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Role for NUP62 depletion and PYK2 redistribution in dendritic retraction resulting from chronic stress

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Contributed by Bruce S. McEwen, October 3, 2014 (sent for review February 1, 2014; reviewed by James P. Herman)

Genetic evidence suggests cell-type–specific functions for certain nucleoporins, and gene expression profiling has revealed that nucleoporin p62 (NUP62) transcripts are decreased in the prefrontal cortex of major depressives. Chronic stress, which can precipitate depression, induces changes in the architecture and plasticity of apical dendrites that are particularly evident in the CA3 region of the hippocampus. Genetically targeted translating ribosome affinity purification revealed a selective reduction in translated Nup62 transcripts in CA3 of chronically stressed mice, and the Nup62 protein content of nuclei extracted from whole hippocampus was found to be decreased in chronically stressed rats. In cultured cells, phosphorylation of a FAK/proline-rich tyrosine kinase 2 (PYK2) consensus site in the alpha-helical domain of NUP62 (human Y422) is shown to be associated with shedding of NUP62 from the nuclear pore complex (NPC) and/or retention of NUP62 in the cytoplasm. Increased levels of phospho-Y425 Nup62 were observed in cytoplasmic fractions of hippocampi from chronically stressed rats, and immunofluorescence microscopy revealed redistribution of activated Pyk2 to the perinuclear region of stressed pyramidal neurons. Depletion of Nup62 from cultured embryonic day 18 rat hippocampal and cortical neurons resulted in simplification and retraction of dendritic arbors, without disruption of axon initial segment integrity. Thus, at least two types of mechanisms—one affecting expression and the other association with the NPC—could contribute to loss of NUP62 from CA3 pyramidal neurons during chronic stress. Their combined actions may account for the enhanced responsiveness of CA3 apical dendrites to chronic stress and may either be pathogenic or serve to protect CA3 neurons from permanent damage.

nucleoporin p62 | proline-rich tyrosine kinase 2 | chronic stress | dendrite retraction | hippocampus

Substructures of the nuclear pore complex (NPC) are visible by electron microscopy and include an inner scaffold that interfaces the nuclear envelope, a central channel, and two asymmetric rings that project into either the cytoplasm or the nucleus (1). Selective nucleocytoplasmic transport through the NPC is mediated by phenylalanine–glycine (FG)-repeat–containing nucleoporins in the central channel and asymmetric ring structures. Some FG-repeat nucleoporins, including the central channel nucleoporin p62 (NUP62), function in transcription and chromatin organization independently of their roles in transport (2–4). Tissue-specific genetic diseases arising from mutations in nucleoporins have suggested that the NPC and/or its components can mediate cell-type–specific functions (5, 6). For example, the Q391P mutation in NUP62 causes autosomal recessive infantile bilateral striatal necrosis (IBSN), a fatal degeneration of the corpus striatum that occurs during early childhood (7). Although Nup62 null mice are embryonic day 7 lethal (8), human Q391P homozygotes develop past term, suggesting that the mutation affects a specialized function that NUP62 performs in striatal and potentially other neurons.

Chronic psychosocial or restraint stress causes retraction and simplification of apical dendrites in hippocampal neurons in a number of animal models (9–12), an effect that corresponds to impairment of hippocampal-dependent memory tasks (11). Changes in dendritic architecture resulting from chronic stress do not represent an aggregate result of repeated episodes of acute stress, but rather emerge after a threshold level of repeated acute stress is reached. While prompting the hypothalamus pituitary–adrenal axis to increase secretion of adrenal glucocorticoids, stress promotes in hippocampal neurons elevated activity of excitatory amino acids, modifications of cytoskeletal proteins, secretory vesicle depletion, and alterations in expression and receptor activity of neurotrophic factors (12). Intriguingly, impaired neuronal plasticity is thought to be a factor in clinical depression, and gene-expression profiling has revealed that NUP62 transcripts are reduced to 82% of control levels in the prefrontal cortex of major depressives (13).

Here, genetically targeted translating ribosome affinity purification (TRAP) and RNA shotgun sequencing (RNA-Seq) analyses show that Nup62 transcript translation is reduced in the CA3 subfield of hippocampi from chronically stressed mice. Subcellular fractionation reveals that the Nup62 protein content of hippocampal nuclei is reduced in chronically stressed rats. Activated proline-rich tyrosine kinase 2 (PYK2) is found to redistribute to the perinuclear region of the soma of stressed hippocampal neurons, and evidence is presented that tyrosine phosphorylation of NUP62 by PYK2 has a role in reducing hippocampal nuclear NUP62 content. Forced reduction of Nup62

Significance

Associated with depression and cognitive impairment, chronic stress causes reversible dendritic shrinkage particularly evident in the hippocampal CA3 region in several animal models. In further elucidating the molecular events leading to dendritic shrinkage, this study reveals two unexpected mechanisms reduction in translated transcripts of a nuclear pore complex protein, nucleoporin p62 (NUP62), and tyrosine phosphorylation of NUP62, that appear to act cumulatively in chronic stress to reduce NUP62 content in CA3 neurons. Subcellular redistribution of activated proline-rich tyrosine kinase 2 in chronically stressed pyramidal neurons suggests a mechanism for stress-induced tyrosine phosphorylation of NUP62. Furthermore, evidence from cultured hippocampal neurons shows that diminishing the content of NUP62, which functions in nucleocytoplasmic transport and chromatin organization, results in simplification and shortening of dendritic arbors.

