

BAG1 plays a critical role in regulating recovery from both manic-like and depression-like behavioral impairments

Sungho Maeng*, Joshua G. Hunsberger*, Brandon Pearson*, Peixiong Yuan*, Yun Wang*, Yanling Wei*, Joseph McCammon*, Robert J. Schloesser*, Rulun Zhou*, Jing Du*, Guang Chen*, Bruce McEwen^{†‡}, John C. Reed[§], and Husseini K. Manji*[¶]

*Laboratory of Molecular Pathophysiology and Experimental Therapeutics, Mood and Anxiety Disorders Program, National Institute of Mental Health, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892; [†]Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10021; and [§]Burnham Institute for Medical Research, La Jolla, CA 92037

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Recent microarray studies with stringent validating criteria identified *Bcl-2*-associated athanogene (*BAG1*) as a target for the actions of medications that are mainstays in the treatment of bipolar disorder (BPD). *BAG1* is a Hsp70/Hsc70-regulating cochaperone that also interacts with glucocorticoid receptors (GRs) and attenuates their nuclear trafficking and function. Notably, glucocorticoids are one of the few agents capable of triggering both depressive and manic episodes in patients with BPD. As a nexus for the actions of glucocorticoids and bipolar medications, we hypothesized that the level of *BAG1* expression would play a pivotal role in regulating affective-like behaviors. This hypothesis was investigated in neuron-selective *BAG1* transgenic (TG) mice and *BAG1* heterozygous knockout (+/−) mice. On mania-related tests, *BAG1* TG mice recovered much faster than wild-type (WT) mice in the amphetamine-induced hyperlocomotion test and displayed a clear resistance to cocaine-induced behavioral sensitization. In contrast, *BAG1*+/− mice displayed an enhanced response to cocaine-induced behavioral sensitization. The *BAG1* TG mice showed less anxious-like behavior on the elevated plus maze test and had higher spontaneous recovery rates from helplessness behavior compared with WT mice. In contrast, fewer *BAG1*+/− mice recovered from helplessness behavior compared with their WT controls. *BAG1* TG mice also exhibited specific alterations of hippocampal proteins known to regulate GR function, including Hsp70 and FKBP51. These data suggest that *BAG1* plays a key role in affective resilience and in regulating recovery from both manic-like and depression-like behavioral impairments.

FKBP51 | lithium | valproate | mood disorders | resilience

Bipolar disorder (BPD) is one of the most severely debilitating medical illnesses and affects the lives and functioning of millions worldwide. A number of studies indicate that for a large percentage of patients, outcome is quite poor (1), with high rates of relapse, chronicity, lingering residual symptoms, subsyndromes, cognitive and functional impairment, psychosocial disability, and diminished well being (2–4). Suicide is estimated to be the cause of death in up to 15% of individuals with BPD, and in addition to suicide, many other deleterious health-related effects are increasingly being recognized (5). In light of this tremendous morbidity and mortality, it is striking that no new treatments have been developed specifically for BPD in 40 years. With the exception of lithium, every alternative treatment currently used for BPD was initially developed for other illnesses (most notably epilepsy or schizophrenia) and then subsequently used in BPD (1, 6). This lack of specific medication development for BPD is undoubtedly due, at least in part, to the fact that our understanding of the underlying neurobiology of the disorder is in its infancy.

Syndromically and symptomatically, BPD is a complex illness encompassing various degrees of disturbances of emotions, behav-

ior, thought and cognition, and hedonic and motoric drive (1). Indeed, the hallmark of BPD appears to be the predilection to “overshoot” into full-blown affective episodes in the face of stressors (6). Thus, a number of stressors (including psychological, hormonal, and pharmacological) that induce modest, transient perturbations in healthy individuals are capable of inducing full-blown, sustained mood episodes in individuals with a bipolar diathesis. At present, the precise cellular mechanisms underlying this loss of homeostasis and clinical manifestation of affective symptomatology, that is, mania and/or depression, remain to be fully elucidated. However, it is noteworthy that considerable clinical data have shown that glucocorticoids are one of the few agents capable of triggering both depressive and manic episodes in patients with BPD (reviewed in ref. 6).

