Regulation of the Neuronal Proteasome by Zif268 (Egr1)

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Key words: Egr-1; 26S proteasome; Lmp2; immunoproteasome; plasticity; proteasome activity

Introduction

Proteasomes are multi-subunit protein complexes that provide the major route of targeted protein degradation in cells. The subunit composition of the proteasome is thought to be constant across most cell types, although in immune cells, transcriptional induction of “inducible” subunit genes, the psmb8 (Lmp7), psmb9 (Lmp2), psmb10 (MECL-1), psme1 (PA28α), and psme2 (PA28β) genes, can change the subunit composition to form the “immunoproteasome” and hence increase proteasome activity (Harris et al., 2001; Cascio et al., 2002).

Although modifications in proteasome structure and function in the immune system have been studied intensively, little is known regarding proteasome adaptations to serve the highly specialized and polarized characteristics of neurons. However, the subunit composition of the proteasome varies in different brain regions (Noda et al., 2000; Ding and Keller, 2001). Interestingly, the pyramidal neurons of the cerebral cortex and hippocampus show the highest proteasome levels (Mengel et al., 1996) and express the inducible psmb9 and psmb8 subunits of the immunoproteasome constitutively (Díaz-Hernández et al., 2003), linking psmb8 and psmb9 to the function of these glutamatergic neurons. Proteasome subunits are located in neuronal dendrites (Rezvani et al., 2003), implicating the proteasome in synaptic signaling.

The late phase of most, if not all, forms of synaptic plasticity is dependent not only on protein synthesis (Morris, 2004) but also on limited protein degradation. Inhibition of proteasome activity facilitates various forms of plasticity (Obin et al., 1999; Speese et al., 2003; Zhao et al., 2003; Juo and Kaplan, 2004). The proteasome regulates the levels of specific proteins with important roles in synaptic communication: NMDA receptor subunits, postsynaptic density scaffolding molecules (Colledge et al., 2003; Ehlers, 2003; Pak and Sheng, 2003), and synaptic glutamate transporters (Boehmer et al., 2003, 2004).

One of the few proteins with a confirmed role in many different forms of plasticity is the transcription factor (TF) Zif268. Zif268 is induced during periods of plasticity [i.e., during learning tasks or induction of long-term potentiation (LTP)] in the hippocampus, visual or temporal cortex, and spinal cord, in diverse species from primates to songbirds (Cole et al., 1989; Widdgen et al., 1990; Jarvis et al., 1998; Kaczmarek et al., 1999; Mello et al., 2004; Morris, 2004). The threshold and duration of hippocampal LTP correlates with the degree of Zif268 induction (Richardson et al., 1992; Worley et al., 1993), and mice with targeted disruption of the Zif268 gene show deficits in late-phase...
LTP and in a range of memory tests (Jones et al., 2001), confirming the importance of Zif268 induction for long-term plasticity. In peripheral cells, Zif268 mediates transcriptional suppression or induction of various genes (Cao et al., 1993; Li et al., 1996; Beckmann and Wilce, 1997; Thottassery et al., 1999; Bahouth et al., 2002; Virolle et al., 2003; Zhang and Liu, 2003; Wang et al., 2005). We have recently identified candidate target genes of Zif268 in neurons (James et al., 2005). A remarkable finding was that many of the genes identified encoded either components of the proteasome or proteins with functions closely allied to the proteasome. In this study, we test the hypothesis that the expression of proteasome subunits and functionally related genes is regulated by Zif268.

Materials and Methods

Expression constructs. An overview of the plasmid constructs used during this study is provided in supplemental Table 1 (available at www.jneurosci.org as supplemental material). The Zif268 expression vector pZif268 has been described previously (Groot et al., 2000). As a control expression construct, an N-terminal deletion mutant (Δ3–372 aa) of pZif268, termed ptrZif268 (truncated Zif268), was constructed (James et al., 2005). This construct retains the 5′ and 3′ noncoding DNA sequence of the full-length Zif268 insert.