Author contributions: Y.K., R.G.H., J.D.G., B.S.M., D.L.B., and D.S.K. designed research; Y.K., R.G.H., J.D.G., R.M., and D.S.K. performed research; R.G.H., J.D.G., R.M., B.S.M., D.L.B., and D.S.K. contributed new reagents/analytic tools; Y.K., R.G.H., J.D.G., B.S.M., D.L.B., and D.S.K. analyzed data; and B.S.M., D.L.B., and D.S.K. wrote the paper.

Reviewers included: J.P.H., University of Cincinnati.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1418896111/-/DCSupplemental.

content in cultured embryonic day 18 (E18) rat hippocampal neurons diminished dendrite length and complexity, consistent with a functional role for loss of NUP62 in altering dendritic architecture in chronic stress.

Results

Decreased NUP62 Content in Hippocampal Neurons Exposed to Chronic Restraint Stress. Rodents were restraint-stressed for 6 h per day for 21 d and killed 18 h after the last restraint to discount effects from acute stress. Reduction of the thymus-to-body-weight ratio was observed in stressed rats (Fig. 1*A*), indicating prolonged exposure to corticosteroids, a consequence of chronic stress. Microarray expression comparison of RNA isolated from chronically stressed or control total mouse hippocampus (14) revealed no significant differences in Nup62 or any other nucleoporins. TRAP analysis of the murine CA3 subfield was performed as described (15), by using a transgenic mouse strain containing a BAC construct with the murine G Protein-Regulated Inducer of Neurite outgrowth 3 (Gprin3) upstream region driving expression of enhanced green fluorescent protein–L10a fusion protein (eGFP–L10a). In the hippocampus, expression of eGFP–L10a from this construct was concentrated in the CA3 subfield (Fig. S1). Hippocampi were resected for TRAP analysis, and transcripts isolated by TRAP were compared by RNA-Seq analyses (Figs. S2–S4). Significant decreases in translated transcripts of the central channel nucleoporins Nup62 and Nup58, and of Seh1, were observed in chronic stress, whereas 26 nucleoporins from other pore substructures remained unchanged or were increased significantly.

Hippocampi resected from control and chronically stressed rats were processed into whole-cell, cytoplasmic, and nuclear extracts. In Fig. 1*B*, the lanes were loaded with extracts derived from equal amounts of starting material to compare relative content in each extracted compartment. Total Nup62 (central channel), Nup98 (ring substructures), and Nup133 (inner scaffold) partitioned with the nuclear fractions, indicating that nuclei were extracted with intact NPCs. Glyceraldehyde 3-phosphate dehydrogenase (Gapdh; a cytoplasmic enzyme) was enriched in the cytoplasmic extracts, indicating effective separation of cytoplasmic and nuclear proteins. Fractionated samples of hippocampus from seven chronically stressed and seven control animals were analyzed by Western blot. Whole-cell and nuclear extracts from stressed animals displayed a significant reduction in Nup62 signals compared with controls (Fig. 1*C*), whereas signals for Nup98 or Nup133 were not reduced (Fig. 1*E*). These data indicate that nuclear Nup62 content was reduced in stressed animals, whereas base components of NPCs remained constant. By inference, the Nup62 content of a population of NPCs in the hippocampus is reduced in chronically stressed animals, and the absence of diminished Nup62 transcripts in microarray analyses of total hippocampus suggests a role for post-translation regulation.

