reduced HSC70 binding and abolished *in vitro* activity. For HSP70s, the major portion of subdomain II is sufficient for binding to HAP46/BAG-1, although the affinity is greatly reduced (Brive *et al*, 2001). Phage display and peptide scanning yielded several potential contact regions on HSC70 in two main areas, one on each subdomain: one corresponds to the site detected by X-ray analysis and NMR, whereas the other lies on the opposite side of the cleft (Petersen *et al*, 2001). It is not known at present whether HAP46/BAG-1 requires more than one point of contact to exert an effect on the HSP70s.

An important aspect of the HAP46/BAG-1 interaction with HSP70s is the inhibition of their ATP-dependent chaperone activity (Gebauer *et al*, 1997; Takayama *et al*, 1997; Zeiner *et al*, 1997), which is concomitant with a reduction in the binding of HSP70s to misfolded proteins (Gebauer *et al*, 1998). However, HAP46/BAG-1 has also been described as a positive co-chaperone (Terada & Mori, 2000), and so the effect on HSP70s has still not been definitively determined. Apart from protein folding, HAP46/BAG-1 also promotes the ADP/ATP exchange reaction and/or enhances the ATPase activity of the chaperone (Höhfeld & Jentsch, 1997; Gebauer *et al*, 1998; Terada & Mori, 2000; Brehmer *et al*, 2001; Gässler *et al*, 2001). However, HAP46/BAG-1

has a less pronounced effect on nucleotide exchange than does GrpE (Brehmer *et al*, 2001), which is the prokaryotic counterpart of HAP46/BAG-1 but has a different structure. X-ray analysis of GrpE in complex with the ATP domain of *Escherichia coli* hsp70 protein DnaK disclosed contacts on both subdomains and showed a substantial opening of the central cleft (Mayer *et al*, 2001). It therefore seems reasonable that HAP46/BAG-1 might also use two contacts to produce a similar opening (Petersen *et al*, 2001) and binding might favour an alternative structure of the HSP70 ATP domain with a more open conformation (Osipiuk *et al*, 1999).

The interaction of HAP46/BAG-1 with HSP70 chaperones has an additional aspect relating to cellular regulation. The protein kinase RAF-1 is one of the few proteins known thus far to interact with HAP46/BAG-1 independent of HSP70 chaperones. The contact site is located in the C-terminal portion of HAP46/BAG-1, partially overlapping with the BAG domain (Song *et al*, 2001). This then explains competitive binding of HAP46/BAG-1 and RAF-1 to HSC70, thereby providing a molecular switch in RAF-1/ERK signalling under conditions of increased HSP70 expression (Song *et al*, 2001).

HAP46/BAG-1 isoforms

By carefully inspecting human HAP46/BAG-1 cDNAs, Packham *et al* (1997) discovered the noncanonical translational start codon CUG upstream of the previously identified AUG. This gives rise to a polypeptide of 345 residues; that is, it contains a 71-amino-acid extension (Fig 1) and begins with leucine. This larger form has been detected in human and mouse cell extracts (Packham *et al* 1997; Takayama *et al*, 1998; Yang *et al*, 1998). The apparent molecular size is ~50 kDa, hence the name HAP50 or BAG-1L (BAG-1-long). The amino-terminal end is rich in arginine, therefore raising the calculated pI from 5.1 in HAP46/BAG-1 to 8.3. A notable feature of human and mouse HAP50/BAG-1L is a cluster of basic

Fig 1 | Isoforms of human HAP46/BAG-1M and domain structure. Green: potential nuclear localization signal (NLS) (SV40 large T and nucleoplasmin types). Red: DNA-binding domain (appears brown in HAP50/BAG-1L owing to overlap with the potential NLS). Sequence in HAP46/BAG-1M: (1)MKKKTRRRST(10). Light blue: acidic hexa-repeat domain (consensus sequence Thr-Arg-Ser-Glu-Glu-X). Pink: ubiquitin-like domain. Dark blue: BAG domain, which is depicted here as originally defined by Takayama *et al* (1999).