Of relevance to the ability of glucocorticoids to trigger both manic and depressive episodes, Wei *et al.* (7) recently examined the behavioral phenotype of glucocorticoid receptor (GR)-overexpressing transgenic (TG) mice. These mice displayed a significant increase in depression-like behaviors relative to wild-type (WT) (control) mice. Additionally, the mice showed enhanced sensitization to cocaine and were also supersensitive to antidepressants. Together, these intriguing data suggest that these mice show an enhanced propensity toward the development of both depression-like and manic-like episodes, thereby recapitulating certain facets of BPD.

Moreover, studies have shown that two pharmacologic agents that are the mainstay of treatment for BPD, lithium and valproate (VPA), regulate the expression of a protein known to modulate GR function (8), despite the fact that these agents have highly dissimilar chemical structures (a monovalent cation and a fatty acid). Specifically, chronic lithium or VPA, when administered in therapeutically relevant paradigms, robustly up-regulate the GR chaperone protein *BAG1* (8). Consistent with the known actions of *BAG1* on GR trafficking and function, previous studies have demonstrated that lithium/VPA-induced *BAG1* up-regulation attenuates GR nuclear translocation and also attenuates the activity of a reporter gene driven by GRs (8). Notably, these effects were only observed

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[†]To whom correspondence may be addressed. E-mail: mcewen@mail.rockefeller.edu.

[¶]To whom correspondence may be addressed at: Mood and Anxiety Disorders Program, National Institute of Mental Health, Building 35, 1C-912, 35 Convent Drive, Bethesda, MD 20892. E-mail: manjih@mail.nih.gov.

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differences were observed between *BAG1* TG and WT mice with respect to levels of dopamine transporter (DAT), catechol-*O*-methyltransferase (COMT), and transcription factors previously implicated in control of expression of these genes: Fos B and phospho-CREB (cAMP-responsive element binding protein) (Fig. S3).

Discussion

In this report, we present evidence that the level of neuronal BAG1 expression plays an important role in regulating affective-like behaviors, a finding with translational implications for BPD. In BPD, both physical and psychosocial stresses are capable of destabilizing the illness and inducing sustained manic or depressive episodes. Thus, a number of stressors that induce modest, transient perturbations in healthy individuals are capable of inducing sustained mood episodes in individuals with a bipolar diathesis. The primary function of *BAG1* concerns its interaction with *Hsp70* family molecular chaperones, and thus BAG1 can be considered to be an anti-cell stress protein. *BAG1* displays a variety of cytoprotective activities and effects on signal transduction, transcription, and cell-surviving pathways, all suggestive of a role in overcoming cellular stress (reviewed in refs. 9, 24, and 25).

BAG1 is known to interact with GRs and attenuate their nuclear trafficking and function (23, 26). In this context, it is noteworthy that considerable clinical data have shown that glucocorticoids are one of the few agents capable of triggering both depressive and manic episodes in patients with BPD (reviewed in ref. 6). Furthermore, GR-overexpressing mice show an enhanced predilection toward the development of both depression-like and manic-like episodes (7). Finally, chronic treatment with lithium or VPA, which enhance recovery from spontaneous as well as stress-induced affective episodes (discussed in ref. 27), robustly up-regulate the GR chaperone protein BAG1 (8). In the present work, we demonstrated a role for neuronal BAG1 overexpression in regulating recovery from manic-like and depression-like behavioral states that are known to be enhanced by stress. In keeping with its role in regulating GR nuclear translocation and transactivation, we also demonstrated that BAG1 overexpression resulted in higher hippocampal levels of Hsp70 and lower levels of FKBP51 without changing overall GR levels or levels of Bcl-2, ERK, or pERK. Our findings thus provide additional support for an important role of GRs in mania and depression, although other GR-independent functions of BAG1 may also contribute.

