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Protein Kinase CK2 Enhances *Mcl-1* Gene Expression Through the Serum Response Factor-Mediated Pathway in the Rat Hippocampus

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The protein kinase CK2 (casein kinase 2) is a ubiquitous serine/threonine protein kinase that suppresses apoptosis. CK2 is composed of catalytic and regulatory subunits, and CK2-dependent phosphorylation is a global mechanism in the inhibition of caspase signaling pathways. The serum response factor (SRF) is an important regulator of cell growth and differentiation. Although CK2 has been shown to phosphorylate SRF in vitro, the biological relevance of this interaction remains largely unclear. We observed increased SRF phosphorylation and increased *Mcl-1* gene expression in hippocampal CA1 neurons following transfection with a plasmid expressing the wild-type CK2α (CK2αWT) protein, whereas transfection with a plasmid expressing a catalytically inactive mutant of CK2α (CK2α156A) reduced *Mcl-1* gene expression. Cotransfection with a plasmid expressing the inactive SRF99A mutant inhibited the CK2αWT-induced upregulation of *Mcl-1* gene expression. The expression of either the CK2α156A or the SRF99A mutant also inhibited the glutamate-induced upregulation of Mcl-1 protein expression in PC12 cells. Our results suggest that CK2-mediated signaling represents a cellular mechanism that may aid in the development of alternative therapeutic strategies to attenuate apoptosis in hippocampal neurons. © 2013 Wiley Periodicals, Inc.

Key words: protein kinase CK2; serum response factor; Mcl-1; antiapoptosis

The protein kinase CK2 (casein kinase 2) is a ubiquitous serine/threonine protein kinase composed of α and/or α′ catalytic subunit and β regulatory subunits, which associate to form α2β2, α′2β2, and αα′β2 heterotetramers (Litchfield, 2003). Catalyzing more than 300 substrates, CK2 is involved in various cellular functions, including gene transcription and signal transduction (Meggio and Pinna, 2003). It is a key suppressor of apoptosis, and the inhibition of CK2 activity has been shown to induce apoptosis in cancer cells (Ahmad et al., 2008; Hanif et al., 2010). The CK2-dependent phosphorylation is thought to be a global mechanism for inhibiting caspase signaling pathways during apoptosis (Duncan et al., 2011), and CK2 reduces cleavage of the proapoptotic protein Bid by phosphorylation, thereby diminishing Bid-induced cell death (Desagher et al., 2001). The ARC protein (an apoptosis repressor) is also phosphorylated by CK2, which increases its inhibitory effect on caspase activity (Li et al., 2002). Moreover, CK2 enhances cell survival through the phosphorylation of the transcription factor NF-κB and the subsequent activation of NF-κB-mediated pathways (Manna et al., 2007; Chao et al., 2011). However, whether other transcription factors mediate the antiapoptotic effects of CK2 remains largely unclear.

The serum response factor (SRF) is a transcription factor that is activated by serum growth factors and intracellular calcium. The SRF mediates the rapid activation of the immediate early gene *c-fos* in resting cells (Herdegen and Leah, 1998). Downstream SRF-mediated signaling occurs primarily through the mitogen-activated protein kinase, Ca^{2+}/calmodulin-dependent kinase, and Rho/actin signaling cascades (Knöll and Nordheim, 2009) through SRF binding to the serum response element (SRE) in the respective promoter regions (Chai and Tarnawski, 2002). In addition to the role of SRF in the regulation of cell growth and differentiation, the in vitro effects of SRF deficiency have been shown to parallel the apoptotic effects of impaired *Bcl-2* and *Bcl-xL* gene expression in murine embryonic stem cells (Schratt et al., 2004).

Although the phosphorylation of SRF by CK2 has been shown to enhance the DNA binding activity of SRF in vitro (Manak and Prywes, 1991; Marais et al., 1992), the biological relevance of these interactions

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remains largely unclear. The aim of our study was to determine whether CK2 mediates the in vivo expression of the antiapoptotic gene *Mcl-1* in the rat hippocampus by influencing SRF binding of the SRE in the promoter of *Mcl-1* (Vickers et al., 2004).

MATERIALS AND METHODS

Animals

Adult male Wistar rats (250–350 g) were purchased from BioLASCO (Taipei, Taiwan) and housed individually in a temperature-controlled (22°C ± 2°C) room with 12-hr light/dark cycles (lights on at 7:30 AM) and food and tap water continuously available. All animal care and experimental procedures were conducted in accordance with the University Committee on the Use and Care of Animals at National Cheng Chi University and the *Guide for the care and use of laboratory animals* as adopted and promulgated by the National Institutes of Health (NIH-80-23).