Promoter–reporter chloramphenicol acetyl transferase constructs. Functional analysis of the serum/glucocorticoid-regulated kinase (SGK) (−1595 to +28), proteasome β9 subunit (psmb9) (−539 to +18), and Tap1 (−566 to −10) promoters (whereby +1 denotes the transcription start site [TSS]) was performed using plasmids containing the wild-type promoters cloned upstream of the chloramphenicol acetyl transferase (CAT) gene in pBLCAT3. The pTap1-CAT and pPsmb9-CAT constructs, whereby the rat Tap1/psmb9 intergenic region was subcloned in either orientation upstream of the CAT gene, were a generous gift from Prof. P. Charnay (Institut National de la Santé et de la Recherche Médicale, Paris, France) (Topilko et al., 1998). We did not assess their behavior, but all animals were in the same behavioral conditions before they were killed. The anterior portion of the cerebral cortex (frontal cortex) incorporating prefrontal, cingulate, and motor areas was dissected for analysis.

Transient transfections. Transient transfection of confluent PC12 cells has been described previously (James et al., 2004, 2005). Endotoxin-free DNA (2 μg of pZif268 or ptrZif268) was combined with Lipofectamine 2000 (LF200; Invitrogen, Paisley, UK) in OPTI-MEM 1 medium incubated at room temperature for 20 min. RNA was extracted 48 h after transfection.

GeneChip data analysis. 3 GeneChips were used for each group. The average difference and expression level of genes were calculated according to absolute and comparison analysis algorithms according to Affymetrix (Santa Clara, CA) protocols. Normalization was performed using BioConductor (http://biowww.dci.harvard.edu/~biconductor/). Significant changes in expression levels for the pZif268 transfection treatment relative to the control, ptrZif268, treatment were calculated using Significance Analysis of Microarrays (SAM) (Tusher et al., 2001) (www.stat.stanford.edu/~tibs/SAM/). SAM uses permutations of the repeated measurements to provide an accurate estimate of the percentage of genes identified by chance, the false discovery rate (FDR), and hence avoids the usual problems of a large number of false positive results because of the performance of multiple uncorrected statistical tests. The FDR was set at a threshold of 0.05 in our analysis, so few false positive results are anticipated.

In silico inspection of promoter regions of candidate target genes. The program Gene2Promoter (Genomatix Software, Munich, Germany) was used to extract the promoter regions from a list of accession numbers. Typically, a region of 1101 bp (−1000 to +100) was extracted. Zif268 binds to consensus DNA sequences known as Egr response elements (ERE’s). The program MatInspector (Quandt et al., 1995) was used to locate putative EREs in a DNA sequence. Core similarity was set at the default level of 0.75. A selection of randomly chosen genes was obtained using the random gene selection tool at the Regulatory Sequence Analysis Tools web site provided by the Service de Conformation des Macromolécules Biologiques et de Bioinformatique (l’Université Libre de Bruxelles, Bruxelles, Belgium).

Reverse transcription–PCR. Where possible, at least one of the primer pairs used for PCR was designed to span an exon–intron boundary. Primer sequences are provided in Table 1. RNA was extracted from PC12 cells, mouse frontal cortex, or rat striatum using the RNeasy Mini kit (Qiagen, Chatsworth, CA). A single PCR cycle within the logarithmic phase of the PCR was selected. Band intensity was normalized to the corresponding signal for the "housekeeping" gene glyceraldehyde-3-phosphate dehydrogenase (Gapdh), and differences between the groups were assessed using the two-sample t test (striatal/cortical studies) and the Mann–Whitney U test (PC12 validation study).

Table 1. RT-PCR primer sequences used during this study

| Gene target | Sense Gene/locus accession number | Amplicone size (bp) |
|-------------|---------------------------------|--------------------|
| Gapdh       |                                 |                    |
| psmb2       |                                 |                    |
| Mouse psmb2 |                                 |                    |
| psmb9       |                                 |                    |
| SGK         |                                 |                    |
| Tap1        |                                 |                    |

*Primers targeted rat DNA template unless otherwise denoted.

*Where possible, at least one of the primer pairs were predicted to span an exon–intron boundary. The exon number for which a section of the primer sequence is predicted to target are denoted. For primers predicted to span exon–intron boundaries, the portions of the primer sequence that target distinct exons are characterized by alternating type style (roman or italic/underline).

