Heat Shock Protein 70 kDa (Hsp70) family molecular chaperones play critical roles in protein folding and trafficking in all eukaryotic cells. The mechanisms by which Hsp70 family chaperones are regulated, however, are only partly understood. BAG-1 binds the ATPase domains of Hsp70 and Hsc70, modulating their chaperone activity and functioning as a competitive antagonist of the co-chaperone Hip. We describe the identification of a family of BAG-1-related proteins from humans (BAG-2, BAG-3, BAG-4, BAG-5), the invertebrate Caenorhabditis elegans (BAG-1, BAG-2), and the fission yeast Schizosaccharomyces pombe (BAG-1A, BAG-1B). These proteins all contain a conserved ~45-amino acid region near their C termini (the BAG domain) that binds Hsc70/Hsp70, but they differ widely in their N-terminal domains. The human BAG-1, BAG-2, and BAG-3 proteins bind with high affinity ($K_D \approx 1–10 \text{ nM}$) to the ATPase domain of Hsc70 and inhibit its chaperone activity in a Hip-repressible manner. The findings suggest opportunities for specification and diversification of Hsp70/Hsc70 chaperone functions through interactions with various BAG-family proteins.

The chaperone activity of mammalian Hsc70/Hsp70 is regulated by partner proteins that either modulate the peptide binding cycle or that target the actions of these chaperones to specific proteins and subcellular compartments. DnaJ-family proteins (Hdj-1/Hsp40; Hdj-2; Hsj-1) stimulate the ATPase activity of Hsc70/Hsp70, resulting in the ADP-bound state that binds tightly to peptide substrates (1–3). The Hip protein collaborates with Hsc70/Hsp70 and DnaJ homologues in stimulating ATP hydrolysis and, thus, also stabilizes Hsc70/Hsp70 complexes with substrate polypeptides, whereas the Hop protein may provide co-chaperone functions through interactions with the C-terminal peptide binding domain (4–6).

Specialized functions for Hsc70-family chaperones are promoted by their interactions with particular co-chaperones. For instance, the DnaJ-family proteins Ydj1p of yeast and human hdj2 have C-terminal CAAX prenylation motifs that account for their post-translational modification with lipids which anchor them in intracellular membranes, thereby allowing them to recruit Hsp70/Hsc70-family chaperones into protein-folding reactions involved in mitochondrial protein import (7, 8). Yeast deficient in Ydj1p membrane targeting exhibit defects in protein import into mitochondria and endoplasmic reticulum (9).

Similarly, hdj2 supports mitochondrial protein import in vitro, whereas hdj-1 does not (10). In contrast, hdj-1 and its yeast counterpart Sis1p are uniquely associated with ribosomes among known DnaJ-family members, collaborating with Hsp70/Hsc70-family proteins in folding of nascent polypeptides during translation (11, 12).

BAG-1 and its longer isoforms BAG-1M (Rap46) and BAG-1L are recently described Hsc70/Hsp70-regulating proteins (13–17). BAG-1 competes with Hip for binding to the Hsc70/Hsp70 ATPase domain and promotes substrate release (13–15). BAG-1 also reportedly stimulates Hsc70-mediated ATP hydrolysis by accelerating ADP/ATP exchange, analogous to the prokaryotic GrpE nucleotide exchange protein of the bacterial Hsc70 homologue, DnaK (15). Gene transfection studies indicate that BAG-1 proteins can influence a wide variety of cellular phenotypes through their interactions with Hsc70/Hsp70, including increasing resistance to apoptosis, promoting cell proliferation, enhancing tumor cell migration and metastasis, and altering transcriptional activity of steroid hormone receptors (18–28).

We describe here the identification of an evolutionarily conserved family of BAG-like proteins. Biochemical characterization of two members of this family, BAG-2 and BAG-3, suggests that they interact with and modulate Hsp70/Hsc70 similarly as to BAG-1. The findings reveal unexpected diversity in the protein networks available for targeting Hsp70/Hsc70 functions in cells.