Plasmid Construction and DNA/Polyethyleneimine Complex Preparation

The HA-tagged CK2α expression plasmid contained the full-length rat *CK2α*-coding sequence. The cDNA of *CK2α* was amplified by PCR using the forward primer, 5′-CGGAATTCTCGGGACCCGTGCCAAGCAG-3′, which incorporated an EcoRI cleavage site (underlined) and removed the ATG start codon, and the reverse primer, 5′-GCTCTAGATTACTGCTAGCGCCAGCGG-3′, which incorporated an XbaI cleavage site (underlined) and maintained the TAA stop codon, as described previously (Chao et al., 2006). The PCR product was subcloned through the EcoRI and XbaI sites of the mammalian expression vector pcDNA3. The CK2α156A expression plasmid was generated by nucleotide base substitution of the *CK2α* cDNA template using the QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA). The Flag-tagged SRF99A expression plasmid was constructed using the pCMV-Tag 2B plasmid (Agilent Technologies), and was kindly provided by Dr. Eminy H.Y. Lee (Tyan et al., 2008). Polyethyleneimine (PEI; 25 kDa; Sigma Aldrich, St. Louis, MO) was used as the transfection agent for experiment animals. The PEI molecule is a cationic, highly branched organic polymer that is nontoxic to neurons and has been used to introduce recombinant DNA into mammalian brain cells (Abdallah et al., 1996; Chao et al., 2011). Before injection, the plasmid DNA solutions and the PEI were diluted to 2.77 μg/μl and 0.1 M, respectively, in 5% glucose. Immediately before injection, the PEI and DNA solutions were combined at a ratio of 6 and 10 PEI nitrogen per DNA phosphate for the CK2α and SRF plasmids, respectively. The mixture was vortexed for 30 sec and equilibrated for 15 min.

Intrahippocampal DNA Transfection

Rats were anesthetized with Zoletil 50 (40 mg/kg) intraperitoneally and subjected to stereotactic surgery. The cannulae were implanted bilaterally into the dorsal hippocampal CA1 areas at the following coordinates: 3.5 mm posterior to the bregma, 2.5 mm lateral to the midline, and 3.4 mm ventral to the skull surface. Transfection was performed by injecting 1.5 μg/μl plasmid DNA. For the cotransfection experiments, a mixture of 0.75 μg of each plasmid was used for injection. The injection volume was 0.8 μl for each CA1 area, and the injection rate was 0.4 μl/min. The injection needle was left in place for 2 min to limit the diffusion of the injected molecules. The rats were sacrificed 48 hr posttransfection. The brain was removed, and two 2-mm-thick hippocampal tissue slices were resected using a brain slicer. The hippocampal tissue containing the major CA1 area and the transfected area was further resected using a punch with 1.4-mm inner diameter, as described previously (Chao et al., 2011), to obtain the hippocampal CA1-area tissue samples for the mRNA and Western blot analyses.

Reverse Transcription and Real-Time Quantitative PCR

Total RNA was isolated from the CA1 tissue using the RNAspin Mini kit (GE Healthcare, Waukesha, WI). The cDNA was generated from 0.5 mg of the total RNA using 0.5 μg of oligo-dT and the MMLV reverse transcriptase (Epicentre Biotechnologies, Madison, WI). Real-time quantitative PCR (Q-PCR) analysis was performed using the SYBR Green (Life Technologies, Carlsbad, CA) method in an Applied Biosystems 7300 Real-Time PCR System (Life Technologies). The forward and reverse primer sequences, 5′-CTTATTTCTTTTGGTGCCTTTGTG-3′ and 5′-TCGATGCAGCTTTCTTGGTTT-3′, respectively, were used for *Mcl-1* mRNA quantification. The endogenous gene hypoxanthine phosphoribosyl transferase (*HPRT*) was used as an internal control. The forward and reverse primer sequences used were 5′-GCCGACCGGTTCTGTCAT-3′ and 5′-TCATAACCTGGTTCATCATCACTAATC-3′, respectively. The amounts of *Mcl-1* and *HPRT* mRNA from each sample were measured simultaneously in separate reactions. The PCR thermal conditions were 55°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 sec and 60°C for 1 min.