The Entrez Gene ID is provided. The exon–intron structure information and sequence template for primer design were also obtained from the ENSEMBL database (www.ensembl.org).
Microarray analysis of genes encoding proteasome subunits and functionally related genes after overexpression of Zif268 in PC12 cells

After increased expression of Zif268 in PC12 neurons, we assessed the changes in the pattern of gene expression using Affymetrix GeneChips to profile a large proportion of the neuronal transcriptome. In a previous report, we noted that a number of proteasome and related genes were prominent in the list of putative neuronal Zif268 target genes (James et al., 2005). The proteasome comprises a 20S core consisting of 28 subunits composed of structural proteasome (psm) α subunits (psma1–7) and proteolytic β subunits (psmb1–7), with a 19S regulator at each end consisting of ATPase subunits (psmc1–6) and non-ATPase subunits (psmd1–14). In the immune system, interferon-mediated transcriptional induction of the psme1 (PA28α) and psme2 (PA28β) genes replaces the 19S regulator at one or both ends with the 11S or PA28 regulator (Cascio et al., 2002). Immune challenge also increases transcription of the inducible β subunits psmb5, psmb6, and psmb7, which then replace the constitutive β subunits psmb5, 6, and 7. The presence of the 11S regulator and the psmb8 and psmb9 subunits in the immunoproteasome then increases proteasome activity (Harris et al., 2001; Cascio et al., 2002). Detailed analysis revealed that increased expression of Zif268 suppressed the expression of a number of proteasome subunit genes, including psme4, psmb8, psmb9, psme1, and psme2 (Fig. 1a).

In addition, expression of a number of proteasome accessory and regulatory genes was also decreased, including proteasome-related kinases such as SGK, E2 and E3 ubiquitin ligases, which are key intermediaries in the tagging of proteins for proteasomal degradation, and genes encoding components of the COP9 signalosome (Fig. 1b).

The levels of the mRNAs derived from the three genes encoding subunits of the proteasome-associated antigen peptide transporter (Tap) complex, Tap1, Tap2, and tapasin, also appeared decreased. However, the level of expression of other proteasome subunit genes was not affected by the increased expression of Zif268 (Fig. 1c).
Bioinformatic analysis of Zif268-regulated proteasome genes

Bioinformatic analysis of gene promoters, using software trained as follows: ERE, TGCGTRGGCGK; Sp1, NGGGGGCGGGGYN; NFκB, SGGRRNNTTCE. Note that bioinformatic predictions are not in themselves indicative of functional significance.

Table 2. Putative Zif268-regulated proteasome genes and randomly selected genes used for the bioinformatic analysis

| Proteasome genes | ERE | Sp1 | NFκB | Randomly selected genes | ERE | Sp1 | NFκB |
|------------------|-----|-----|------|-------------------------|-----|-----|------|
| anpse1           | 2   | 2   | 0    | NM_001916.2              | 0   | 1   | 1    |
| cdc5-likl        | 2   | 5   | 3    | BB04                     | 0   | 4   | 2    |
| cap1             | 6   | 5   | 1    | C14orf15                 | 0   | 3   | 2    |
| Herc3            | 4   | 5   | 1    | C0Orf26                  | 0   | 11  | 0    |
| mdm2             | 4   | 1   | 5    | DKFp76716058             | 1   | 3   | 2    |
| Hedd4            | 2   | 5   | 1    | FLZ02043                 | 2   | 1   | 1    |
| Hedd8-UB1        | 8   | 7   | 4    | HEG                      | 0   | 4   | 2    |
| psma5            | 1   | 8   | 1    | KAA1958                  | 4   | 5   | 0    |
| psmb8            | 5   | 4   | 1    | LIN7C                    | 1   | 2   | 1    |
| psmb9/Top1       | 4   | 6   | 3    | LOC148003                | 0   | 1   | 1    |
| psmc4            | 2   | 13  | 2    | LOC882594                | 0   | 2   | 1    |
| psme1            | 3   | 1   | 0    | LOC888617                | 0   | 18  | 0    |
| psme2            | 8   | 14  | 2    | LOC923539                | 0   | 14  | 0    |
| Rhizin2          | 3   | 2   | 2    | LOC401352                | 2   | 3   | 2    |
| SAK              | 3   | 0   | 0    | LOC402055                | 1    | 27  | 0    |
| SGK              | 7   | 9   | 0    | MAC30                    | 2   | 2   | 2    |
| tgt              | 3   | 1   | 1    | PGH3A10                  | 0   | 11  | 0    |
| Tap2             | 3   | 1   | 0    | RON2                     | 6   | 1   | 1    |
| tapasin          | 7   | 11  | 3    | ZNF26                    | 2   | 1   | 0    |
| abc7             | 5   | 1   | 1    | ZNF530                   | 1    | 1   | 1    |
| UBE2F1           | 2   | 6   | 2    |                            |     |     |      |
| ubiquitin        | 5   | 2   | 0    |                            |     |     |      |
| ubiquitinD       | 2   | 2   | 1    |                            |     |     |      |
| vhl              | 4   | 3   | 1    |                            |     |     |      |