Therefore, therapies aimed at enhancing *BAG1* function may ultimately be capable of treating both the manic and depressive phases of BPD. In this regard, the up-regulation of BAG1 by mood stabilizers represents a finding that meets several important criteria for assessing potential clinical therapeutic relevance (8). Specifically, (i) the effect of mood stabilizers on BAG1 protein expression and GR trafficking are common to both lithium and VPA; (ii) BAG1 up-regulation by lithium and VPA occurs in the hippocampus, a brain region known to be involved in critical affective neuronal circuits; (iii) BAG1 up-regulation occurs in the CA3 subfield, a region where robust protection against stress-induced apical dendritic retraction by lithium was recently observed (VPA was not examined in that study) (28); (iv) the effect of lithium and VPA on BAG1 and GR trafficking/function occurs at therapeutic concentrations both *in vivo* and *in vitro*; (v) similar to the clinical therapeutic effects, the changes in BAG1 protein expression and GR trafficking/function are observed only after chronic (and not after acute) administration; (vi) the effects are specific to the mood stabilizers lithium and VPA; chronic administration of an antidepressant, a psychostimulant, or an antipsychotic does not affect BAG1; and (vii) the effects of BAG1 overexpression on GR function were manifest at high (pathophysiologic) glucocorticoid levels (8).

The *BAG1* TG mice appeared normal in terms of physical appearance and performance on a variety of neurological, sensory, locomotor, and learning and memory tests. On behavioral tests relevant to affective-like behavior, these mice showed increased exploratory activity in relatively stressful conditions, enhanced spontaneous recovery from helplessness, faster recovery from amphetamine-induced hyperactivity, and resistance to cocaine-induced behavioral sensitization (Figs. 1–4). Collectively, the behavioral pattern of *BAG1* TG mice suggests a phenotype associated with enhanced recovery from certain affective-like behaviors associated with BPD. In contrast, we found that the *BAG1*+/- mice showed an enhanced sensitivity to cocaine (Fig. 4). Furthermore, fewer *BAG1*+/- recovered spontaneously from helplessness (Fig. 2 *D* and *F*).

Because BAG1 emerged as a candidate gene from previous microarray studies of lithium and VPA, the question of whether or not rodents treated with lithium and VPA show behavioral similarities to *BAG1* TG mice is important. Despite some methodological differences between previous pharmacologic studies and this report, the overall data do suggest strong similarities between lithium-treated (and to a lesser extent VPA-treated) mice and BAG1 TG mice (reviewed in ref. 6).

Studies of suppression of GR action by the BAG1 proteins have identified two primary modes of action: (i) inhibition of GR translocation to the nucleus and (ii) a more direct nuclear action at the level of regulation of the transactivation function of the receptor (23, 29). Consistent with this mode of action, we have observed in studies with lithium and VPA an attenuation of GR nuclear translocation and an inhibition of the activity of a reporter gene driven by GRs (8). Notably, these effects were only observed when using high (pathophysiologic) glucocorticoid concentrations. Similarly, GR function at lower glucocorticoid levels was unaffected by BAG1 up-regulation (8). Chronic lithium and VPA (both of which up-regulate BAG1) have been shown to enhance recovery from both the depressive and manic episodes associated with exogenous or endogenous (i.e., Cushing disease) elevations of glucocorticoids (discussed in ref. 6). The data presented here suggest that the interaction between GRs and BAG1 contributes to enhanced recovery from manic- and depression-like behavioral impairments observed in TG mice overexpressing BAG1 in neurons.

Because BAG1 regulates GR function primarily by attenuating its nuclear translocation and transactivation functions, it is not altogether surprising that we did not observe changes in total GR protein levels. However, we did observe alterations in the levels of two GR chaperone proteins: Hsp70 and FKBP51. We found that *BAG1*-overexpressing mice had higher hippocampal levels of Hsp70. BAG1 is known to bind to and stabilize Hsp70, thereby leading to Hsp70 accumulation in cells (10). Notably, BAG1-induced activation of Hsp70 has been postulated to be important for neuroprotection (26). Thus, BAG1-overexpressing TG animals have increased levels of Hsp70 and exhibit relative resistance to *in vitro* and *in vivo* insults (10, 26).

