Essential Role of the Unusual DNA-binding Motif of BAG-1 for Inhibition of the Glucocorticoid Receptor*

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BAG-1 is involved in the regulation of a diversity of physiological processes, e.g. apoptosis, tumorigenesis, and neuronal differentiation (1–4), by virtue of its ability to interact with numerous regulatory proteins, including nuclear receptors. BAG-1 was originally identified as an associating factor of the anti-apoptotic factor bc2 (5) and, independently, as a protein called RAP46 that associates with the glucocorticoid receptor (GR) (6) and other steroid hormone receptors (6). It also is termed "hap46," hsp70- and hsp70-interacting protein (hip). hsp40 enhances the chaperone activity of hsp70/hsp90 organizing protein (hip) (17–19), which bridges hsp70 and hsp90 via its tetra-tripeptide repeat domains (20). hsp90, hsp70, and hop, possibly by the involvement of hsp40 (21), form an intermediate complex with GR (15, 22), from which hsp70 and hop presumably dissociate to allow entry of p23 and one of the large immunophilins to the final complex, where GR gains hormone binding competence.

The chaperone activity of hsp70 can be modulated by BAG-1 and also by hsp40, C terminus of hsp70-interacting protein, and hsp70-interacting protein (hip). hsp40 enhances the ATPase activity of hsp70 in vitro (23) and the hsp70-dependent refolding in mammalian cells (24, 25). hip also has been identified as a positive regulator of hsp70 chaperone activity (26, 27). In contrast, the C terminus of hsp70-interacting protein has been found to inhibit the ATPase activity of hsp70 and to interfere with stable hsp70-substrate complexes (28). All isoforms of BAG-1, i.e. the 50-kDa BAG-1L, the 46-kDa BAG-1M, and the 33/29-kDa BAG-1S, which derive from different translation initiation sites localized on the same gene (29–31), have been described in several studies (27, 32–35) to inhibit hsp70-dependent refolding activity in vitro and in vivo. Moreover, BAG-1 was found to compete with the stimulatory action of hip (27), hip, in turn, opposes the negative effect of BAG-1 on steroid binding of GR (36). The negative effect of BAG-1 on steroid binding (37) was not observed by others (38). This seeming discrepancy could be explained by different protein concentrations of BAG-1 present in these assays, as the protein level of BAG-1 has been shown to be crucial for its inhibitory action (37).

Although all these reports strongly suggest a cytosolic effect of BAG-1 on GR folding activity, there are also data supporting a nuclear function of BAG-1. For example, BAG-1 has been reported to bind to DNA in a nonsequence-specific manner and to stimulate DNA transcription (39). Deletion of, or mutations within, the N-terminal 10 amino acids of BAG-1 abolish its DNA binding (39). In addition, it has been shown that BAG-1 is transported into the nucleus upon steroid binding and nuclear translocation of GR (38). This nuclear translocation is

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[The on-line version of this article (available at http://www.jbc.org) contains sequences of the oligonucleotides.

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‡ The abbreviations used are: GR, glucocorticoid receptor; MMTV, mouse mammary tumor virus; HA, hemagglutinin; FCS, fetal calf serum; Ni-NTA, nickel-nitrilotriacetic acid; RAR, retinoic acid receptor; hsp, heat shock protein; hip, hsp70-interacting protein; hsp, hsp70/hsp90 organizing protein.
dependent on the C-terminal hsp70 binding domain of BAG-1. Moreover, the inhibiting effect of BAG-1 on DNA binding of GR (13) can be overcome in a cell-free system by supplementing with increasing amounts of hsp70 (40).

Both C- and N-terminal deletions deprive BAG-1 of its ability to inhibit GR function (38). Whereas an hsp70 interaction domain has been identified and characterized by crystallography and NMR analysis in the C-terminal region (41, 42), the function of the N-terminal part seems less clear. It has been speculated that a serine- and threonine-rich E_X domain may be necessary for the inhibitory function of BAG-1 (38). However, it also is conceivable that the described DNA binding domain is important for inhibition of GR function.