**MATERIALS AND METHODS**

**Two-hybrid Screens—**Yeast two-hybrid library screening of a human Jurkat cell cDNA library was performed as described previously (13, 27) using EGY48 strain yeast transformed with pGilda-Hsc70/ATPase (67–377 amino acids) and the lacZ reporter plasmid pSh18–34. Of the resulting $5 \times 10^5$ transformants, 112 Leu+ colonies were obtained after 1-week incubation at 30 °C. Assay of β-galactosidase activity of these colonies resulted in 96 clones. Mating tests were then performed using RFY206 yeast strain transformed with pGilda, pGilda mBAG-1 (1–219), or pGilda Hsc70/ATPase. Of these, 66 displayed specific interactions with Hsc70/ATPase. The pGL4–5 cDNAs were recovered using KCl Escherichia coli strain that is auxotrophic for Trp, DNA sequencing revealing three partially overlapping huBAG-1, four identical and one overlapping cDNAs encoding BAG-2, and two partially overlapping BAG-3 clones.

**Recombinant Protein Production—**The cDNAs encoding residues 5–211 of huBAG-2 (clone 11) and the C-terminal 135 amino acids of huBAG-3 (clone 28) were subcloned into the EcoRI/Xhol sites of pGEX4T-1 (Amersham Pharmacia Biotech). These plasmids, as well as pGEXX-T-1-BAG-1, pGEXX-T-1-BAG-1(D), and pGEXX-T-1-XL plasmids (13, 29), were expressed in XL-1 Blue strain E. coli (Stratagene, Inc.). Briefly, a single colony was inoculated into 1 liter of LB medium containing 50 μg/ml ampicillin and grown at 37 °C overnight. The culture was then diluted by half with fresh LB/ampicillin and cooled to room temperature for 1 h before inducing with 0.4 mM IPTG for 6 h at 30 °C.

**The abbreviations used are: IPTG, isopropyl-1-thio-β-D-galactopyranoside; PMSF, phenylmethanesulfonyl fluoride; TA, trans-activation; ORF, open reading frame; GST, glutathione S-transferase; PAGE, polyacrylamide gel electrophoresis.
Cells were then recovered and incubated with 0.5 mg/ml lysozyme in 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Tween 20, 0.1% 2-mercaptoethanol, 5 mM EDTA, 1 mM PMSF, and a mixture of other protease inhibitors (Boehringer Mannheim 1697498) at room temperature for 0.5 h, followed by sonication. Cellular debris were pelleted by centrifugation at 27,500 g for 10 min, and the resulting supernatants were incubated with 30 ml of glutathione-Sepharose (Amersham Pharmacia Biotech) at 4 °C overnight. The resin was then washed with 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20, and 0.1% 2-mercaptoethanol until A280 nm reached ~1.0. For removal of GST, the resin with immobilized GST-fusion protein was incubated overnight with 10 units of thrombin (Boehringer, Inc.) in 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20, 0.1% 2-mercaptoethanol, and 2.5 mM CaCl2. Released proteins were then purified on Mono Q (HR10/10, Amersham Pharmacia Biotech) by fast protein liquid chromatography using a linear gradient of 0.5M NaCl at pH 8.0 and then dialyzed into chaperone assay buffer. Hip was purified as His 6 protein (30).

Protein Interaction Assays—Immunoprecipitation and in vitro GST-protein binding assays were performed as described (13), using pcI-Neo flag or pcDNA3-HA into which huBAG-3, BAG-4, BAG-5, and C. elegans (C.e.) BAG-2. The overall predicted amino acid sequences of the C. elegans BAG-1 and S. pombe BAG-1A proteins are ~18% identical (~61% similar) and ~17% identical (~64% similar), respectively, to human BAG-1. C. elegans and human BAG-2 share ~34% amino acid sequence identity (~70% similarity) with each other, over their C-terminal 225 amino acids that encompass both the BAG domain and upstream region. B. the amino acid sequence of the BAG-regions are aligned. Black and gray shading represent identical and similar amino acids, respectively. The C. elegans BAG-1A protein contains a 5–7-amino acid insert in its BAG-domain relative to the human, murine, and yeast BAG-1 homologues and, thus, is more similar to BAG-2 in this particular regard. The human BAG-2 protein contains a 9-amino acid insert in its BAG-domain relative to its C. elegans counterpart.