residues (positions 68–79) that is characteristic of contiguous nuclear-localization signals (NLSs). To distinguish better the isoforms, human HAP46 was then named BAG-1M in which the M stands for medium (Takayama *et al*, 1998). Smaller isoforms, often called BAG-1S, originate from AUG codons further downstream. The main difference between human and mouse mRNAs is that the latter do not contain the AUG codon that gives rise to human HAP46/BAG-1M. Instead, an intermediate form originates from a start codon further downstream (Packham *et al* 1997; Takayama *et al*, 1998) and is shorter by 55 residues. This is the original mouse Bag-1 (Takayama *et al*, 1995), which corresponds to human HAP33 (Fig 1). Generally, this form is present in cells at higher concentration than other forms. Importantly, all isoforms contain the same C-terminal BAG domain and interact equally with HSP70s to affect their chaperoning activities.

HAP50/BAG-1L enhances androgen-receptor action (Knee *et al*, 2001). This requires the BAG domain, so HSP70s are involved. HAP50/BAG-1L also cooperates with the vitamin D receptor, but the measured response has been variable (Guzey *et al*, 2000; Witcher *et al*, 2001). The N-terminal portion of HAP50/BAG-1L is required for *in vitro* binding to the vitamin D receptor and HAP46/BAG-1M does not interact (Witcher *et al*, 2001).

Intracellular localization and expression

The effects of HAP46/BAG-1M on protein folding indicate that it is localized in the cytosol and this has now been established by different experimental approaches (Takayama *et al*, 1998; Zeiner *et al*, 1999; Nollen *et al*, 2000; Knee *et al*, 2001). For example, HeLa cells that express HAP46/BAG-1M coupled to the green fluorescent protein showed fluorescence that was equally distributed between the cytoplasm and the nucleus at 37 °C. However, exposing the same cells to heat shock resulted in redistribution of the protein to the nucleus, which is a response that has been seen with several cell types (Zeiner *et al*, 1999). The potential bipartite NLS of HAP46/BAG-1M (Fig 1) possibly becomes active only under specific conditions, such as heat stress. By contrast, HAP50/BAG-1L is almost exclusively localized in the cell nucleus at 37°C (Packham *et al*, 1997; Takayama *et al*, 1998; Knee *et al*, 2001; Niyaz *et al*, 2001).

Even though the *BAG-1* gene is expressed differentially in various mammalian tissues it is expressed minimally in almost all cell types. Notable changes in tissue distribution were observed during early mouse development (Crocchi *et al*, 2000), which are undoubtedly biologically significant. It will be exciting to see what developmental defects are observed in mice in which the *BAG-1* gene is disrupted.

Expression of the *BAG-1* gene is often increased in cancer cells relative to normal cells and the levels of the isoforms also differ. In general, there is a rough correlation between HAP46/BAG-1 protein levels and cell proliferation, which indicates that it might be suitable as a prognostic marker (Townsend *et al*, 2003a). High levels in tumour cells might be due to increased expression of the *BAG-1* gene by p53 mutants (Yang *et al*, 1999).

DNA binding and transcriptional stimulation

The N-terminal end of HAP46/BAG-1M contains a prominent cluster of basic residues that was thought to interact with other molecules. Indeed, HAP46/BAG-1M readily binds to DNA even though the protein as a whole is acidic. Neither the origin of the

DNA nor its size is important, although a minimum length is required. In addition, not only linear fragments but also supercoiled plasmid DNA bind to the protein, which indicates a lack of sequence specificity (Zeiner *et al*, 1999; Niyaz *et al*, 2003). The deletion of ten N-terminal residues abolished DNA binding. HAP46/BAG-1M can simultaneously interact with DNA and HSP70s, yielding trimeric complexes of the type DNA•HAP46/BAG-1M•HSP70 (Zeiner *et al*, 1999; Niyaz *et al*, 2003) in which the substrate-binding site of HSP70/HSC70 remains available for further interactions. The large form HAP50/BAG-1L contains the same basic DNA-binding region, although this overlaps with a putative NLS (Fig 1).