Western Blotting

The hippocampal CA1 tissue was lysed by sonication in a lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, and 1% IGEPAL CA-630RIPA, pH 7.4) with protease inhibitor cocktail set I (Calbiochem, San Diego, CA) and protein phosphatase inhibitor cocktail set IV (Calbiochem) and clarified by centrifugation at 14, 000*g* for 20 min at 4°C. Equal amounts of lysate samples (15 μg protein) were subjected to 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Billerica, MA). The PVDF membranes were blocked with 2% bovine serum albumin (BSA) in Tris-buffered saline with 0.05% Tween-20 before incubation with one of the following primary antibodies: anti-pS103SRF (1:1,000; Cell Signaling Technology, Danvers, MA), anti-SRF (1:1,000; EMD Millipore), anti-Mcl-1 (1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA), anti-CK2α (1:4,000; Abcam, Cambridge, MA), anti-HA tag (1:500; Roche, Nutley, CA), or anti-β-actin (1:10,000; EMD Millipore) antibody. A horseradish peroxidase (HRP)-conjugated secondary antibody (Perkin Elmer, Waltham, MA) was used for all Western blots in our

study. The protein bands were visualized using a chemiluminescent HRP substrate and the XLite Image System (Avegene, Taipei, Taiwan). The density of each band was quantified in Image J (http://rsbweb.nih.gov/ij/; National Institutes of Health).

Immunoprecipitation

For the immunoprecipitation of CK2α and Flag-tagged proteins, 500 μg of CA1 tissue lysate was incubated with 10 μl of anti-CK2α antibody (Abcam) or anti-Flag tag antibody (EMD Millipore) overnight at 4°C, respectively. The immune complexes were precipitated using protein-G magnetic beads (EMD Millipore) at 4°C for 2 hr. After being washed twice with phosphate-buffered saline (PBS), the precipitate was combined with a sample loading buffer before SDS-PAGE and Western blotting.

Immunohistochemistry

At 48 hr posttransfection, rats were euthanized with an intraperitoneal injection of pentobarbital (100 mg/kg) and perfused with ice-cold PBS, followed by 4% paraformaldehyde. The brains were removed and postfixed in a solution of 30% sucrose and 4% paraformaldehyde for 24 hr before freezing. The frozen brains were cut into 30-mm sections on a cryostat, and the sections were mounted on gelatin-coated slides. The brain sections were rinsed with PBS for 10 min and made permeable by immersion in a prechilled solution of 95% ethanol and 5% acetic acid for 10 min, followed by three rinses in PBS for 10 min. The sections were incubated in a blocking buffer (3% normal goat serum, 3% BSA, and 0.2% Triton X-100 in PBS) for 2 hr, followed by three rinses in PBS for 10 min. For immunofluorescence detection of HA- or Flag-tagged proteins, tissue sections were incubated with a mouse monoclonal anti-HA tag IgG antibody (1:100; Roche) or a mouse anti-Flag tag IgG antibody (1:100; EMD Millipore) in a blocking buffer overnight at 4°C. The sections were rinsed three times in PBS before incubation with an anti-mouse FITC-conjugated antibody or an anti-mouse Texas red-conjugated antibody (1:100; Sigma Aldrich) in PBS for 1 hr. The sections were rinsed three times in PBS before mounting with a mounting medium.

Statistical Analysis

The data for Western blotting, and real-time Q-PCR were evaluated using a Student's *t*-test or one-way analysis of variance, followed by a post hoc Dunnett's *t*-test or Newman-Keul multiple-comparisons test, the results of which are expressed as the tD or q value, respectively.

RESULTS

CK2 Enhanced SRF Phosphorylation and *Mcl-1* Gene Expression

We first examined the effect of transfection with a wild-type CK2α (CK2αWT) expression plasmid on the CK2α protein level in the hippocampal CA1 area. The Western blotting results showed that the total CK2α protein level increased in the CA1 tissue transfected with the CK2αWT expression plasmid ($t_{1,12} = 8.12$, $P < 0.01$ by Student's *t*-test; Fig. 1A). The results from immunohistochemistry using an anti-HA antibody showed immunostaining of the HA-tagged CK2αWT protein in the CA1 area (Fig. 1B, upper panel). Immunoprecipitation of the HA-tagged CK2α protein was also performed with an anti-CK2α antibody, and Western blot analysis of the precipitate with an anti-HA antibody confirmed that the HA-tagged CK2αWT protein was expressed in the hippocampal cells (Fig. 1B, lower panel).