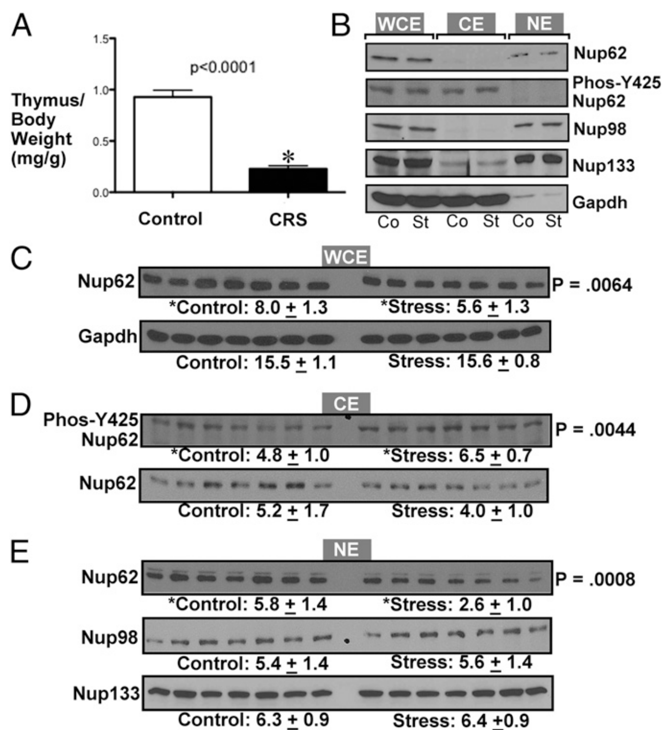

Fig. 1. Chronic stress reduces nuclear Nup62 content and enhances accumulation of cytoplasmic phospho-Y425 Nup62 in hippocampi of male rats. Rats were restrained 6 h per day for 21 d and killed 18 h after the last restraint treatment. (*A*) Weights of thymuses from control and chronically stressed animals (CRS) were normalized to body weight. *Indicates statistically significant data. (*B*) Whole-cell (WCE), cytoplasmic (CE), and nuclear (NE) extracts from the hippocampus of a control (Co) or chronically stressed (St) rats were analyzed by Western blot with antibodies to total Nup62, Phos-Y425 Nup62 (rat position for Y422), total Nup98, total Nup133, and Gapdh. To compare protein content in different compartments, loaded samples were derived from equivalent amounts of starting tissue. (*C*–*E*) Whole hippocampi from seven control or seven chronically stressed (Stress) rats were fractionated and analyzed by Western blot. Relative densities of Western blot signals were compared. Only *P* values <0.05 are shown.

Phosphorylation of a FAK/PYK2 Site on NUP62 Is Induced by Stress Signaling Pathways. Tyrosine phosphorylation of human NUP62 at residue Y422 (rat Y425 and mouse Y426) and ubiquitin conjugation of surrounding sites within the alpha-helical domain (Fig. 2*A*) have been detected in proteomic analyses curated by PhosphoSitePlus (16). Analysis of residues adjacent to Y422 on NUP62 by NetPhos (Version 2.0; ref. 17) revealed consensus with sites phosphorylated by focal adhesion kinase (FAK)/PYK2, and these residues are highly conserved in rat and mouse. A polyclonal antibody was generated to a synthetic phosphopeptide that mimics phospho-Y422 and the conserved surrounding residues on NUP62. The phospho-Y422–specific antibodies were isolated by phosphopeptide affinity chromatography and nonphosphorylated peptide exclusion chromatography. Intriguingly, phospho-Y425 Nup62 partitioned to the cytoplasmic rather than nuclear compartment in rat hippocampal extracts (Fig. 1*B*). Accumulation of phospho-Y425 Nup62 in the cytoplasmic extracts of hippocampi from chronically stressed animals was significantly increased over that of controls (Fig. 1*D*), suggesting a role for phosphorylation at this site in relegating Nup62 to the cytoplasm during chronic stress.

Certain effects of chronic restraint stress, including dendritic remodeling in the CA3, can be mimicked by daily injection of glucocorticoids (9, 18). Hippocampal neurons respond rapidly to glucocorticoids via a G-protein–coupled membrane receptor, and downstream effectors include activation of Pyk2 (19). Activation and nuclear translocation of Pyk2 also has been observed in cultured cells treated with pervanadate [protein tyrosine phosphatase inhibitor (20, 21)] and in hippocampal slices treated with depolarizing concentrations of K^+ (22). To determine how these agents affect Y422 phosphorylation of NUP62, human neuroblastoma cells were treated with 1 μM dexamethasone (a corticosteroid), 30 mM K^+ (depolarizing), or 100 μM pervanadate in the presence or absence of PF562271 [a specific FAK/PYK2 inhibitor (23)]. Western blot analyses of NUP62 immunoprecipitates with phospho-Y422 NUP62 antibody revealed induction of NUP62 at this site after treatment with dexamethasone and weaker induction after treatment with depolarizing concentrations of K^+ or with pervanadate. Phosphorylation of Y422 in these assays was inhibited by PF562271 (Fig. 2*B*). Treatment of neuroblastoma cells with dexamethasone, 30 mM K^+, or pervanadate affected the distribution of NUP62 between cytoplasmic and nuclear compartments, with enhanced accumulation of NUP62 observed in the cytosolic fractions of treated cells, and these effects were blocked by PF562271 (Fig. 2*C* and Fig. S5). The increase in