With respect to FKBP51, this immunophilin is known to attenuate GR function (22). It is a potent inhibitor of GR binding, and glucocorticoid resistance in three New World primates is associated with overexpression of FKBP51 (22). Most recently, it has been demonstrated that a combination of a transcriptionally incompetent GRs and overexpression of the GR cochaperone FKBP51 (22) contributes markedly to glucocorticoid resistance in squirrel monkeys. Together, the data suggest that the decreases in hippocampal FKBP51 observed in *BAG1*-overexpressing mice may well represent a compensatory change in response to attenuated GR function.

Overall, this work shows that brain overexpression of BAG1, a previously identified biochemical target for structurally dissimilar mood stabilizers, is associated with enhanced recovery from certain affective states. We are not aware of any human genetic studies that

implicate *BAG1* as a gene associated with mood disorders. However, *BAG1* is a chaperone protein that interacts with GRs, Hsp70, and, thus, FKBP5. Binder *et al.* (30) investigated single nucleotide polymorphisms (SNPs) in the *FKBP5* gene (6p21.321.2) and seven other genes thought to play a role in *HPA* axis regulation. Two SNPs in *FKBP5* were associated with rate of treatment response in two independent samples. Most recently, a completely independent study replicated an association between *FKBP5* markers and treatment response to antidepressant treatment when using the categorical “responder” and “remitter” outcomes (31). Indeed, this study also found an association between the *FKBP5* gene and depression itself. Finally, very recent data have also shown that variants in the *FKBP5* gene are associated with an increased likelihood of developing PTSD (32); these findings fit nicely with the putative role of these molecules in mediating affective resilience. Ongoing studies are attempting to elucidate the complex interactions between *FKBP5* and *BAG1* in the pathophysiology and treatment of mood disorders.

In conclusion, the data presented here suggest that therapies designed to enhance *BAG1* function may lead to treatments for both the manic and depressive phases of BPD, as well as modulating the effects of stress. Further investigation of the role of *BAG1* will help clarify the mechanisms of interaction between mood stabilizers and glucocorticoids in mood disorders.

Materials and Methods

Animals. Mice expressing FLAG-mouse *BAG-1* (mBAG-1) under the control of the neuron specific enolase promoter and WT FVB/n mice used in the study are described in a previous report (10). All these mice had the same genetic background and were bred in-house. *BAG1* heterozygous knockout (+/-) mice were generated as described (25) and backcrossed to mice of C57BL/6J (Jackson Laboratories) background for seven generations. All animals were housed under constant temperature (22 ± 1°C) and 12 h light/dark cycle with access to water and food ad libitum. All of the behavioral experiments were performed between 8 and 11 weeks of age. All animal treatments, procedures, and care were approved by the National Institute of Mental Health Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Animals*.

General Evaluation. Assessment of physical appearance, growth and development, and neurological and sensory functions were performed following guidelines established by Crawley (33).