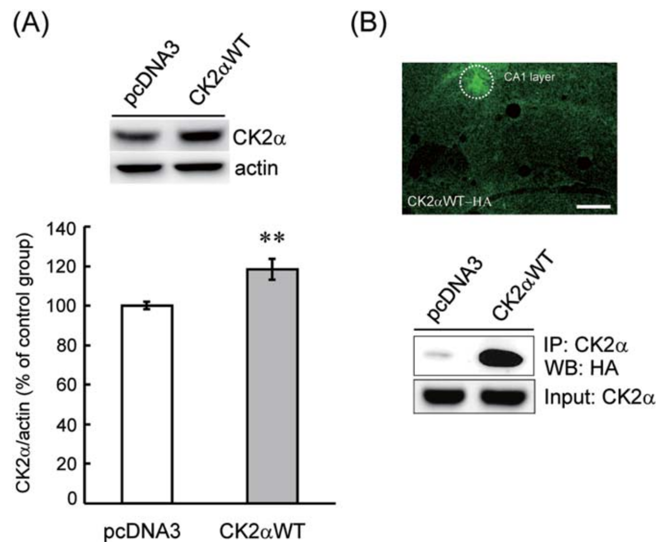

Fig. 1. Expression of transfected CK2α protein in the rat hippocampal CA1 area. **A:** Western blot analysis of the CK2α protein level at 48 hr following bilateral transfection of the hippocampal CA1 areas using 1.5 μg of the CK2αWT expression plasmid or the pcDNA3-HA (control) plasmid (n = 7). The data were quantified, and the mean ± SEM of CK2αWT expression is shown relative to that of the control. **$P < 0.01$ by Student's *t*-test. **B:** Immunohistochemical staining of the HA-tagged CK2αWT protein following DNA transfection of the hippocampal CA1 area (upper panel). An anti-HA-tag primary (IgG) antibody and an FITC-conjugated anti-IgG secondary antibody were used for visualization. Circle indicates the punch area (diameter about 1.4 mm) of the transfection. The HA-tagged CK2αWT protein was immunoprecipitated from 1 mg CA1 tissue lysate using an anti-CK2α antibody. The precipitate was separated by SDS-PAGE and analyzed by Western blotting using an anti-HA primary antibody (lower panel). Scale bar = 300 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

To demonstrate that the phosphorylation of SRF at Ser99 (identical to Ser103 of human SRF) is mediated by CK2α, we measured the differences in the levels of Ser99 phosphorylation in the SRF protein in the hippocampal CA1 area in transfection experiments using the pcDNA3-HA control plasmid, the CK2αWT expression plasmid, or a plasmid that expressed the catalytically inactive mutant CK2α156A. The results showed that the expression of the CK2αWT protein in the hippocampal CA1 area increased the phosphorylation of SRF at Ser99 ($F_{2,15} = 60.02$, $P < 0.001$; tD = 5.70, $P < 0.01$ by Dunnett's *t*-test; Fig. 2A) and that the expression of the CK2α156A mutant reduced the phosphorylation of SRF at Ser99 (tD = 5.25, $P < 0.01$). To demonstrate that the