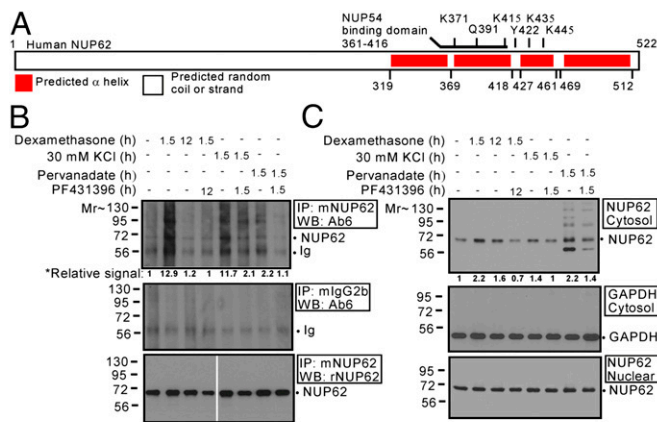

Fig. 2. Induction of protein tyrosine phosphorylation of NUP62 at Y422 by FAK/PYK2 in human neuroblastoma cells enhances accumulation of cytoplasmic NUP62. (*A*) Positions of alpha-helical domain, NUP62–NUP54 heterotrimer binding region, FAK/PYK2 phosphorylation site (Y422), and potential ubiquitination sites (K371, K415, K435, and K445) on NUP62 (16, 53). (*B*) Human neuroblastoma cells were treated with 1 μM dexamethasone, 30 mM KCl, 100 μM pervanadate, and/or PF562271 for the indicated times. Immunoprecipitates (IP:) were derived by using a monoclonal antibody to total NUP62 (mNUP62) or a control monoclonal IgG2b (mIgG2b). Immunoprecipitates were analyzed by Western blot (WB:) with rabbit phosphospecific antibody to NUP62 phosphorylated at Y422 (Ab6) or rabbit antibody to total NUP62 (rNUP62). (*C*) Human neuroblastoma cells were treated with 1 μM dexamethasone, 30 mM KCl, 100 μM pervanadate, and/or 1 μM PF562271 (23) for the indicated times. Nuclear and cytoplasmic extracts representing equal numbers of cells were loaded and analyzed by Western blot with a monoclonal antibody to total NUP62 (NUP62) or to GAPDH. Western blots were processed in parallel, and the NUP62 blot of nuclear fractions was exposed for one-seventh of the time used for the cytoplasmic fractions to facilitate comparison.

cytosolic NUP62 observed in these experiments represented only a small fraction of total NUP62, and a reduction in nuclear NUP62 signal was not detected. Chronic stimulation of these pathways may lead to sufficient cumulative redistribution of NUP62 to result in detectable reduction of NUP62 content in NPCs.

Intracellular Redistribution of Phospho-Y402 PYK2 in Pyramidal Neurons Is Associated with Chronic Stress. Activation of PYK2 is marked by autophosphorylation of Y402 (24). Phospho-Y402 Pyk2 in whole-cell, cytosolic, or nuclear fractions of hippocampus was detected at equivalent levels in control and stressed rats, and total Pyk2 was detected at equivalent levels in whole-cell or cytosolic fractions from control and stressed rats (Fig. S6). These observations suggest that enhanced phosphorylation of NUP62 at Y422 in response to stress signaling may be mediated by changes in the subcellular distribution of the pool of active PYK2. Immunofluorescence microscopy of the CA3 region of the hippocampus from control mice revealed granular phospho-Y402 Pyk2 immunolabeling distributed throughout the molecular and cellular layers (Fig. 3). In stressed mice, immunolabeling of the molecular layers was reduced, whereas diffuse immunolabeling in the perinuclear region of the soma of the cellular layers was enhanced (Fig. 3 and Fig. S7). Redistribution of phospho-Y402 Pyk2 was noted in other fields of the hippocampus but was most apparent in CA3 where dendritic shrinkage is greatest (ref. 11; Fig. S8).

Forced Reduction of NUP62 Content in Cultured Rat Hippocampal Neurons Induces Dendritic Retraction and Simplification. Survival of cultured E18 rat hippocampal neurons transfected after 1 d in vitro (DIV) with NUP62-specific or control small interfering RNAs (siRNAs) was assessed at 10 DIV. A slight reduction in the number of adhered neuronal cells was detected, but it was not significant at this sample size (Fig. 4*A*). To assess how diminished Nup62 affected accumulation of axonal or dendritic proteins, NUP62-specific or control siRNAs were transfected into cultured E18 rat hippocampal neurons at 7 DIV, and cultures were analyzed at 10 DIV by Western blot. Neurons transfected with NUP62 siRNA displayed ~70% decrease in the signal for total cellular Nup62 protein in comparison with neurons transfected with control siRNA (Fig. 4*B*). A mild reduction in the cytoplasmic enzyme glyceraldehyde 3-phosphate dehydrogenase (Gapdh) was observed, an effect that may be associated with dendritic shrinkage and/or diminished cytoplasmic volume (described below). Reductions in Map2 and tau phosphorylated at serine 202 (designation from human tau isoform 2), both of which accumulate preferentially in dendritic processes (25, 26), were observed in neurons transfected with NUP62 siRNA. In contrast, reduction of nonphosphorylated tau (Tau-1 antibody binding), which accumulates preferentially in extended axonal processes (27), was not observed (Fig. 4*B*).