Here we present a detailed mutational analysis of the N-terminal part of BAG-1 with the aim to 1) describe the unusual DNA binding domain in more detail, and 2) dissect the contributions of the E_X motif domain and the DNA binding domain to the inhibition of GR-dependent transcription. We demonstrate here that, surprisingly, the first eight amino acids of BAG-1 are required for the inhibitory effect of BAG-1 on GR, whereas the E_X domain is dispensable. Moreover, the binding of BAG-1 to DNA is because of the positive electric charge at the N terminus. Mutations that inhibit binding to DNA cannot be functionally rescued by overexpressing BAG-1 with a point mutation abolishing its interaction with hsp70, indicating that the DNA binding and the hsp70 interaction domain must be present in cis. Here we discuss a model in which the nonspecific DNA-binding motif of BAG-1 is essential for inhibiting GR function and becomes specific due to interaction with other factors like GR.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfection—Human neuroblastoma SK-N-MC cells (ATCC HTB-10) and COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS), 36 mg/liter sodium pyruvate, 100 units/ml penicillin, 100 mg/ml streptomycin sulfate, 0.25% MgCl₂, and 0.8% glucose at 37 °C and 10% CO₂.

Two days before transfection, cells were seeded into medium containing 10% charcoal-stripped, steroid-free FCS. Dextan T-70 (Amersham Biosciences) was used for charcoal-stripping of FCS (43). Cells were harvested at about 70–90% confluency, and about 0.5 to 1 × 10⁶ cells were resuspended in 400 µl of electroporation buffer (50 mM K₂HPO₄, 20 mM KCl, pH 7.35, 250 mM MgCl₂). 0.5 µg of steroid-responsive firefly luciferase reporter plasmid MTV-Luc (44), 4 µg of pRKTGR that expresses human GR (45), and 50 µg of pMD18T vector (46) were added, and the reaction was incubated at 37 °C. After 10–30 min, absorption was measured at 405 nm in a multimodector (DynaTech MR5000).

When using the Renilla luciferase expression plasmid in combination with the firefly reporter plasmid, cell extracts were scraped in 200 µl of lysis buffer provided and prepared according to the manufacturer’s recommendation (Promega Inc.). firefly and Renilla luciferase activities were measured on a 96-well plate in 50 µl of cell extract in the automatic luminescence on a 96-well plate.

Protein Expression and Purification—Histidine-tagged BAG-1 encoding plasmids were grown in pBl2 lys bacteria (Invitrogen). Lysis and purification was performed using Ni-NTA-agarose according to the manufacturer’s recommendations (Qiagen). The histidine tag was removed by cleavage with tobacco edge virus protease for 6 h at 30 °C (Invitrogen). The cleaved proteins were rebound to Ni-NTA-agarose columns again to remove traces of uncleaved proteins and purified afterward with Bio-Spin columns (Bio-Rad) to remove dithiothreitol from cleavage buffer and traces of liquid columns. Protein concentrations were determined using the BCA Protein Assay kit (Pierce).

DNA Binding and Gel Shift Assays—DNA binding of BAG-1 was essentially performed as described (39). Briefly, 1 or 2 µg of purified and tobacco edge virus-cleaved BAG proteins (isoforms or mutants) were incubated with 0.2 ng of 32P-end-labeled 125-bp MIIHID DNA fragment (128), and 0.2 µg of the middle binding buffer (38) was added. The proteins were resolved by native gel electrophoresis. Protein DNA complexes were resolved on native 5% acrylamide gels in TBE buffer and visualized after overnight incubation on x-ray films (Eastman kodak).

Immunodetection of GR and BAG-1 Proteins—25 µg of total protein from whole lysates used for luciferase assays was separated by SDS-PAGE under denaturing conditions (Lambli buffer containing 10% SDS). The proteins were transferred to polyvinylidene difluoride membrane (Schleicher & Schuell). Nonspecific binding to the membrane was blocked by 5% nonfat milk in Tris-buffered saline/Tween buffer, and then specific antibodies were added. BAG protein was detected by addition of a monoclonal anti-BAG antibody (anti-BAG-C18, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) or a monoclonal anti-BAG antibody (Boehringer Ingelheim) followed by addition of horseradish peroxidase-conjugated goat anti-rabbit antibody (Sigma). The HA tag on GR was detected by a monoclonal anti-HA antibody (Boehringer Ingelheim) followed by addition of horseradish peroxidase-conjugated goat anti-rabbit antibody (Sigma). Signals were visualized by enhanced chemiluminescence system solution (Amerham Biosciences).