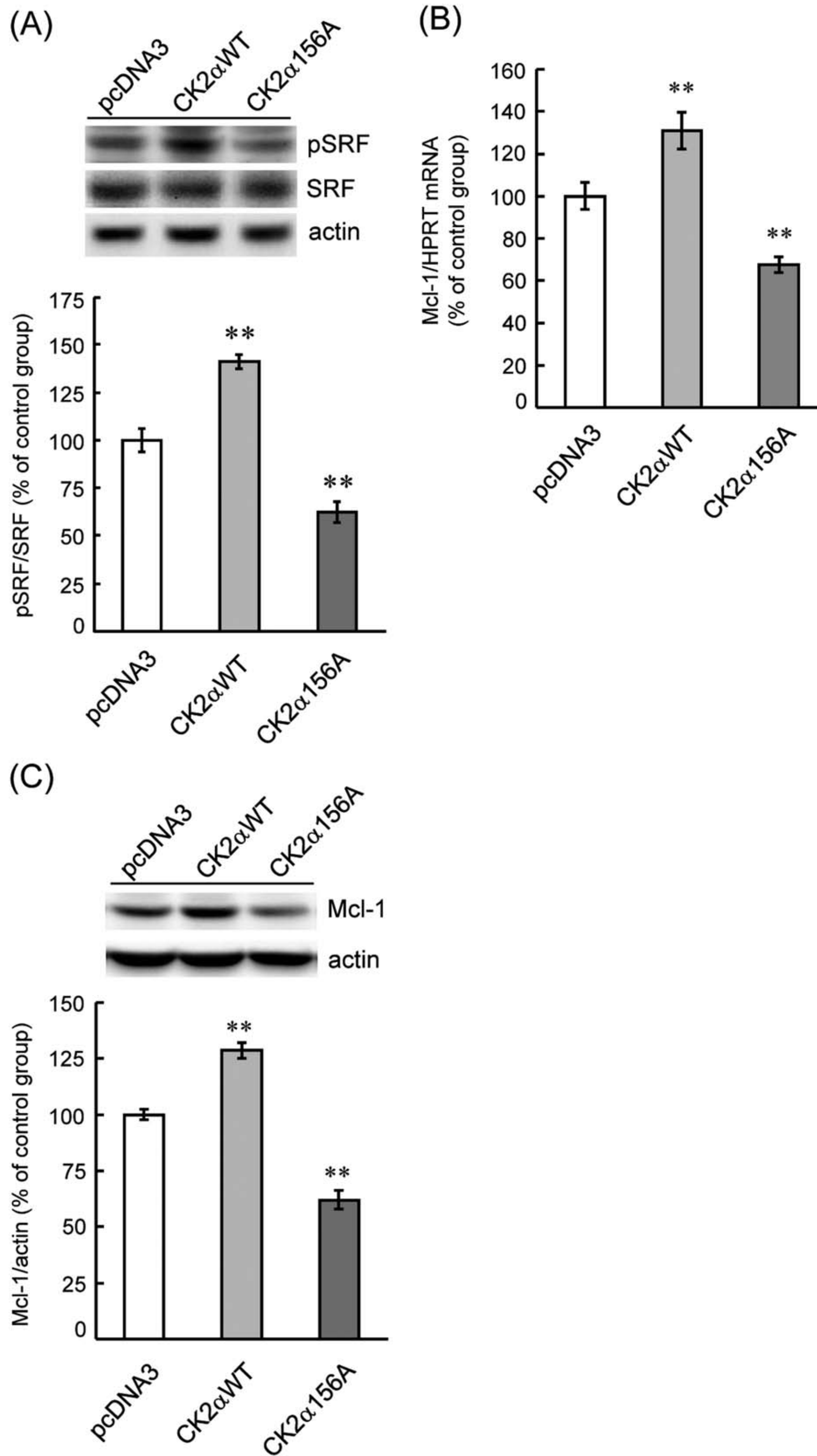

Fig. 2. CK2α phosphorylation of SRF results in increased *Mcl-1* mRNA and protein expression. The effects of wild-type CK2αWT and mutant CK2α156A protein expression on SRF phosphorylation at Ser99 (**A**), *Mcl-1* mRNA level (**B**) and Mcl-1 protein level (**C**) in the CA1 area were examined. The CA1 areas of rats were bilaterally transfected with 1.5 μg of the pcDNA3-control plasmid or the CK2αWT or CK2α156A expression plasmid, and the rats were sacrificed 48 hr later (n = 6–8). The data are mean ± SEM. Statistical significance was evaluated by one-way analysis of variance followed by the Dunnett's *t*-test (**$P < 0.01$).

phosphorylation of SRF influences downstream gene expression, the expression of the antiapoptotic gene *Mcl-1* was examined by Q-PCR. The results of Q-PCR analysis showed that the level of *Mcl-1* mRNA was higher in the CA1 tissue that was transfected with the CK2αWT expression plasmid compared with the control ($F_{2,21} = 25.76$, $P < 0.001$; tD = 3.61, $P < 0.01$) and that the level of *Mcl-1* mRNA was lower in the CA1 tissue that was transfected with the CK2α156A expression plasmid compared with the control (tD = 3.57, $P < 0.01$; Fig. 2B). Similarly, the Mcl-1 protein level was also higher for the CK2αWT transfection experiment ($F_{2,21} = 60.42$, $P < 0.001$; tD = 6.14, $P < 0.01$) and lower for the CK2α156A transfection experiment (tD = 4.83, $P < 0.01$) compared with the control (Fig. 2C).