1. Belmaker RH (2004) Bipolar disorder. *N Engl J Med* 351:476–486.
2. Fagioliini A, *et al.* (2005) Functional impairment in the remission phase of bipolar disorder. *Bipolar Disord* 7:281–285.
3. Revicki DA, Matza LS, Flood E, Lloyd A (2005) Bipolar disorder and health-related quality of life: Review of burden of disease and clinical trials. *Pharmacoeconomics* 23:583–594.
4. Tohen M, *et al.* (2003) The McLean–Harvard First-Episode Mania Study: Prediction of recovery and first recurrence. *Am J Psychiatry* 160:2099–2107.
5. Kupfer DJ (2005) The increasing medical burden in bipolar disorder. *J Am Med Assoc* 293:2528–2530.
6. Goodwin FK, Jamison KR (2007) *Manic-Depressive Illness: Bipolar and Recurrent Unipolar Disorders* (Oxford Univ Press, New York).
7. Wei Q, *et al.* (2004) Glucocorticoid receptor overexpression in forebrain: A mouse model of increased emotional lability. *Proc Natl Acad Sci USA* 101:11851–11856.
8. Zhou R, *et al.* (2005) The anti-apoptotic, glucocorticoid receptor co-chaperone protein *BAG-1* is a long-term target for the actions of mood stabilizers. *J Neurosci* 25:4493–4502.
9. Kermer P, *et al.* (2002) *Bag1* is a regulator and marker of neuronal differentiation. *Cell Death Differ* 9:405–413.
10. Kermer P, *et al.* (2003) *BAG1* over-expression in brain protects against stroke. *Brain Pathology* 13:495–506.
11. Kurt M, Arik AC, Celik S (2000) The effects of sertraline and fluoxetine on anxiety in the elevated plus-maze test in mice. *J Basic Clin Physiol Pharmacol* 11:173–180.
12. Petit-Demouliere B, Chenu F, Bourin M (2005) Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology (Berlin)* 177:245–255.
13. Maier SF (1984) Learned helplessness and animal models of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 8:435–446.
14. Chourbaji S, *et al.* (2005) Learned helplessness: Validity and reliability of depressive-like states in mice. *Brain Res Brain Res Protoc* 16:70–78.
15. Itoh T, Tokumura M, Abe K (2004) Effects of rolipram, a phosphodiesterase 4 inhibitor, in combination with imipramine on depressive behavior, CRE-binding activity and BDNF level in learned helplessness rats. *Eur J Pharmacol* 498:135–142.
16. Camacho A, Akiskal HS (2005) Proposal for a bipolar-stimulant spectrum: Temperament, diagnostic validation and therapeutic outcomes with mood stabilizers. *J Affect Disord* 85:217–230.
17. O'Donnell KC, Gould TD (2007) The behavioral actions of lithium in rodent models: leads to develop novel therapeutics. *Neurosci Biobehav Rev* 31:932–962.
18. Post RM, Weiss SR (1989) Sensitization, kindling, and anticonvulsants in mania. *J Clin Psychiatry* 50(Suppl):23–30; discussion 45–27.
19. Post RM, Weiss SR, Pert A (1988) Cocaine-induced behavioral sensitization and kindling: implications for the emergence of psychopathology and seizures. *Ann N Y Acad Sci* 537:292–308.

Behavioral Tests. The open-field test (OFT), the active and passive avoidance tests, the EPM, the FST, the learned-helplessness paradigm, the amphetamine-induced hyperlocomotion test, and cocaine-induced behavioral sensitization were conducted in WT and *BAG1* TG mice as specified in *SI Methods*. The Ethovision system (Noldus) was used to analyze data from the EPM, the FST, the amphetamine-induced hyperlocomotion test, and cocaine-induced behavioral sensitization. The learned-helplessness paradigm and cocaine-induced behavioral sensitization were also conducted in WT and *BAG1*+/- mice as specified in *SI Methods*. The Clever Systems (CleverSys, Inc.) was used to analyze data from the cocaine-induced behavioral sensitization experiment.

Hippocampal Cellular Fractionations, Striatal Sample Preparation, and Immunoblotting. Hippocampus and striatum were removed from drug-naïve WT and *BAG1* TG mice, and the cellular fractionation of hippocampal samples was conducted as specified in *SI Methods*. Frozen striatal tissues were homogenized in lysing buffer containing protease inhibitor and phosphatase inhibitor cocktails (Sigma) and then spun at 12,000 × *g* for 10 min to remove debris. Immunoblotting of fractionated hippocampal samples and striatal protein extracts was conducted as described previously (34), using antibodies from Santa Cruz Biotechnology for *BAG-1*, Hsp70, Bcl-2, DAT, Fos B, and COMT, from Abcam for GRs and FKBP51, and from Cell Signaling for phospho-ERK, total-ERK, and phospho-CREB. An equal amount of total protein from different animals was loaded on the gel for comparison. The amount of protein loaded on the gel was within a linear detection range for the protein being detected. The immunocomplex was detected by using chemiluminescence (ECL kit; GE Healthcare). Quantitation of the immunoblots was performed by densitometric scanning of the film by using a Kodak Image Station (4000R Digital Imaging System). An aliquot of pooled “standard” mouse brain (hippocampus) was run on one lane of each gel. Data were normalized against the standard mouse brain to minimize between-blot variability.