**FIG. 1. Topology of BAG-family proteins and alignment of BAG domain sequences.** A, the topologies of the BAG-family proteins are depicted, showing the relative positions of the BAG domains (black), ubiquitin-like regions (gray), WW domain (stripes), and nucleoplasmin-like nuclear localization sequence. The complete ORFs have not yet been deduced for huBAG-3, BAG-4, BAG-5, and C. elegans (C.e.) BAG-2. The overall predicted amino acid sequences of the C. elegans BAG-1 and S. pombe BAG-1A proteins are ~18% identical (~61% similar) and ~17% identical (~64% similar), respectively, to human BAG-1. C. elegans and human BAG-2 share ~34% amino acid sequence identity (~70% similarity) with each other, over their C-terminal 225 amino acids that encompass both the BAG domain and upstream region. B. the amino acid sequence of the BAG-regions are aligned. Black and gray shading represent identical and similar amino acids, respectively. The C. elegans BAG-1A protein contains a 5–7-amino acid insert in its BAG-domain relative to the human, murine, and yeast BAG-1 homologues and, thus, is more similar to BAG-2 in this particular regard. The human BAG-2 protein contains a 9-amino acid insert in its BAG-domain relative to its C. elegans counterpart.

**25 °C.** Cells were then recovered and incubated with 0.5 mg/ml lysozyme in 50 mM Tris (pH 8.0), 150 mM NaCl, 1% Tween 20, 0.1% 2-mercaptoethanol, 5 mM EDTA, 1 mM PMSF, and a mixture of other protease inhibitors (Boehringer Mannheim 1697498) at room temperature for 0.5 h, followed by sonication. Cellular debris were pelleted by centrifugation at 27,500 x g for 10 min, and the resulting supernatants were incubated with 30 ml of glutathione-Sepharose (Amersham Pharmacia Biotech) at 4 °C overnight. The resin was then washed with 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20, and 0.1% 2-mercaptoethanol until A280 nm reached <0.01. For removal of GST, the resin with immobilized GST-fusion protein was incubated overnight with 10 units of thrombin (Boehringer, Inc.) at 4 °C in 20 mM Tris (pH 8.0), 150 mM NaCl, 0.1% Tween 20, 0.1% 2-mercaptoethanol, and 2.5 mM CaCl2. Released proteins were then purified on Mono Q (HR10/10, Amersham Pharmacia Biotech) by fast protein liquid chromatography using a linear gradient of 0.5 M NaCl at pH 8.0 and then dialyzed into chaperone assay buffer. Hip was purified as His6 protein (30).

**Protein Interaction Assays—**Immunoprecipitation and in vitro GST-protein binding assays were performed as described (13), using pcI-Neo flag or pcDNA3-HA into which huBAG-2 (clone 11) or huBAG-3 (clone 28) had been subcloned for in vitro translation of L-[^35S]methionine-labeled proteins or expression in 293T cells.

**Surface Plasmon Resonance—**Kinetic measurements were performed using a BIAcore-II instrument with CM5 sensor chip and Amine Coupling Kit (Pharmacia Biosensor AB, Sweden), as described in Refs. 29 and 31. The rate constants kₐ and kₛ were generated with BIAevaluation, Version 3.0 software (Pharmacia Biosensor AB, Sweden).

**Chaperone Assays—**Dna-J-dependent refolding assays were performed as described (10). Hsp40-dependent refolding assays were performed as described (32), using recombinant luciferase (Promega, QuantumLuc™) that was heat-denatured at 42 °C for 10 min, 1.8 μM Hsc70 (Sigma; purified from bovine brain), 0.9 μM Hsp40 (kindly provided by O. Minami, Oita Medical College), and various recombinant purified proteins as indicated. Luciferase activity was measured (Promega luciferase assay kit) using a luminometer (EG&G Berthold, MicroLumat luminometer, model LB960 P). All results were normalized relative to non-denatured luciferase subject to the same conditions. Control reactions lacking ATP, Hsc70, or Hsp40 resulted in negligible luciferase refolding.
RESULTS