To analyse further the DNA-binding region, the three lysines (positions 2–4) and the three arginines (positions 6–8) in HAP46/BAG-1M were exchanged *en bloc* for neutral residues (Zeiner *et al*, 1999; Schmidt *et al*, 2003). In both cases, DNA binding was destroyed, which indicates that some basic residues within both clusters are essential.

HAP46/BAG-1M supplemented with a nuclear extract stimulates *in vitro* transcription independent of whether prokaryotic or eukaryotic templates are used or whether these contain promoter elements (Zeiner *et al*, 1999; Niyaz *et al*, 2003). The transcripts have no distinct length, which indicates random initiation. Termination probably occurs at the end of linearized DNAs. Transcriptional stimulation also occurs in intact cells when HAP46/BAG-1M is translocated to the nucleus owing to heat stress. Whereas heat shock drastically diminishes general message levels in normal cells, the overexpression of HAP46/BAG-1M almost reverses this, and the induction of HSP70 and HSP40 is enhanced (Zeiner *et al*, 1999). The expression of the *BAG-1* gene, however, is unaltered by this treatment (Townsend *et al*, 2003b). Transcription is similarly stimulated in cells that overexpress HAP50/BAG-1L, and this mainly affects endogenous genes that are subject to regulation rather than highly expressed genes, such as those that encode actin or tubulin (Niyaz *et al*, 2001). The stimulatory effect is apparently specific for RNA polymerase II.

To test whether HSP70s participate in transcriptional stimulation, the BAG domain was deleted from HAP46/BAG-1M and stimulation was greatly diminished as a result (Niyaz *et al*, 2003). This delineates a new cellular function of the HSP70 system in transcription and corresponds to the proposed involvement of HSP70 in chromatin remodelling (Schmidt *et al*, 2003). It might be relevant, however, that the above deletion variant still produced some stimulation, which indicates that this process involves not only the BAG domain of HAP46/BAG-1M but also, possibly, the DNA-binding domain.

HAP46/BAG-1M interacts with transcription factors

To find a biochemical reason for the effect on transcription, a series of transcription factors was studied. Several steroid hormone receptors, cAMP responsive element binding protein (CREB) and core binding factor (CBFA1/RUNX2) readily associated with HAP46/BAG-1M when HSC70 was present, but these interactions were destroyed by ablating the BAG domain (Niyaz *et al*, 2003). This is clear evidence for the involvement of HSP70/HSC70. For glucocorticoid receptors, this observation confirmed previous data (Schneikert *et al*, 2000), whereas it was the first direct evidence of trimeric complexes of oestrogen receptors containing HSC70 (Niyaz *et al*, 2003).

These data do not exclude the possibility that HAP46/BAG-1M interacts with other transcription factors independent of HSP70s, as was observed for the homeodomain factor GAX (Niyaz *et al*, 2003). The vitamin D receptor is another candidate for direct interaction, as binding to HAP50/BAG-1L required the N-terminal part in addition to the BAG domain (Guzey *et al*, 2000; Witcher *et al*, 2001). Therefore, there are two different types of interaction between transcription factors and the transcription-stimulating protein HAP46/BAG-1M.

Possible mechanism for transcriptional stimulation

The above observations can be incorporated into a model that explains the transcription-stimulating effect of HAP46/BAG-1M (Fig 2). It postulates that HAP46/BAG-1M binds to DNA in areas of active chromatin and, through HSP70s as bridging molecules, recruits transcription factors and possibly other components of the transcription machinery into functional complexes. This might then affect the expression of the respective gene.