Cultured E18 rat hippocampal neurons at 7 DIV were transfected with NUP62 or control siRNA. Neurons were fixed and analyzed at 10 DIV by immunofluorescence microscopy with antibodies to ankyrin G (Ank3), a marker for the axon initial segment (AIS), and Map2. Dendritic arbors were analyzed quantitatively from deconvolved image stacks. Transfection of hippocampal neurons with NUP62 siRNA at 25 nM for 3 d resulted in moderately reduced total dendritic length, no significant change in the number of primary dendrites, and a significant reduction in dendritic branch vertices (Fig. 5 *A*, *B*, *F*, and *G*). Transfection of hippocampal neurons with NUP62 siRNA at a concentration of 50 nM resulted in a profound reduction in total dendritic length, with remaining dendrites appearing thinner (Fig. 5 *A*, *C*, and *H*). To determine whether the effects of NUP62 depletion on dendritic arbors are shared by other types of cortical neurons, E18 rat neocortical neurons were transfected at 7 DIV with 50 nM NUP62 siRNA and fixed and analyzed at 10 DIV. Reduced overall dendritic length was observed, although the difference was less severe than was observed for hippocampal neurons (Fig. 5 *D*, *E*, and *G*). Rapid and irreversible disassembly of the AIS, including loss of ankyrin G, precedes cell death in cultured neurons (28). Transfection of NUP62 siRNA at DIV 7 did not result in disassembly of the AIS by 10 DIV (Fig. 5 *C* and *E*), and axonal processes remained evident in these cultures.

Rescue experiments were performed by cotransfecting NUP62 siRNA with a plasmid expression construct for V5 epitope-tagged

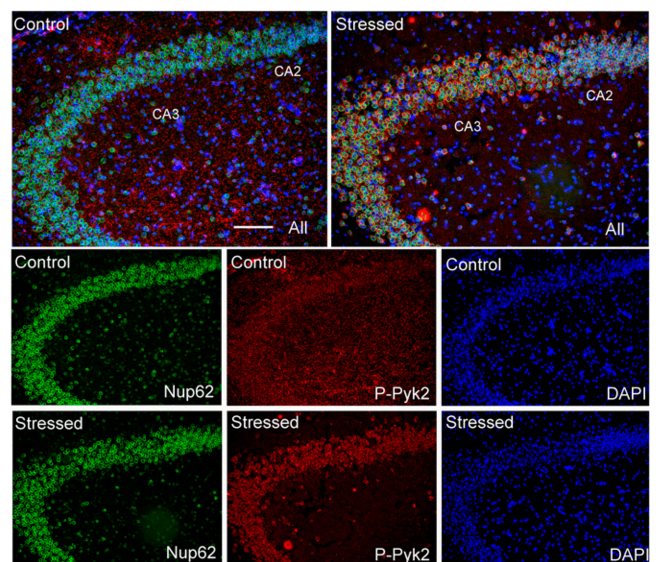

Fig. 3. Changes in the intracellular redistribution of phospho-Y402 Pyk2 in hippocampal neurons after chronic stress. Immunofluorescence microscopy of Nup62 (green pseudocolor) and Pyk2 phosphorylated at Y402 (P-Pyk2; red pseudocolor) in hippocampus of control and chronically stressed mouse brain. Nuclei were stained with DAPI (blue). (Bar: 100 μm.)

Fig. 4. Reduced Nup62 content in cultured E18 rat hippocampal neurons is associated with reductions in MAP2 and phosphorylated tau. (*A*) Survival of E18 hippocampal neuronal cells transfected at 1 DIV with control (Con.) or NUP62 siRNA assessed at 10 DIV. Plated neuronal cells (Plated) were counted before transfection at 1 DIV. Neuronal cells in random 450 × 335-μm fields were counted, with *P* values for Student's *t* test comparisons shown. (*B*) Western blot analyses of whole-cell lysates from neuronal cultures treated with control (C) or NUP62 (N) siRNAs. Lysates from equivalent cell number were loaded per lane. Membranes were processed with antibodies to total Nup62, Gapdh, total tau, nonphosphorylated tau (Non-Phos), tau phosphorylated at serine 202 (Phos S202), and Map2.