Using the ATPase domain of Hsc70 as a “bait” in yeast two-hybrid screens, several human cDNAs were cloned that encode portions of BAG-1 or two other BAG-1-like proteins, which were arbitrarily named BAG-2 and BAG-3. The longest of the cDNAs for BAG-2 and BAG-3 contained open reading frames (ORFs) of 207 and 162 amino acids, respectively, followed by stop codons. All BAG-1, BAG-2, and BAG-3 cDNAs obtained by two-hybrid library screening with Hsc70/ATPase contained a conserved domain of 30–50 amino acids, which we have termed the “BAG” domain (Fig. 1).

A λ-phage cDNA library was screened using the longest of the BAG-2 and BAG-3 clones derived from two-hybrid screening as hybridization probes. DNA sequencing of huBAG-2 cDNA clones revealed an ORF encoding a predicted 211-amino acid protein, preceded by an in-frame stop codon (Fig. 1 A). The predicted ATG initiation codon resides in a favorable context for translation. The longest of the 23 obtained huBAG-3 λ-phage cDNA clones contained a continuous ORF of 682 amino acids followed by a stop codon, but without an identifiable start codon. Thus, the 5’ end of this ORF remains to be defined. Though BAG-1, BAG-2, and BAG-3 all contain a homologous BAG domain near their C termini, the N-terminal regions of these proteins are dissimilar. The BAG-2 N-terminal region contains potential kinase phosphorylation sites but otherwise shares no apparent similarity with other proteins or functional domains. In contrast, the predicted N-terminal region BAG-3 contains a WW domain (Fig. 1 A).

bBLAST and FASTA searches of the translated GenBank™ data base also identified human expressed sequence tags that appear to represent additional BAG-family proteins, which we have tentatively termed BAG-4 (accession numbers AA693697 and N74588) and BAG-5 (accession numbers AA456862 and N34101). The putative BAG-4 and BAG-5 proteins contain BAG domains that share the greatest sequence similarity with the BAG domain of BAG-3, having 62% identity (81% similarity) to BAG-4 and 51% identity (75% similarity) to BAG-5 (Fig. 1 B).

Probable BAG-family orthologues or homologues were also identified by computer-based searches in the nematode Caenorhabditis elegans and the fission yeast Schizosaccharomyces pombe. The C. elegans genome encodes two apparent BAG-family proteins, which are most similar in their overall sequences to the human BAG-1 (Afo39713, gi:2773211) and BAG-2 (Afo668719, gi:3168927), whereas S. pombe contains two BAG-family proteins that share the greatest overall sequence similarity with human BAG-1 (Alo23854, gi:3133105 and Alo23634, gi:3150250). The human and C. elegans BAG-1 proteins as well as S. pombe BAG-1A all have ubiquitin-like domains of unknown function near their N termini (Fig. 1 A).

Evolutionary tree prediction algorithms suggest that human and C. elegans BAG-2 represent a distinct branch of the BAG-
family that is more evolutionarily distant from the other BAG-family proteins. None of the predicted BAG-family proteins contain recognizable regions analogous to those found in other Hsc70 regulatory proteins, such as the J-domains and G/F-domains of DnaJ-family proteins and the Tetra tricopeptide Repeat (TR) domains of Hip/Hop family proteins.

Hsc70/ATPase Domain Specifically Interacts with BAG-1, BAG-2, and BAG-3 in Vitro and in Cells—To confirm that the BAG-2 and BAG-3 proteins interact specifically with Hsc70/ATPase, the longest of the cDNAs obtained for each of these proteins were expressed with N-terminal transactivation (TA) domains in yeast and tested by two-hybrid assay for interactions with fusion proteins consisting of Hsp70/ATPase or a variety of unrelated proteins (Fas, Siah, Fadd) containing N-terminal LexA DNA-bindings domains. TA-BAG-2 and TA-BAG-3 displayed positive interactions with LexA-Hsc70/ATPase, resulting in transactivation of a lacZ reporter gene under the control of LexA operators, whereas no interactions with LexA-Fas (cytosolic domain), LexA-Siah, LexA-Fadd, or LexA were detected by this method (Fig. 2A). BAG-2 and BAG-3 also failed to interact with BAG-1 or a deletion mutant of BAG-1(ΔC), which is missing part of its C-terminal domain required for Hsp70/Hsc70 binding (13), suggesting that these proteins do not form heterodimers. Specific two-hybrid interactions between Hsc70/ATPase and either BAG-2 or BAG-3 were also observed when BAG-2 and BAG-3 were expressed as LexA DNA-binding domain fusion proteins, and Hsc70/ATPase was fused with a TA domain (Fig. 2A, right panel), further confirming the validity of the measured interactions.