The candidate Zif268-regulated proteasome genes were compared with a randomly selected set of genes (see Materials and Methods) for the number of potential EREs, Sp1 sites, and NFκB sites present in the upstream regions. For details of procedures, see Materials and Methods. The consensus sequences are significant (Table 2). Analyses revealed, in all cases, the presence of a consensus NFκB site in the upstream region of these proteasome-related genes, which is a remarkable concentration of potential EREs in the promoter regions of these genes.

The predicted ERE abundance was substantially greater in the upstream regions of the proteasome genes relative to the randomly selected genes, suggesting that this finding did not occur by chance (Fig. 2a). To complement this result, we used another approach to assess the representation of EREs in the promoter regions of the proteasome-related genes identified as Zif268 targets. Rather than looking for specified consensus TF recognition sites in individual promoter sequences, the “overrepresented TF binding site prediction” method (Zheng et al., 2003) searches, with no a priori assumptions, for significantly overrepresented cis elements in a group of sequences and performs post hoc matches to the TRANSFAC database. This method identified an enrichment of Sp1 sites \([P(M,t) = 2.8 \times 10^{-10}]\) and EREs \([P(M,t) = 3.5 \times 10^{-8}]\) in the promoter regions of the proteasome-related genes. No enrichment of Sp1 sites or EREs was detected in the randomly selected promoter sequences.

We also assessed the distribution of the EREs in these putative promoter regions, because, in many known Zif268 target genes, EREs are located close to the TSS. We found a notable concentration of the putative EREs immediately upstream of the TSS from the proteasome genes (Fig. 2b). Because some proteasome genes are thought to be regulated at the transcriptional level by members of the nuclear factor κB (NFκB)/Rel family of TFs (Chatterjee-Kishore et al., 1998; Whitehouse and Tisdale, 2003; Marques et al., 2004), and because Zif268 is known to interact with and modulate NFκB/Rel transcriptional activity (Cogswell et al., 1997; Chapman and Perkins, 2000), we similarly monitored the abundance of predicted NFκB/Rel binding sites in the 1 kb upstream regions of the proteasome-related genes. These sites were also enriched in the upstream regions of the proteasome genes relative to the randomly selected genes (Fig. 2c, Table 2), although, interestingly, the relative abundance of predicted EREs was even greater than that of the NFκB/Rel binding sites (Fig. 2a,c).

The TF Sp1 has also been implicated in the regulation of transcription of proteasome genes, and there is considerable evidence to suggest that many actions of Zif268 involve competition or interaction with Sp1 (Ebert et al., 1994; Srivastava et al., 1998; Fukuda and Tonks, 2001; Davis et al., 2003; Tan et al., 2003). We therefore assessed the association between predicted EREs and Sp1 sites in the upstream regions of the proteasome genes. We found that 20 of 24 proteasome-related genes (83%) contained overlapping EREs and Sp1 sites in these upstream regions compared with 2 from 20 randomly selected genes (10%). The relationship between the Sp1 sites and EREs for four prototypical genes is shown in supplemental Fig. 1 (available at www.jneurosci.org as supplemental material). To test the hypothesis that the ability of Zif268 to alter the expression of these genes might involve coordinate interaction between Sp1, NFκB/Rel, and Zif268, we searched for possible modules of TF binding sites containing these three motifs in close proximity (<30 bp). We found that 8 from 24 proteasome genes showed the presence of one or more copies of this module in their upstream region compared with none of the randomly selected genes (Fig. 2d). It is thought that NFκB/Rel proteins and Zif268 can interact over larger distances (Wright et al., 1995). Although we did not analyze more widely separated Sp1, NFκB/Rel, and Zif268 modules in detail, it was clear that a less conservative criterion (>30 bp) would have revealed the presence of this combination of motifs in many more proteasome gene upstream regions.