NUP62 [V5–NUP62 (29)]. Consistent with other reports, expression of transfected expression plasmids in primary hippocampal neurons was observed at low frequency (30). In rare expressing neurons, V5–NUP62 accumulated to high levels and was detected both in the nuclear envelope (the typical perinuclear ring labeling observed for NPCs) and in the processes of expressing neurons (Fig. S9). Cytoplasmic accumulation of V5–NUP62 made it possible to distinguish processes originating from expressing neurons, and they were found to greatly exceed in length processes originating from nonexpressing neurons in the same cultures, as well as those of neurons transfected with control siRNA (Fig. S10). Although the processes appeared beaded, forced V5–NUP62 expression in primary neurons treated with NUP62 siRNA significantly enhanced their overall growth.

To determine whether depletion of NUP62 affects neuronal differentiation and prevents outgrowth and/or promotes retraction of dendrites, neurons were transfected at 1, 3, or 7 DIV with siRNA, and dendrite length was assessed at 10 DIV. A reduction in total dendritic processes was apparent in all cultures treated with NUP62 siRNA compared with those treated with control siRNA (Fig. 5*I*). The AIS remained intact, and extensive branching axon processes were visible in the cultures transfected with NUP62 siRNA (Fig. S11). Together with the Western blot data (Fig. 4), the results suggest that depletion of Nup62 impairs dendritic development, but does not affect other aspects of neuronal differentiation, such as axon projection. The lengths of dendrites at 10 DIV were similar for neurons transfected with NUP62 siRNA at different culture ages, with the shortest dendrites observed in neurons transfected at 3 DIV. If Nup62 reduction only blocked neurite outgrowth, then the length of dendrites would have been greater at later transfection times. Hence, the data indicate that decreased Nup62 content promotes dendrite retraction as well as preventing outgrowth.

Discussion

Stress Signaling and the Transition from Acute to Chronic Stress Adaptive Responses. Chronic stress induces retraction and simplification of apical dendrites in CA3 neurons and less severe changes in neurons of CA1 and the dentate gyrus (9, 31). The effects of chronic stress on dendritic arbors results from the actions of corticosteroids (9) combined with other neurochemical signaling systems in the hippocampus. These systems include glutamate release, NMDA receptors (NMDARs), calcium channels, the serotonin system, GABA receptors, opioids, and collapsin-response mediator proteins (12, 32). In chronic stress, pharmacological evidence indicates that glutamate functions through NMDARs (33), and chronic-stress–induced dendritic retraction is not evident in the CA3 or CA1 neuronal fields of mice engineered to lack functional NMDAR in CA3 neurons (34). The mechanisms that mediate the transition from acute to chronic stress adaptation, as well as those responsible for the relative vulnerability of CA3 neurons, are not well understood.

Autophosphorylation of Y402 renders Pyk2 active, creating a high-affinity binding site for the SH2 domain of Src that, when bound, alters the conformation of Src from a low- to high-activity state (24). PYK2 is a component of the NMDAR complex (35) and is activated by NMDAR stimulation (36). In addition, a rapid response of hippocampal neurons to glucocorticoids includes activation of Pyk2 via a putative G-protein–coupled receptor (19). We show here that the subcellular distribution of activated Pyk2 (phospho-Y402 Pyk2) in hippocampal pyramidal neurons is altered in chronically stressed mice. Immunofluorescence microscopy of control animals revealed granular phospho-Y402 Pyk2 immunolabeling distributed throughout the hippocampus.

Fig. 5. Reducing Nup62 content results in shortened dendritic arbors in cultured E18 rat hippocampal and neocortical neurons. (*A*–*E*) Cells at 7 DIV were transfected with control (*A* and *D*), 25 nM NUP62 (*B*), or 50 nM NUP62 (*C* and *E*) siRNAs for 3 d. Cells were fixed and processed for immunofluorescence microscopy with antibodies to ankyrin-G (Ank3; green pseudocolor) and Map2 (red pseudocolor). Dendrites appear red/orange, and axons appear green. Nuclei were stained with DAPI. (Bar: 100 μm.) (*F*) Hippocampal neurons at 7 DIV were treated with control or 25 nM NUP62 siRNAs and analyzed at 10 DIV. (*Upper*) Dendrites from several fields quantified as average total length per neuron. (*Lower*) Average number of primary dendrites per cell. (*G*) Branch vertices per dendrite were quantified in hippocampal neuronal cultures after treatment with siRNAs as described in *F*. Distributions of vertex number per dendrite are shown. (*H*) Hippocampal and neocortical neuronal cultures were transfected with control or 50 nM NUP62 siRNA at 7 DIV and then analyzed at 10 DIV. Dendrites were quantified as average total length per neuron. (*I*) Hippocampal neurons were cultured for one (day 1), three (day 3), or seven (day 7) days and then transfected with control or 50 nM NUP62 siRNAs. At 10 DIV, dendrites from several fields were quantified as average total length per neuron.