Association of BAG-2 and BAG-3 with Hsc70/ATPase was also tested by an in vitro protein binding assay wherein Hsc70/ATPase or BAG-family proteins were expressed in bacteria as glutathione S-transferase (GST) fusion proteins, immobilized on glutathione-Sepharose, and tested for binding to 35S-labeled in vitro translated (IVT) proteins. 35S-Hsc70/ATPase bound in vitro to GST-BAG-1, GST-BAG-2, and GST-BAG-3 but not to GST-BAG-1(ΔC) or a variety of negative control proteins such as GST-CD40 cytosolic domain (Fig. 2B). Similarly, GST-Hsc70/ATPase bound to 35S-BAG-1, BAG-2, and BAG-3 but not to BAG-1(ΔC) or several other control proteins (Fig. 2B; and not shown). BAG-1, BAG-2, and BAG-3 also exhibited little or no binding to themselves or each other, suggesting that these proteins do not strongly homo- or hetero-dimerize or oligomerize.

Next, the ability of the BAG-2 and BAG-3 proteins to interact in cells with Hsc70 was explored by co-immunoprecipitation assays, expressing these proteins with N-terminal Flag epitope tags in 293T human epithelial cells. For these experiments, cDNAs encoding the旗-phage cloned regions of BAG-2 and BAG-3 were subcloned in-frame into pcDNA3-Flag. Anti-Flag immune complexes prepared from 293T cells after transfection with plasmids encoding Flag-BAG-1, Flag-BAG-2, or Flag-BAG-3 were analyzed by SDS-PAGE/immunoblot assay, revealing the presence of associated Hsc70, as detected with an anti-Hsc70 specific antiserum. In contrast, control immune complexes prepared with IgG, as well as anti-Flag immune complexes prepared from cells transfected with Flag-tagged control proteins such as Daxx and Apaf-1 did not contain associated Hsc70 protein (Fig. 1C).

BAG-family Proteins Bind ATPase Domain of Hsc70 with High Affinity—BAG-1 binds tightly to the ATPase domain of Hsc70 (31). To contrast the affinity and kinetics of binding of Hsc70/ATPase to BAG-1 with that of BAG-1, BAG-2, and BAG-3 proteins, we used a surface plasmon resonance technique (BIAcore) (29, 31). For these experiments, BAG-1, BAG-2, and BAG-3 proteins were produced in bacteria and purified to near homogeneity (Fig. 3A) and then immobilized on biosensor chips and tested for interactions with Hsc70 in the soluble phase. Addition of Hsc70 to chips containing BAG-1, BAG-2, or BAG-3 resulted in concentration-dependent binding, as reflected by an increase in the response units (Fig. 3B, RU; and data not shown) measured at the chip surface. In contrast, Hsc70 failed to display interactions in BIAcore assays with a variety of control proteins (not shown) as well as a mutant of BAG-1 lacking a C-terminal portion of the BAG domain that is required for Hsc70-binding (Fig. 3B) (13). Moreover, flowing various control proteins such as GST, BSA, and Bcl-XL over the BAG-1, BAG-2, or BAG-3 chips resulted in negligible interac-
BAG-family Regulators of Hsp70/Hsc70