These results suggest that Zif268 may exert a previously un-
suspected regulatory control on proteasome gene expression in neurons and could interact in some cases with the relatively well-characterized NFκB/Rel and Sp1 regulatory pathways. This control appears to be exerted at multiple levels of proteasome function: on genes encoding components of the proteasome, proteins mediating peptide export to the class I MHC, ubiquitin ligase enzymes, and proteins involved in proteasome regulation.

Validation of the influence of Zif268 on expression of proteasome subunit genes and proteasome-related genes

Thus far the data clearly implicated Zif268 in the coordinate regulation of a number of proteasome subunit and proteasome-related genes. Potential regulation of proteasome subunit genes and associated E2 and E3 ligases is striking in light of the emerging concept that altered proteasome function affects synaptic plasticity. The altered expression of inducible proteasome subunits such as psmb9 and psme2, which encode proteasome subunits known to modulate proteasome activity; Tap1, which is physically associated with the proteasome and has been implicated in synaptic plasticity; and SGK, which regulates the access to the proteasome of certain proteins involved in plasticity and is also itself degraded by the proteasome. We validated the differential expression of these four candidate Zif268 target genes (psmb9, psme2, SGK, and Tap1) after Zif268 transfection of PC12 cells using reverse transcription-PCR (RT-PCR) (Fig. 3a,b). The levels of all the mRNAs tested were suppressed by Zif268 transfection, consistent with the results from the microarray experiment. We also monitored the levels of psmb9 protein immunoreactivity by Western blotting. Although there was greater variability in the levels of protein compared with the mRNA levels, the results suggested that the levels of psmb9 protein were also regulated by Zif268 transfection (Fig. 3c).

The level of expression of these genes was also tested in cerebral cortex tissue from mice with a targeted deletion of the Zif268 gene (Zif268 knock-out mice) and wild-type control mice. The levels of psmb9, psme2, SGK, and Tap1 mRNAs were all found to be increased in the tissue from the Zif268 knock-out mice (Fig. 4a,b). In the case of psmb9, we also tested whether this regulation of expression at the mRNA level was mirrored by altered expression at the protein level. In cortical tissue from the Zif268 knockout mice, there was an increase in psmb9 protein levels relative to wild-type mice (Fig. 4c,d). We also obtained evidence for elevated SGK expression in the hippocampus from Zif268 knock-out mice relative to controls (data not shown).

Assessment of ability of Zif268 to transactivate the SGK, Tap1, and psmb9 promoters

Although the psme2 promoter is not well characterized, the structures of psmb9, SGK, and Tap1 promoters have been described previously (Webster et al., 1993; Proffitt and Blair, 1997; Waldegger et al., 1998). We therefore focused on these three genes, each of them with a potentially interesting role in synaptic plasticity. Promoter–reporter CAT constructs for the potential Zif268 target genes [all of which contain a putative ERE site(s) in their promoter regions] were prepared (Fig. 5a) to test the ability of Zif268 to alter the activity of the promoters. PC12 cells were cotransfected with these constructs and either full-length Zif268 or control, truncated Zif268. In the presence of full-length Zif268, promoter activity was suppressed for each of the three genes selected (Fig. 5b).

Note that although the bioinformatic analysis supports the possibility of direct actions in each case, it should be remembered that there is a possibility that the effects of Zif268 could be indirect, reflecting the induction of other TFs by Zif268, which then themselves act on the SGK, Tap1, and psmb9 promoters. However, whether the effects of Zif268 are direct or indirect, our results clearly show that altered expression of proteasome and related genes is likely to be a consequence of the elevated Zif268 expression that occurs during neuronal plasticity.