In chronically stressed animals, immunolabeling of the molecular layers was reduced, and immunolabeling in the somata and perinuclear region of the CA3 pyramidal cell layer was markedly enhanced. Subcellular redistribution of phospho-Y402 Pyk2 represents a previously unidentified mechanism of modulating the profile of substrates phosphorylated by Pyk2 and possibly associated Src kinases. Accumulation of Pyk2 and phospho-Y402 Pyk2 in proximal dendrites, somata, and intranuclear compartments of rat hippocampal slices has been observed after depolarization in vitro (22, 37).

Reduction of NUP62 Content in Hippocampal Neurons Under Chronic Stress. RNA-Seq analyses of RNAs isolated by TRAP from CA3 neurons revealed significantly less mRNA for central channel nucleoporins Nup62 and Nup58 in chronically stressed mice than in control mice. The same analyses revealed that TRAP mRNAs for 26 other nucleoporins either increased in chronically stressed mice or did not display significant differences from control mice. The one exception was SEC13 homologue 1 (Seh1), which is involved kinetochore function in mitotic cells. The data suggest that CA3 neurons specifically down-regulate either transcript accumulation or translation of Nup62 and Nup58 in response to chronic stress independently of or inverse to regulation of other nucleoporins. Consistent with these results, Nup62 content was reduced in nuclear fractions of whole hippocampus of chronically stressed rats, whereas the Nup98 (ring substructures) and Nup133 (inner scaffold) content remained constant. The RNA-Seq and these results together indicate that the outcome of down-regulation of NUP62 may be the generation of NPCs that have reduced NUP62 content. Several studies have suggested that NUP62-depleted NPCs function differently from the general NPC population. For example, yeast NPCs with reduced Nsp1p (NUP62 ortholog) content are retained in the maternal cell, whereas NPCs containing sufficient Nsp1p are sorted to budding daughter cells (38). Depletion of Nsp1p in yeast appears to promote detachment of chromatin from the NPC and may act as an alarm mechanism for cellular stress (38), whereas reduction of NUP62 content in some human cancer cells alters their response to chemotherapeutic stress (39). In hippocampal neurons, accumulation of NUP62-depleted NPCs in response to chronic restraint stress may signal adaptive changes in dendritic architecture.

Microarray expression analyses of total hippocampus did not reveal significant differences between control and chronically stressed mice in the transcript levels for 29 nucleoporins, including Nup62. The reduction of Nup62 expression that was observed by TRAP in the CA3 region of stressed mice may be obscured in microarray analysis of total hippocampus by dilution with less severely affected neuronal fields (11). Nonetheless, a significant reduction in Nup62 protein content was observed in fractionated total hippocampus from chronically stressed rat, suggesting that posttranslational regulatory mechanisms may have a role in reducing Nup62 protein accumulation. A FAK/PYK2 consensus site within the alpha-helical domain of NUP62 (Y422) was reported to be phosphorylated in an online phospho-proteomic database, and several ubiquitinated residues were identified near this site (16). We found that NUP62 is phosphorylated at Y422 in response to membrane depolarization by K^+ and to treatment with dexamethasone, a potent synthetic glucocorticoid, both of which are positively associated with stress signaling. Induction of NUP62 phosphorylation at Y422 resulted in enhanced accumulation of NUP62 in the cytoplasm of cultured neuroblastoma cells and was blocked by a specific FAK/PYK2 inhibitor. Enhanced accumulation of phospho-Y425 Nup62 (rat ortholog to Y422) was observed in cytosolic extracts from hippocampi of chronically stressed rats, potentially a downstream effect of redistribution of activated Pyk2 to the perinuclear region of pyramidal neurons.

A Role for NUP62 Depletion in Dendrite Maintenance and Pathogenesis. A primary function of the NPC is nucleocytoplasmic transport, and FG-repeat–containing nucleoporins play a direct role in transport by binding cargo or karopherins. Reduction of NUP62 content in the NPC could negatively affect neuronal health or dendritic complexity by interfering with transport of certain cargo, particularly those that interact with NUP62 such as the glucocorticoid receptor (40). Turnover of nucleoporins in terminal neurons is slow, and during aging, the NPC deteriorates from oxidative damage and loss of nucleoporins, including those containing FG repeats. Such deterioration has been shown to compromise the integrity of the nuclear/cytoplasmic barrier, allowing diffusion of cytoplasmic proteins into the nucleus (41). Loss of NUP62 in chronic stress could have a similar impact on hippocampal neurons, resulting in degeneration of neuronal function and architecture. Alternatively, given the reversibility of CA3 dendritic remodeling, simplification and shortening of dendrites in response to chronic stress may reflect adaptive and protective, rather than degenerative, processes, with reduction of NUP62 content selectively and reversibly altering NPC transport of cargo involved in regulating dendrite morphology (11).