Chapter 4: BAG-1, BAG-2 and BAG-3 similarly modulate Hsc70 chaperone activity. A, refolding of chemically denatured luciferase by Hsc70 plus DnaJ was measured in the absence (lane 1), or presence of BAG-1 (lane 2), BAG-1(ΔC) (lane 3), BAG-2 (lane 4), or BAG-3 (lane 5) added in equimolar amounts relative to Hsc70 (1.8 μM). Various controls in which ATP (not shown) or chaperones (lane 6) were omitted (bovine serum albumin used to normalize protein) confirmed minimal spontaneous refolding of denatured luciferase. B, representative data are presented showing concentration-dependent inhibition of Hsc70-mediated refolding of heat-denatured luciferase by BAG-1 (●), BAG-2 (○), and BAG-3 (▲), but not BAG-1(ΔC) (▲). C, Hsc70/Hsp40-mediated refolding of heat-denatured luciferase was assayed in the presence of (black bars) or absence of (striped bars) of 1.8 μM Hip, with (lanes 3-10) or without (lanes 1 and 2) various BAG-family proteins (1.8 μM) as indicated (mean ± S.E.; n = 3). A control (CNTL) is shown (lane 1) in which Hsc70 was replaced with an equivalent amount of BSA.

The rates of Hsc70 binding to BAG-1, BAG-2, and BAG-3 were similar, following pseudo first-order kinetics with estimated association rate constants (kₐ) of 2.1, 2.1, and 2.4 × 10⁹ M⁻¹ sec⁻¹, respectively. After allowing binding of Hsc70 to immobilized BAG-1, BAG-2, or BAG-3 to reach plateau levels, the chaperone was removed from the flow solution, and the dissociation rate was monitored. BAG-1 and BAG-2 exhibited similar dissociation rates, with relatively slow loss of Hsc70 from the chip surface, resulting in estimated dissociation rate constants (k₉) of 3.0 and 5.0 × 10⁻⁴ sec⁻¹, respectively (Fig. 3B). In contrast, Hsc70 dissociated more rapidly from biosensor chips containing BAG-3 (Fig. 3B), yielding an estimated k₉ of 1.7 × 10⁻³ sec⁻¹. From the kinetic data, the apparent affinities (Kᴰ = k₉/kₐ) were calculated for binding of Hsc70 to BAG-1 (Kᴰ = 1.4 nm), BAG-2 (Kᴰ = 2.4 nm), and BAG-3 (Kᴰ = 7.4 nm). We conclude, therefore, that interactions of Hsc70 with these BAG-family proteins occur with apparent affinities sufficient for physiological relevance.

BAG-family Proteins Suppress Hsp70/Hsc70 Chaperone Activity in Vitro—BAG-1 has been shown to inhibit Hsp70/Hsc70-dependent refolding of denatured protein substrates in vitro (13–15). We, therefore, contrasted the effects of BAG-1 with BAG-2 and BAG-3 using in vitro protein refolding assays similar to those employed previously for assessing BAG-1 function. In one assay (10), purified components were employed consisting of human Hsc70 and bacterial DnaJ. The combination of Hsc70 (1.8 μM) and DnaJ (0.9 μM) resulted in ATP-dependent refolding of chemically denatured firefly luciferase, with function of over half the denatured enzyme restored in a 90-min reaction, as monitored by a chemiluminescence assay. In contrast, neither Hsc70 nor DnaJ alone was competent to induce substantial refolding of denatured luciferase (not shown), and very little spontaneous restoration of luciferase activity was seen with control proteins such as BSA, GST, or Bel-XL (Fig. 4A, and not shown). Addition of recombinant purified BAG-1, BAG-2, or BAG-3 to these assays in amounts equimolar to Hsc70 (1.8 μM) resulted in striking inhibition of luciferase refolding, with BAG-2 and BAG-3 displaying somewhat greater inhibitor activity than BAG-1 (Fig. 4A). In contrast, the BAG-1(ΔC) protein that fails to bind Hsc70 (Fig. 4A), as well as several other control proteins (not shown), had no effect in this refolding assay.

In another refolding assay (32), purified Hsc70 and the human DnaJ homolog Hdj-1 (Hsp40) were employed with additional cofactors provided in reticulocyte lysates (5% v/v) to produce a system competent to refold denatured luciferase. Various amounts of purified BAG-1, BAG-2, or BAG-3 were added relative to Hsc70, resulting in concentration-dependent inhibition of Hsc70 chaperone activity. As in the other assay, BAG-2 and BAG-3 inhibited Hsc70 chaperone activity at least as potently as BAG-1. In contrast, the BAG-1(ΔC) mutant (Fig. 4B) as well as a variety of control proteins (not shown) did not suppress Hsc70-mediated refolding of denatured luciferase.