Zif268-regulated proteasome genes and CNS plasticity

We extended our analysis of the regulated expression of these genes using a well-characterized model of CNS plasticity, the
response of rat striatal neurons to elevated corticostriatal activity after D2 receptor blockade in vivo. Systemic administration of drugs with D2 dopamine receptor antagonist properties, such as haloperidol, increases glutamate release from corticostriatal terminals and produces LTP of corticostriatal transmission (Cala-bresi et al., 1997; Cepeda et al., 2001), with dramatic induction of striatal Zif268 (with a peak expression of ~1–2 h) (Nguyen et al., 1992; Simpson and Morris, 1994; Keefe and Gerfen, 1995). We confirmed induction of Zif268 after haloperidol administration (Fig. 6a). We also determined the relative expression levels of the SGK, psmb9, and Tap1 mRNAs 6 h after haloperidol administra-

![Figure 3](image_url)  
**Figure 3.** Validation of psmb9, psme2, Tap1, and SGK regulation by Zif268 in PC12 cells. Semiquantitative RT-PCR was used to determine the relative expression levels of the genes. a, Representative RT-PCR product band intensities in psmb9-, psme2-, Tap1-, and SGK-transfected PC12 cells. Note the stronger band intensities for Tap1, psmb9, SGK, and psme2 in the cells transfected with the truncated Zif268 expression vector ptrZif268. b, Relative amounts of target cDNA were determined (see Materials and Methods) 48 h after transfection. Results from five independent experiments are shown from ptrZif268 (□) and ptrZif268 (■)-transfected cells (means ± SEM). *p < 0.05, **p < 0.01, ***p < 0.001, expression levels lower in pZif268-transfected PC12 cells compared with ptrZif268-transfected cells (Mann–Whitney U test). c, Levels of psmb9 protein immunoreactivity (psmb9-ir) in pZif268- and ptrZif268-transfected PC12 cells. The results suggested a corresponding regulation of protein levels.

![Figure 4](image_url)  
**Figure 4.** Validation of psmb9, psme2, Tap1, and SGK regulation by Zif268 in Zif268 knock-out mice. Semiquantitative RT-PCR was used to determine the relative expression levels of the genes. a, Representative RT-PCR product band intensities for tissue from wild-type (+/+) and Zif268 knock-out (−/−) mice. b, Relative mRNA expression in cortical tissue isolated from age-matched Zif268 wild-type (□□) and Zif268 knock-out (□□) mice. c, Western blot bands showing psmb9 immunoreactivity in cortical tissue from wild-type (+/+) and Zif268 knock-out (−/−) mice. d, Relative amounts of psmb9 immunoreactivity in cortical tissue isolated from age-matched Zif268 wild-type (□□) and Zif268 knock-out (□□) mice. The results are normalized pixel intensity (relative to Gapdh signal intensity) (b) or integrated band optical density (d) and are mean ± SEM: *p < 0.05, **p < 0.01, ***p < 0.001, expression levels greater in knock-out mice compared with wild-type mice (Mann–Whitney U test).

Assessment of ability of Zif268 to modify proteasome activity
Proteasome activity in transfected PC12 cells was monitored using the fluorogenic synthetic proteasome substrate LLVY-AMC. Generation of fluorescent AMC in these studies was completely blocked by the proteasome inhibitor MG132 (N-carbobenzoxy-Leu-Leu-leucinal) (10 μM) (data not shown). It was found that full-length Zif268 induced a small but clear decrease in the generation of AMC by the proteasome relative to truncated, inactive Zif268 (Fig. 7a,b).

Proteasome activity was similarly monitored in cortical tissue from Zif268 knock-out mice (Topilko et al., 1998) and corresponding wild-type controls. Proteasome activity was substantially elevated in the tissue from the Zif268 knock-out mice (Fig. 7c,d).

Discussion
We report here the novel concept that Zif268, which is induced during many different forms of neuronal plasticity, regulates the expression of a subset of proteasome genes.