RESULTS

Deletion of the N-terminal DNA Binding Domain of BAG-1 Abolishes Its Inhibitory Function on GR, Whereas Deletion of the E_X Motif Domain Does Not—BAG-1, the short isoform of BAG-1, is unable to inhibit the transcriptional activity of GR (38). The N-terminal amino acid stretch missing in BAG-1 is compared with BAG-1M contains a serine- and threonine-rich E_X repeat domain and a recently described DNA binding domain (Table I) (39). To clarify which features in the N terminus are required for inhibition of GR, we created two deletion mutants, either missing the E_X domain (BAG-1M Δ11–17, Table I) or the putative DNA binding domain (BAG-1M ΔN70, Fig. 1). These mutants were analyzed in transient transfection assays in two cell lines, COS-7 cells and the neuroblastoma cell line SK-N-MC. Cells were expressed with a reporter plasmid carrying the luciferase gene driven by the GR-sensitive mouse mammary tumor virus (MVT) promoter, a Renilla luciferase reference plasmid, and either an empty expression vector or a vector expressing one of the BAG-1M mutants. The
light units measured for the reporter enzyme were normalized by the light units of the control enzyme (Renilla luciferase). Stimulation after incubation with 100 nM dexamethasone was quantitated with a luminometer and presented as percent activity with the activity of control vector-transfected cells set as 100%. Results represent mean ± S.E. of eight independent experiments performed in sextuplicate. B and C. Representative Western blot of either GR or BAG protein. Whole cell extracts used for luciferase assays were prepared for SDS-PAGE and immunoblotting. Antibodies were directed against either the C terminus of BAG-1 or the HA tag on GR.

TABLE I

| Structure of BAG-1M, BAG-1S, and the mutants BAG-1M ΔN10 and BAG-1MΔ11–67 |
|---------------------------------|---------------------------------|
| DNA, DNA-binding domain; UBI, ubiquitin-like-domain; hsp70-1AD, Hsp70-interacting-domain; E_4X_4, E_4X_4-repeat motif domain. |

| N'  | DNA | UBI | Hsp70-1AD | E_4X_4 | C' |
|-----|-----|-----|-----------|-------|----|
| 1   | 1   | 10  | 67        | 275   |    |
| BAG-1M |
| BAG-1S |
| BAG-1MΔ11–67 |

TABLE II

| Structure of BAG-1 mutated in the DNA binding domain |
|------------------------------------------------------|
| DNA, DNA-binding domain; UBI, ubiquitin-like-domain; hsp70-1AD, Hsp70-interacting-domain; E_4X_4, E_4X_4-repeat motif domain. |

| N'  | DNA | UBI | Hsp70-1AD | E_4X_4 | C' |
|-----|-----|-----|-----------|-------|----|
| 1   | 1   | 10  | 67        | 275   |    |
| BAG-1M |
| BAG-1MΔ9–67 |

**FIG. 1.** Effect of wild type BAG-1M, ΔN10, and Δ11–67 on GR-dependent transcription. A. SK-N-MC cells were transiently transfected with 3.5 μg of GR-responsive MMTV-luciferase (MTV-Luc) reporter gene, 1 μg of an internal control plasmid encoding Renilla luciferase under the control of the SV40 promoter, and 4 μg of a human GR-encoding plasmid (pRK7GRHA). In addition, 6 μg of either an empty expression vector (C = control) or the mutants BAG-1M ΔN10, BAG-1M Δ11–67, or the isoforms BAG-1M and BAG-1S were cotransfected. Cells were transfected using electroporation and replated and cultured for 24 h in fresh medium either with or without 100 nM dexamethasone. Luciferase activities were corrected by Renilla luciferase activities and are presented as percent activity with the activity of vector-transfected cells set as 100%. Results represent mean ± S.E. of eight independent experiments performed in sextuplicate. B and C. Representative Western blot of either GR or BAG protein. Whole cell extracts used for luciferase assays were prepared for SDS-PAGE and immunoblotting. Antibodies were directed against either the C terminus of BAG-1 or the HA tag on GR.