Previously, it has been shown that BAG-1 competes with Hip for binding to Hsc70, with these proteins having opposing effects on Hsc70-mediated protein refolding (15). Addition to refolding assay reactions of purified Hip protein (Fig. 3A) at equimolar concentrations relative to BAG-1, BAG-2, or BAG-3 (1.8 μM) completely negated the inhibitory effects of these BAG-family proteins on refolding of denatured luciferase (Fig. 4C). This observation demonstrates that the suppression of Hsc70 chaperone activity by BAG-1, BAG-2, and BAG-3 is reversible, and suggests that Hip antagonizes the effects not only of BAG-1, as previously reported (15), but also BAG-2 and BAG-3.

Though BAG-1 reportedly stimulates ADP/ATP exchange (15) and shares some limited sequence similarity with the prokaryotic nucleotide exchange factor GrpE (31), we observed only an ~2-fold increase in nucleotide exchange induced by BAG-1 (not shown), suggesting that its mechanism is not analogous to GrpE.

DISCUSSION

Hsc70/Hsp70 family molecular chaperones require partner proteins for dictating their functions in specific cellular compartments and cellular processes (1–3). The discovery of a family of BAG-1-like proteins thus reveals additional potential diversity in the regulation of Hsc70/Hsp70 chaperones. BAG-family proteins all contain an ~45-amino acid conserved region that mediates binding to the ATPase domain of Hsc70/Hsp70, but differ markedly in their N-terminal unique regions. These non-conserved regions of BAG-family proteins may participate in targeting of Hsc70/Hsp70 to other proteins or protein complexes or may control their subcellular distribution. For instance, isoforms of the human BAG-1 protein have been identified that differ in their N-terminal regions, with the BAG-1L protein containing a nuclear localization sequence that preferentially targets it to the nucleus (16, 17). BAG-1L has been shown to collaborate with androgen receptors, enhancing their
transactivation function, whereas BAG-1 does not (28), demonstrating an important functional difference between these proteins.

It seems likely, therefore, that the unique regions of other BAG-family proteins, such as the WW domain (33) found in BAG-3, will be responsible for mediating interactions with specific proteins in various subcellular compartments. It is also likely that additional diversity can be generated through tissue-specific control of BAG-family gene expression (17), analogous to the DnaJ-family protein wherein at least one member, Hsj-1, is expressed primarily in neurons (34).

The ability of BAG-family proteins to modulate the chaperone activity of Hsc70/Hsp70 can have different consequences for the various proteins whose functions are controlled by conformational changes induced by Hsc70/BAG-complexes. For instance, BAG-1 or its longer isoforms BAG-1M and BAG-1L have been reported to form complexes with and enhance the functions of Bcl-2, the kinase Raf, androgen receptors, human growth factor (HGF) receptors, and platelet-derived growth factor (PDGF) receptors, while inhibiting retinoic acid receptors (RAR), glucocorticoid receptors, and Siah-1 (18, 19, 21–28). The mechanisms responsible for either a net enhancement or inhibition of protein function in vivo may not be predictable. The functional antagonisms displayed between BAG-family proteins and Hip suggests that a proper balance of these two types of protein is required for achieving optimal cycles of substrate binding and release required for inducing conformational changes in proteins, with Hip promoting peptide substrate binding by Hsc70/Hsp70 and BAG-family protein promoting dissociation. Thus, while BAG-family proteins exert a measurable inhibitory effect on Hsc70-assisted refolding of denatured luciferase in vitro, this may not be reflective of all protein folding reactions in which BAG-family proteins participate in cells. Future studies of the biological roles played by individual members of the BAG-family, as well as biochemical and structural analysis of their mechanisms, are likely to provide new insights into the repertoire of means used to customize Hsc70/Hsp70 chaperone functions in vivo.

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