The rigorous statistical analysis used for our microarray analysis, with tight control of the false positive rate, ensures that the potential Zif268 targets identified can be viewed with considerable confidence. We confirmed altered expression of four of the genes identified in Zif268-transfected cells by RT-PCR. The magnitude of the change detected by RT-PCR was very similar to that detected by the microarray analysis. Consistent with the microarray analysis, these genes were all downregulated by Zif268. When we assessed the level of expression of the psmb9, psme2, Tap1, and SGK genes in CNS tissue from Zif268 knock-out mice, we found that the mRNA levels were all increased relative to wild-type mice. This is very strong evidence confirming the conclusions from the in vitro cells: that Zif268 is involved in suppressing the transcription of these genes. The results are striking, because these mice exhibit learning and memory deficits, yet this is the first demonstration of genes that show altered CNS expression as a result of the functional deletion of the Zif268 gene.

The general concept of transcriptional suppression by Zif268 is not unprecedented. In fact, the recent data from peripheral cells suggest that a major role of Zif268 is in suppression of gene expression (Cao et al., 1993; Beckmann and Wilce, 1997; Dinkel et
Our results showing regulation of proteasome gene expression by Zif268 are consistent with the evolving concept that proteasome activity is important for neuronal plasticity. The precise role of the proteasome is controversial. On the one hand, proteasome gene products are involved in multiple signaling pathways, such as the regulation of neuronal plasticity. For example, SGK protects synaptic glutamate transporters from proteasomal degradation (Boehmer et al., 2003, 2004; Schniepp et al., 2004), and altered glutamate transporter levels have been suggested to contribute to LTP and memory (Levenson et al., 2002; Shen and Linden, 2005). SGK can reportedly be either upregulated or downregulated in the rodent hippocampus after performance of learning tasks (Donahue et al., 2002; Tsi et al., 2002), and it is proposed that enhanced SGK expression facilitates memory consolidation (Tsi et al., 2002). The oligopeptides generated by proteasomal protein digestion are transported into the endoplasmic reticulum via the antigen transporter complex (Tap1, Tap2, and tapasin), which is physically associated with the proteasome in many cell types (Hwang et al., 2001). Intriguingly, targeted deletion of the Tap1 gene in mice contributes to deficits in synaptic plasticity (Hu et al., 2000). Hence, in both cases, there is support for the hypothesis that regulation of these genes via Zif268 may contribute to synaptic plasticity. The consequences of reduced psmnb9 expression for neuronal function are not clear, and it remains to be determined whether altered expression of psmnb9 directly affects plasticity.

Figure 5. Promoter activity of candidate Zif268 target genes. a, Plasmids used for the promoter activity study (see Materials and Methods). The positions of putative ERE sites are indicated (○). For simplicity, lengthy regions of the SGK promoter devoid of putative EREs are represented as dashed lines. b, Plasmids (2 μg) containing wild-type promoter regions of the psmnb9, SGK, and Tap1 genes were cotransfected in PC12 cells with 2 μg of either pZif268 (expressing Zif268; []). For simplicity, lengthy regions of the SGK promoter devoid of putative EREs are represented as dashed lines. b, Plasmids (2 μg) containing wild-type promoter regions of the psmnb9, SGK, and Tap1 genes were cotransfected in PC12 cells with 2 μg of either pZif268 (expressing Zif268; []). In each experiment, values were corrected for transfection efficiency (see Materials and Methods). Results from at least five independent experiments are shown (means ± SEM). *p = 0.05 versus control (Wilcoxon signed rank test).