Figure 2 assumes that an enhancer protein, such as a steroid receptor, bound to its cognate response element serves as an anchoring component on DNA and forms complexes with HAP46/BAG-1M that involve HSP70s. In this way, long stretches of DNA can be bridged. Other molecules of HAP46/BAG-1M further communicate with the transcription machinery by establishing contact either directly or through HSP70s with the basal transcription factor TFIID and/or RNA polymerase II. Some of these interactions might be transient. The model points to a complex array of interactions of HAP46/BAG-1M with the transcription apparatus and opens up the possibility of mutual potentiation as well as competition. In a similar manner, HAP46/BAG-1M could potentially lead to the repression of transcription of some active genes. The mechanistic details of the effects of HAP46/BAG-1M on transcription are unknown, particularly in view of the fact that ATP and ADP levels affect the protein-binding properties of HSP70 chaperones (Mayer *et al*, 2001).

Physiology

In terms of biology, HAP46/BAG-1M behaves like a chameleon, acting both as a transcriptional stimulator and an HSP70 co-chaperone. It forms a stoichiometric complex with HSP70/HSC70 so that equal concentrations are needed to block protein folding. However, HAP46/BAG-1M is normally present at much lower levels than HSC70, making complete inhibition of folding activity unlikely (Nollen *et al*, 2000). Conversely, in the nucleus, even low levels of HAP46/BAG-1M together with HSP70s might affect transcription. The same applies to HAP50/BAG-1L. There should be well-balanced equilibria in cells under heat or other kinds of stress when excessive amounts of misfolded proteins require chaperoning by cytosolic HSC70. The translocation of HAP46/BAG-1M to the nucleus would release any inhibitory effect on cytosolic chaperoning that could otherwise be operating. HAP46/BAG-1M might therefore act differently depending on physiological conditions and intracellular localization.

In contrast to HAP46/BAG-1M and HAP50/BAG-1L, shorter forms do not contain the basic amino-acid clusters and consequently do not bind to DNA. They function as negative or positive regulators of protein folding depending on specific conditions (Lüders *et al*, 2000b; Nollen *et al*, 2000; Gässler *et al*, 2001). As

Fig 2 | Model depicting possible chaperoning of the transcriptional apparatus to DNA by HAP46/BAG-1M along with HSP70. A simplified version of the initiation complex is shown with, arbitrarily, three molecules of HAP46/BAG-1M bound to DNA. RE, response element on DNA; REBP, specific response-element binding protein.

they contain a potential NLS (Fig 1), they translocate to the nucleus after heat shock (Townsend *et al*, 2003b).

Steroid receptors were the first transcription factors found to form complexes with HAP46/BAG-1M, and HSP70s clearly mediate these interactions. Transcriptional activation by androgen and oestrogen receptors is enhanced by HAP50/BAG-1L but not by HAP46/BAG-1M (Knee *et al*, 2001; Cutress *et al*, 2003), probably because HAP46/BAG-1M is largely cytosolic under normal conditions whereas HAP50/BAG-1L is nuclear. However, under heat stress, HAP46/BAG-1M might similarly stimulate the response to androgen and oestrogen. The effects of HAP46/BAG-1M on glucocorticoid responses are complex. One group found that the overexpression of HAP50/BAG-1L in several cell types promoted glucocorticoid-stimulated expression of a reporter construct and of the receptor gene itself (Niyaz *et al*, 2001). Others have described a negative effect of HAP46/BAG-1M on glucocorticoid-receptor actions. Overexpression of HAP46/BAG-1M reduced receptor binding to DNA and caused decreased hormonal response (Kullmann *et al*, 1998) with both DNA binding and BAG domains being involved (Schneikert *et al*, 2000; Schmidt *et al*, 2003). Such variations in response are possibly the result of using different experimental systems in which either potentiation or inhibition of transcription might prevail (see above).