The fractional reduction in NUP62 content of the NPC associated with chronic stress may not impact nucleocytoplasmic transport significantly, and the resulting effects on dendritic architecture may be associated with other nucleoporin functions. Some FG-repeat nucleoporins, including NUP62, function independently of nucleocytoplasmic transport in chromatin modeling and gene transcription, performing these functions both as components of the NPC and at other sites in the nucleus (2–4). The Q391P mutation that causes IBSN (7) lies within the C-terminal alpha-helical binding domain of NUP62, and as interactions of NUP62 with cargo or karyopherins are directed by the FG-repeat–containing N-terminal domain, it is unlikely to directly modify the transport characteristics of NPCs. The NUP62–NUP54 heterotrimer forms through coiled-coil and bundling interactions of conserved alpha helices in each nucleoporin, and peptide melting studies show that Q391P substitution reduces the stability of these interactions (42). Phosphorylated Y422 presents mainly on NUP62 that has dissociated from the NPC, suggesting that the structural effects of the Q391P mutation and phosphorylation of Y422 may be analogous. Whereas Q391 lies within the NUP54-binding alpha-helical region of NUP62, Y422 lies two residues from its C terminus, and phosphorylation of residues internal and C-terminal to synthetic alpha helices have been shown to destabilize these structures (43). Phosphorylation of Y422 also may promote ubiquitination and proteosomal degradation of NUP62 at sites adjacent to Y422, as has been observed with some cytosolic tyrosine kinase substrates (44).

In summary, at least two types of mechanisms, one affecting expression and the other association with the NPC, may contribute to loss of NUP62 from CA3 and possibly other hippocampal neuronal fields during chronic stress. Their combined actions, so evident in CA3, may explain the exquisite sensitivity of CA3 apical dendrites to chronic stress. Loss of NUP62 may alter nucleocytoplasmic transport and/or chromatin organization and function, leading to reduced dendritic complexity and length. Similar to our observations in chronic stress, NUP62 transcript content in prefrontal cortex of major depressives is reduced to 82% of control levels in the absence of significant changes in other nucleoporins (13). Intriguingly, the therapeutic effect of selective serotonin reuptake inhibitors in treating depression has been shown recently to involve chromatin remodeling through p11/AnxA2 signaling to the SMARCA3 remodeling factor (45). It is an interesting possibility that the therapeutic action of antidepressive agents may involve correcting changes in chromatin organization that result from reductions in NUP62 content.

Materials and Methods

Cell Culture. Hippocampus and neocortex were resected from E18 Sprague–Dawley rat brains and prepared as described (46). Low-density cultures were plated on glass coverslips coated with 1 mg/mL poly-L-lysine (Sigma; P2636) at 2×10^5 cells per 60-mm dish. Cultures were maintained in Neurobasal medium with NS21 supplements (47). The S12 neuroblastoma cell line is a

spontaneously differentiating clone derived from SH-SY5Y (48) and was grown in DMEM (Cellgro) supplemented with 10% (vol/vol) FBS.

Chronic Stress of Rodents. The Rockefeller University Institutional Animal Care and Use Committee approved all experimental procedures involving animals. For chronic stress treatment, rodents were restrained for 6 h a day for 21 d, as described (49). After 21 d, rodents were removed from stressors for 18 h before harvesting. Total body and thymus weights were recorded. For biochemical/molecular analyses, hippocampi were resected and flash frozen. For immunofluorescence studies, animals were anesthetized deeply, perfused transcardially with 10% Dextran 40 in normal saline, followed by 150 mL of 4.0% fresh paraformaldehyde prepared in PBS. Frozen sections were cut at 30–50 μm.

Antibodies and Immunofluorescence Microscopy. Antibodies and immunofluorescence protocols are described in *SI Materials and Methods* and Fig. S5.

TRAP, RNA-Seq, Cell and Tissue Fractionation, Immunoprecipitation, and Western Blot Analyses. TRAP and RNA-Seq analyses were performed as described (15). Subcellular fractionation was performed as described (50). Details of these and analytical procedures are in *SI Materials and Methods*.

siRNA Knockdown. Sequences and procedures used for siRNA knockdown experiments procedures are detailed in *SI Materials and Methods*.

Image Analyses. Immunofluorescence image stacks were generated by using the Zeiss Axioplan 2 microscope and processed by using the 3D deconvolution algorithm of AutoQuant (MediaCybernetics). Neuronal processes were traced and quantified by using the NeuronJ plugin (51) for ImageJ (52).

ACKNOWLEDGMENTS. This work was supported by National Institutes of Health Grants R03CA141318 (to D.S.K.), R01 MH41256 (to B.S.M.), and R01 MH095229 (to D.L.B.); and by the Michael J. Fox Foundation (D.L.B.).

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