Figure 6. Expression of Zif268-target candidate genes during in vivo neuronal plasticity. Semiquantitative RT-PCR was used to determine the relative expression levels of the psmnb9, SGK, and Tap1 genes in striatal tissue isolated from haloperidol-treated rats (hal) compared with corresponding vehicle-treated controls (veh). a, Levels of striatal Zif268 mRNA 30 min after treatment with vehicle ([]) or haloperidol (__). b, Representative RT-PCR product band intensities for candidate genes expressed in striatum tissue for haloperidol- and vehicle-treated rats. c, Relative amounts of target cDNA were determined (see Materials and Methods) 6 h after treatment with vehicle ([]) or haloperidol (__). The results shown are mean ± SEM (n = 3–6). *p = 0.05; **p = 0.01 (two-sample t test).
some inhibitors attenuate learning and memory (Hegde et al., 1997; Chain et al., 1999; Lopez-Salon et al., 2002) and suppress functional plasticity (Moss et al., 2003), whereas compromised proteasome function has been linked with aging (Keller et al., 2000) and with memory impairment in humans (Cooper et al., 2004). This suggests a facilitatory role for the proteasome in plasticity. In contrast, proteasome inhibition enhances neurite outgrowth, presynaptic neurotransmitter release, and postsynaptic glutamate receptor sensitivity, consistent with the proteasome acting to suppress plasticity (Obin et al., 1999; Speese et al., 2003; Zhao et al., 2003; Juo and Kaplan, 2004). Synaptic plasticity probably depends on a precise balance between the synthesis of some proteins and the degradation of others, as has been noted for the guidance of growth cones in response to environmental cues (Campbell and Holt, 2001). Regulation of proteasome genes in the CNS by Zif268 may allow long-lasting but potentially subtle alterations in the activity of the proteasome.

Sustained alterations in proteasome gene expression have been associated with long-term CNS plasticity. Altered expression of a number of proteasome genes, in particular psmb9, psme1, and SGK, has been observed in a variety of plasticity models (El-Khodor et al., 2001; Becker et al., 2003; Blalock et al., 2003; Cirelli et al., 2004). In addition, reduction of the chymotrypsin activity of the proteasome, as would be predicted with reduced psmb9 levels, has been specifically linked with the modulation of neuronal morphology (Fenteany and Schreiber, 1996). Thus, the regulation of proteasome gene expression, and particularly the psmb9 and psme1 genes, is likely to be of major importance for CNS function. A speculative possibility would be that, while interferon and related hormones regulate the expression of inducible proteasome subunits in the immune system, this role is assumed by glutamatergic activity in the CNS, via TFs including Zif268.

Induction or repression of the proteasome subunits psmb8, psmb9, psme1, and psme2 alters the activity of the proteasome (Groетtrup et al., 1995; Harris et al., 2001). Increased Zif268 expression suppressed overall proteasome function in PC12 cells. The degree of inhibition was slight compared with the reductions in psmb9 and psme2 mRNA. However, we observed high basal proteasome activity in PC12 cells, probably because of the relatively high expression of other catalytic β subunits such as psmb3, psmb5, and psmb6 (Fig. 1). Hence, the substantial Zif268-mediated reduction in psmb9 and psme2 expression might not greatly affect overall proteasome activity (Oberdorf et al., 2001; Kazi et al., 2003; Berkers et al., 2005). Nevertheless, the results indicate that Zif268 induction suppresses proteasome activity, with the degree of suppression probably dependent on the level of expression of other catalytic proteasome subunits in the cell at that time.

Conversely, the absence of Zif268 in the gene-targeted mice was associated with substantially elevated cerebral cortex proteasome activity (Fig. 7). In the immune system, transcriptional induction of the psmb8, psmb9, psme1, and psme2 genes enhances proteasome activity, in the absence of any change in expression of the constitutive β catalytic or 19S subunits (Fruh et al., 1994; Yang et al., 1995; Hisamatsu et al., 1996). It is believed that the psmb8 and psmb9 subunits displace the corresponding constitutive β subunits, whereas the psme1 and psme2 subunits compete reversibly with the existing constitutive 19S complex. Thus, elevated proteasome activity after increased expression of the psmb8, psmb9, psme1, and psme2 subset of proteasome subunit genes would be predicted based on extrapolations from the immunoproteasome to the neuronal proteasome. Our data from the Zif268 knock-out mice confirm the importance of Zif268 for the regulation of CNS proteasome activity. They also raise the interesting possibility that abnormal proteasome activity may contribute to the impairment of late-phase LTP and the deficits in behavioral tests of learning and memory that are observed in these mice (Jones et al., 2001).

Overall, our discovery that Zif268 regulates the transcription of proteasome-related genes and hence modulates the activity of the proteasome reveals a new and previously unsuspected dimension to the mechanisms of neuronal plasticity. The regulation of proteasome function at multiple levels provides a new insight into the mechanisms sustaining the late phase of neuronal plasticity and focuses attention particularly on protein degradation and subsequent modification of synaptic transmission as underlying these enduring changes.

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