Statistical Analysis. All results are expressed as mean ± SEM. Statistical comparisons were performed with the use of 1- or 2-way ANOVA with Tukey's post hoc test or a Student *t* test to identify significant differences. In all cases, *P* < 0.05 was considered statistically significant.

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20. Gould TD, *et al.* (2007) Beta-Catenin overexpression in the mouse brain phenocopies lithium-sensitive behaviors. *Neuropsychopharmacology* 32:2173–2183.
21. Swaab DF, Bao AM, Lucassen PJ (2005) The stress system in the human brain in depression and neurodegeneration. *Ageing Res Rev* 4:141–194.
22. Westberry JM, Sadosky PW, Hubler TR, Gross KL, Scammell JG (2006) Glucocorticoid resistance in squirrel monkeys results from a combination of a transcriptionally incompetent glucocorticoid receptor and overexpression of the glucocorticoid receptor co-chaperone FKBP51. *J Steroid Biochem Mol Biol* 100:34–41.
23. Schneikert J, Hubner S, Martin E, Cato AC (1999) A nuclear action of the eukaryotic co-chaperone RAP46 in downregulation of glucocorticoid receptor activity. *J Cell Biol* 146:929–940.
24. Takayama S, *et al.* (1995) Cloning and functional analysis of *BAG-1*: A novel Bcl-2-binding protein with anti-cell death activity. *Cell* 80:279–284.
25. Gotz R, *et al.* (2005) *Bag1* is essential for differentiation and survival of hematopoietic and neuronal cells. *Nat Neurosci* 8:1169–1178.
26. Liman J, *et al.* (2005) Interaction of *BAG1* and Hsp70 mediates neuroprotectivity and increases chaperone activity. *Mol Cell Biol* 25:3715–3725.
27. Boyle MP, Kolber BJ, Vogt SK, Wozniak DF, Muglia LJ (2006) Forebrain glucocorticoid receptors modulate anxiety-associated locomotor activation and adrenal responsiveness. *J Neurosci* 26:1971–1978.
28. Yuan PX, *et al.* (2001) The mood stabilizer valproic acid activates mitogen-activated protein kinases and promotes neurite growth. *J Biol Chem* 276:31674–31683.
29. Kanelakis KC, *et al.* (1999) Differential effects of the hsp70-binding protein *BAG-1* on glucocorticoid receptor folding by the hsp90-based chaperone machinery. *J Biol Chem* 274:34134–34140.
30. Binder EB, *et al.* (2004) Polymorphisms in *FKBP5* are associated with increased recurrence of depressive episodes and rapid response to antidepressant treatment. *Nat Genet* 36:1319–1325.
31. Lekman M, *et al.* (2008) The *FKBP5*-gene in depression and treatment response: An association study in the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) cohort. *Biol Psychiatry* 63:1103–1110.
32. Binder EB, *et al.* (2008) Association of *FKBP5* polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *J Am Med Assoc* 299:1291–1305.
33. Crawley JN (1999) Behavioral phenotyping of transgenic and knockout mice: Experimental design and evaluation of general health, sensory functions, motor abilities, and specific behavioral tests. *Brain Res* 835:18–26.
34. Chen G, Yuan PX, Jiang YM, Huang LD, Manji HK (1998) Lithium increases tyrosine hydroxylase levels both *in vivo* and *in vitro*. *J Neurochem* 70:1768–1771.