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| 1   | 1   | 10  | 67        | 275   |    |
| BAG-1M |
| BAG-1S |
| BAG-1MΔ11–67 |

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| N'  | DNA | UBI | Hsp70-1AD | E_4X_4 | C' |
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The purified and cleaved proteins were used to examine their ability to bind to DNA. The 125-bp fragment of an HindIII cleavage of DNA from bacteriophage λ was chosen as template. Gel shift assays (Fig. 2C) revealed that mutation of lysines 2–4 to alamines abolished DNA binding, consistent with a recent report (39). However, deletion of the E_4X_4 domain (BAG-1M Δ11–67; Fig. 2D) had no influence on DNA binding. Even additional deletion of two N-terminal amino acids retained DNA binding (BAG-1M Δ9–67; Fig. 2C). BAG-1S, as expected, did not bind to DNA. Therefore, it appears that the first 8 N-terminal amino acids are sufficient to confer the ability of BAG-1 to bind to DNA. Moreover, spacing between the positively charged amino acids lysine and arginine, 9–67 is not important for DNA binding, because these mutants bind to
BAG-1 DNA Binding Domain Required for GR Inhibition

Fig. 2. A and B, bacterially expressed BAG-1 and mutants. BAG-1 isoforms and mutants were cloned into the bacterial expression vector pProexHTa (Invitrogen) that delivered a histidine tail to the N terminus of the proteins. BAG-1 isoforms and mutants were expressed in E. coli, purified using Ni-NTA-agarose columns following the QIAexpressionist protocol (Qiagen Inc., Hilden, Germany). The histidine tail was removed by cleaving with tobacco edge virus protease, and proteins were analyzed by SDS-PAGE. Staining was performed with Coomassie Blue R-250. Noncleaved and cleaved proteins were loaded alternately onto gels. M, BAG-1M; KA, BAG-1M KA; ∆T5, BAG-1M−Thr5; ∆Ala5, BAG-1M +Ala5, ∆2Ala5, BAG-1M +2Ala5, S, BAG-1M ∆11–67, BAG-1M ∆11–67, S, BAG-1S, ∆N10, BAG-1M ∆N10, C and D, DNA binding of BAG-1 isoforms and mutants. Radiolabeled 125-bp fragment from phage λ-DNA/HindIII was incubated with either 1 (1st lane of each mutant) or 2 µg (2nd lane of each mutant) of protein for 30 min at 25 °C, and complexes were separated on an acrylamide gel under native conditions. Lane 0, labeled DNA without protein; other lanes = binding reactions, coding as in A and B. Shown are representative autoradiograms.

Table II

Structure of BAG-1 mutated in the DNA binding domain

| Mutant       | DNA Binding | hsp70 Interaction |
|--------------|-------------|-------------------|
| BAG-1M       | Yes         | Yes               |
| KA           | No          | Yes               |
| ∆Ala5        | No          | Yes               |
| ∆2Ala5       | No          | Yes               |

DNA as efficiently as BAG-1M (Fig. 2, C and D). Therefore, BAG-1M apparently contains a short, unusual DNA binding domain.

Mutants That Inhibit DNA Binding of BAG-1 also Abolish Its Inhibitory Function on GR—The results shown in Fig. 1 suggest that DNA binding of BAG-1 is necessary for its inhibitory effect on GR function. We set out to either strengthen this correlation or to prove that, although this domain per se is necessary, it is not DNA binding but some other property of this domain that causes the inhibitory effect. Therefore, we constructed expression clones of all mutants and tested them in our transient reporter gene assay as described in Fig. 2. We find that BAG-1M ∆9–67, BAG-1M−Thr5, BAG-1M +Ala5, and BAG-1M +2Ala5 all are able to inhibit GR-dependent transcription (Fig. 3A). In contrast, BAG-1S and BAG-1M KA lost the inhibitory effect on GR. Again, the protein levels of GR were comparable throughout the experiments (Fig. 3B), as were the levels of the different BAG-1 mutants (Fig. 3C). Similar data were obtained in HeLa cells (data not shown).

Therefore, the data in Figs. 1–3 demonstrate that all mutants of BAG-1M that are able to bind to DNA inhibit the transcriptional activity of GR, whereas those that are unable to bind to DNA have no influence on GR activity.