Overexpression of HAP46/BAG-1M and its isoforms protects various cell types from heat-induced apoptosis (Takayama *et al*, 1997; Zeiner *et al*, 1999; Niyaz *et al*, 2001; Townsend *et al*, 2003b), possibly through the enhanced induction of HSP70 and HSP40 (Zeiner *et al*, 1999). Related to this is the increased growth-factor-dependent proliferation of various cells by HAP46/BAG-1 (Townsend *et al*, 2003a) and the rescue of lymph cells from glucocorticoid-induced apoptosis (Kullmann *et al*, 1998). Cell-protective effects that counterbalance such diverse insults might be due to a stimulation of general transcription and/or enhanced expression of specific genes by HAP46/BAG-1M and HAP50/BAG-1L. In addition, HAP46/BAG-1M might arrest cell death by inhibiting protein phosphatase 1 (PP1), which is associated with the cellular stress response protein GADD34 (Hung *et al*, 2003).

Unresolved problems

Apart from the DNA binding and BAG domains, the functions of the remaining portions of HAP46/BAG-1M are largely unknown. The protein harbours a region of ten acidic hexa-repeats (including one degenerated sequence), whereas HAP33/BAG-1S has only four such repeats. Even though this region may be phosphorylated, this does not affect activity, at least not with respect to steroid-receptor action and binding to HSP70s (Schneikert *et al*, 2000). Deletion of the hexa-repeat region neither destroyed transcriptional stimulation by HAP46/BAG-1M (Niyaz *et al*, 2003) nor glucocorticoid-receptor-dependent transcription in transfected cells (Schmidt *et al*, 2003). Mouse HAP50/BAG-1L contains only six acidic repeats, which are also less conserved.

Moreover, the ubiquitin-like region (Fig 1) remains mysterious. It is much shorter than ubiquitin itself and interacts with proteasomes (Lüders *et al*, 2000a). Conjugation with ubiquitin normally primes proteins for degradation by proteasomes, but HAP46/BAG-1M has a relatively long half-life despite the fact that it is ubiquitylated (Alberti *et al*, 2002). Whereas the prevention of stress-induced growth inhibition by HAP46/BAG-1M and its isoforms requires a conserved lysine in the ubiquitin region (Townsend *et al*, 2003b), it is dispensable for the action of HAP50/BAG-1L on androgen receptors (Knee *et al*, 2001) and of HAP46/BAG-1M on transcription (Y Niyaz, unpublished data).

An exciting question is how HAP46/BAG-1M and HAP50/BAG-1L affect transcription mechanistically. In the context of Figure 2, it will be important to identify the component(s) of the transcriptional apparatus they interact with and whether HSP70s are involved. Mutational analysis will then help to establish biochemical details and functional significance. It will also be important to investigate a more extended panel of transcription factors for their ability to interact with HAP46/BAG-1M.

A key problem concerns the cellular expression of HAP46/BAG-1M and HAP50/BAG-1L. High levels in various cancer cells probably relate to enhanced survival under the adverse and stressful conditions that prevail in tumours and during antitumour treatments. Cells that express a lot of the *BAG-1* gene might be selected for, but defective mutations, particularly in the DNA and BAG domains, might not have immediate selective advantages. A range of cell survival factors have been found to upregulate the *BAG-1* gene in various cell types (Townsend *et al*, 2003a). Also multidrug-resistant cells that are less sensitive to apoptosis compared with their normal counterparts express higher levels of HAP46/BAG-1M and HAP33/BAG-1S (Ding *et al*, 2000), and downregulation of these genes slowed HeLa cell growth (Takahashi *et al*, 2003). Therefore, it will be helpful in the treatment of cancer if levels of HAP46/BAG-1M and HAP50/BAG-1L could be decreased by specific agents or external treatments. Indeed, protein-kinase inhibitors, such as genistein and flavopiridol, and the immunosuppressant rapamycin, downregulate the *BAG-1* gene (Adachi *et al*, 1996; Kullmann *et al*, 1998; Kitada *et al*, 2000). However, decreased levels of HAP50/BAG-1L caused HeLa cells to be less sensitive to anticancer drugs (Takahashi *et al*, 2003). The full importance of HAP46/BAG-1M and its isoforms in the development of various cancers is far from clear, and it is to be hoped that these recent data will stimulate further research in this area.

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