The DNA Binding and the hsp70 Interaction Domain of BAG-1 Need to be Present in cis to Inhibit GR Function—Besides the N-terminal domain, deletion of the C-terminal 70 amino acids also abolishes the effect of BAG-1 on GR (40). Because these amino acids contain the interaction domain with hsp70, it has been proposed that interaction with hsp70 is necessary for the function of BAG-1. This raises the question whether the domains responsible for interaction with hsp70 and for binding to DNA are required to be present in cis. Therefore, we first used a point mutation of BAG-1 that abolishes interaction with hsp70 (R237A BAG-1M (42)). In our transient reporter gene assay, this mutant abolishes the inhibition of GR activity by BAG-1 (Fig. 4A, lane 4). This proves that the previously reported inability of a C-terminally truncated BAG-1 protein to inhibit GR is actually due to the inability to interact with hsp70 rather than to some other concomitant consequence of the deletion. To test whether a BAG-1 protein that is unable to interact with hsp70, but has an intact DNA binding domain, can rescue the inhibitory function of a DNA-binding mutant bearing an intact hsp70 interaction domain, we concomitantly expressed a DNA-binding mutant (KA) with an hsp70 interaction mutant in our reporter assay. Although these proteins are expressed to a comparable level as wild type BAG-1 (Fig. 4B), no inhibition of GR-dependent reporter gene transcription was detected (Fig. 4A, lanes 5 and 6). We also show that the hsp70 interaction mutant does not prevent inhibition of GR by BAG-1M (Fig. 4A, lanes 7 and 8). The levels of GR were the same throughout our experimental conditions (Fig. 4C). We conclude that the N-terminal domain and the hsp70 interaction domain of BAG-1 need to be present at the same time and on the same molecule.

DISCUSSION

BAG-1 displays a remarkable functional versatility by participating in regulation of apoptosis via interaction with Bel-2, by functioning as a modulator of chaperone activity via interaction with hsp70, by enhancing transcription in general (50), by regulating neuronal differentiation (3), by mediating stress signaling via regulating Raf-1/ERK (51), and by modulating nuclear receptor-dependent transcription (shown here and see
mately, it has been reported recently (52) that distinct BAG-1 isoforms have different anti-apoptotic functions. In the present study, we focus on the requirements for BAG-1 to inhibit GR-depen
tendent transcription. It has been speculated that the serine- and threonine-rich E$_X$X$_Y$ motif domain is necessary for the inhibition of GR function by BAG-1 (38). However, we demonstrate that deletion of the entire E$_X$X$_Y$ motif domain does not affect the ability of BAG-1 to counteract GR-dependent transcription. This explains the recent finding that mutations of phosphorylation sites within this domain retain the activity of BAG-1 (40). Against expectation, we discovered that a small DNA binding domain of only 8 amino acids is necessary for inhibition of GR function. This DNA binding domain is uncommon for transcriptional regulatory proteins, because DNA binding appears to be nonspecific (39). The nonspecificity of DNA binding is in line with our observation that spacing of the two positively charged stretches of three lysines and three arginines is not important for DNA binding. This suggests an electrostatic interaction of these positive charges with the negatively charged phosphate backbone of DNA.

Another domain of BAG-1 essential for inhibition of GR-depen
tendent transcription is the hsp70 interaction domain (38). By binding. This suggests an electrostatic interaction of these three lysines and three arginines is not important for DNA binding and function of the protein but that interaction with hsp70 indeed is necessary. Moreover, we demonstrate that binding to hsp70 and binding to DNA have to be linked on the same molecule, i.e. a mixture of two BAG-1 proteins that either carried a mutation in the one or the other domain showed no effect on GR-dependent transcription.

What are the structural requirements of BAG-1 for its effect on other nuclear receptors? In the case of the androgen receptor BAG-1 enhances transcription, but the domains required are similar at first sight, because the N-terminal domain of the long isoform BAG-IL and the hsp70 interaction domain are both required (9). However, it appears that the N-terminal domain of BAG-1L is necessary only for nuclear targeting, because BAG-1M is inefficient unless it is forced to the nucleus by adding a targeting sequence (9). Similarly, the vitamin D receptor also has been reported to be enhanced by BAG-1L but not by BAG-1M. Moreover, the hsp70 interaction domain is required (10). It should be noted however, that more recently an inhibition of vitamin D receptor by BAG-1L has been reported (11). In the case of the retinoic acid receptor, an inhibition by BAG-1M has been demonstrated (12), but no domain analysis was conducted so far.

Thus, taken these reports on the effects of BAG-1 on nuclear receptors together, it seems clear that, although the effect of BAG-1 can be either inhibitory or stimulatory, the N-terminal and C-terminal domains are required. We could prove that the function of the C terminus is indeed the interaction with hsp70. The N terminus of BAG-1L has been suggested to be important for nuclear targeting of BAG-1. However, GR and the retinoic acid receptor are inhibited also by BAG-1M. Moreover, BAG-1M can be translocated into the nucleus upon activation of GR by hormone (40). Thus, we propose that the additional N-terminal amino acids of BAG-1L are necessary only for those receptors that do not promote nuclear transport of BAG-1M. This is also supported by the observation that BAG-1M is effective on vitamin D receptor upon forced nuclear targeting (9). We discovered that the N-terminal 8 amino acids of BAG-1M, which probably represent the entire DNA binding domain, are also required for its function on GR. It will be interesting to evaluate the function of this domain for the effect of BAG-1M on other nuclear receptors.

Regarding the function of the DNA binding domain of BAG-1M, it is of note that BAG-1M binds nonsequence-specifically to DNA. Its intracellular concentration can be estimated from careful analyses in a variety of tumor cell lines (30, 35) to be one molecule per 10$^{10}$–10$^{100}$ kb of DNA. Moreover, it is a predominately cytosolic protein (30, 40). Therefore, other factors must determine when and where BAG-1M binds to DNA. We propose the following model: BAG-1 is transported into the nucleus along with GR upon activation of the receptor with hormone (40). GR interacts with the chromatin at glucocorticoid re
cponse elements. The first step in GR-mediated transactivation presumably is remodeling of the local chromatin structure by cofactors recruited by GR (53). Once the chromatin is restruc
tured, BAG-1 gains access to the DNA, which interferes with the further functions of GR for efficient transactivation. Access of BAG-1 to the DNA might be promoted by the rapid exchange with regulatory sites of GR (54).

The guidance of a nonsequence-specific DNA-binding protein to specific chromosomal loci as suggested here for BAG-1 is not without precedence; Cdc6 is an essential protein in yeast which is recruited to replication origins in the G$_1$ cell cycle phase by another replication factor, origin recognition complex protein 1 (55). It is intriguing not only that cdc6 alone, like BAG-1, binds nonspecifically to DNA but also that site-directed mutagenesis identified the basic protein motif KRKK as essential for DNA binding and function of the protein (56). Apparently, this motif
is very similar to the basic motif of the N terminus of BAG-1 that we demonstrate is not only essential for binding to DNA but also for functional integrity with respect to the inhibition of GR-dependent transcription.

Finally, because we demonstrate that DNA binding and interaction with hsp70 are required in cis, it is likely that hsp70 is required during this process, i.e. hsp70 might be present during chromatin remodeling or even promoting it. In general, chaperones like hsp70 and hsp90 are considered to act in the cytosol. However, nuclear translocation of hsp70 along with hormone-activated GR and BAG-1 has been reported (40). In addition, there is ample evidence that hsp90 is involved in subcellular trafficking and chromatin recycling of GR (57, 58), expanding the original observation that the Hsp90/Hsp70-based chaperone machinery of reticulocyte lysate could dissociate the hormone free GR from DNA and regenerate the non-DNA-binding GR-Hsp90 heterocomplex (59). Moreover, a non-DNA-binding GR-Hsp90 heterocomplex (59). Moreover, a question is whether the described disassembly of transcriptional regulatory complexes by p23 requires ATP (61), as the requirement of hsp90 or hsp70 for the effect of p23 remains to be elucidated.

In summary, overwhelming evidence has accumulated that molecular chaperones and associated proteins like BAG-1 have distinct functions in the nucleus. Obviously, DNA binding of BAG-1 is a clear indication of a nuclear function of this protein. Our model proposed above also implies that BAG-1 can contribute to promote disassembly of transcriptional regulatory complexes by an Hsp70-based process, similarly to p23, which may act via hsp90. This model is also consistent with the observation that DNA-independent transrepression by GR is not inhibited by BAG-1 (38). Future experiments will show whether the DNA binding domain is important for other functions of BAG-1 and whether the model proposed here can also be applied to other nuclear receptors.

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