Mutant SRF99A Inhibits *Mcl-1* Gene Expression

To determine whether Mcl-1 expression positively correlates with the CK2α-mediated ser99 phosphorylation of the SRF protein, we transfected the CA1 areas of rats with a plasmid that expressed a flag-tagged inactive SRF mutant, SRF99A, or the pCMV-flag control plasmid. In our preliminary study, the expression of the mutant SRF99A protein in PC12 cells significantly reduced the SRE-mediated luciferase activity (Supp. Info. Fig. 1). The level of total SRF protein in CA1 tissues was higher for the SRF99A transfection experiment compared with the control ($t_{1,10} = 4.20$, $P < 0.01$ by Student's *t*-test; Fig. 3A, left bar chart) and the level of total ser99-phosphorylated SRF was also lower in CA1 tissues expressing the SRF99A protein compared with the control ($t_{1,10} = 4.70$, $P < 0.01$; Fig. 3A, right bar chart). The immunohistochemistry of the hippocampal CA1 tissues that were transfected with the flag-tagged SRF99A expression plasmid confirmed that the SRF99A protein was expressed in the CA1 cells (Fig. 3B, upper panel). Western blot analysis of flag-tagged proteins that were immunoprecipitated from transfected CA1 tissues also confirmed that the SRF99A protein was expressed in the hippocampal cells (Fig. 3B, lower panel).

The Q-PCR analysis of CA1 tissues that had been transfected with the flag-tagged SRF99A expression plasmid showed that the level of *Mcl-1* mRNA was lower compared with the control ($t_{1,10} = 7.89$, $P < 0.01$; Fig. 4A). Likewise, Western blot analysis showed that the level of the Mcl-1 protein was lower in the transfected CA1 tissues compared with the control ($t_{1,10} = 3.86$, $P < 0.01$; Fig. 4B). In addition, Western blotting results showed that the level of the endogenous CK2α protein was not altered in CA1 tissues transfected with the SRF99A expression plasmid compared with that in CA1 tissues following transfection with pCMV alone ($t_{1,10} = 0.57$, $P > 0.05$; Fig. 4C).

SRF Mediates the Effect of CK2α on *Mcl-1* Gene Expression

To examine further the role of SRF in mediating the effects of CK2α on *Mcl-1* gene expression, the hippocampal CA1 areas of rats were cotransfected with 1) the pCMV and pcDNA3 control plasmids, 2) the SRF99A expression and pcDNA3 control plasmids, 3) the CK2αWT expression and pCMV control plasmids, or 4) the CK2αWT and SRF99A expression plasmids, and subsequent *Mcl-1* gene expression was evaluated by Western blotting and Q-PCR analyses. Although the Ck2α protein level was reduced in the SRF99A + CK2αWT group, compared with that of the pCMV + CK2αWT group, the reduction in CK2αWT expression was not statistically significant (q = 2.58, $P > 0.05$ by Newman-Keul comparison; Fig. 5A), and the CK2αWT protein level remained higher than that of the pCMV + pcDNA3 control group (q = 3.06, $P < 0.05$). The results of Q-PCR showed that the *Mcl-1* mRNA level in the CA1 tissues was lower in the SRF99A + pcDNA3 group compared with the pCMV + pcDNA3 control group ($F_{3,25} = 19.26$, $P < 0.001$; q = 4.64, $P < 0.01$ by Newman-Keul comparison; Fig. 5B) and that the *Mcl-1* mRNA level was higher in the pCMV + CK2αWT group compared with the pCMV + pcDNA3 control group (q = 5.22, $P < 0.01$) but was reduced in the SRF99A + CK2αWT group (q = 7.61, $P < 0.01$).

The Western blotting results were similar to those of Q-PCR analysis, showing that the level of Mcl-1 protein in the CA1 tissues was lower in the SRF99A + pcDNA3 group compared with the pCMV + pcDNA3 control group ($F_{3,24} = 12.49$, $P < 0.001$; q = 4.79, $P < 0.01$; Fig. 5C) and that the level of Mcl-1 protein was higher in the pCMV + CK2αWT group compared with the pCMV + pcDNA3 control group (q = 4.20, $P < 0.01$) but was similarly reduced in the SRF99A + CK2αWT group (q = 5.98, $P < 0.01$).

CK2α-Mediated Signaling Upregulates *Mcl-1* Gene Expression Under Glutamate Insult

To determine whether the CK2α-mediated upregulation of *Mcl-1* gene expression is involved in responses to stress stimuli, we examined this signaling pathway under glutamate insult in PC12 cells. The CK2α156A or SRF99A expression plasmid (1 μg) was transfected into PC12 cells. At 24 hr posttransfection, the cells were treated with 5 mM glutamate and incubated for an additional 24 hr. This subtle dosage of glutamate has been shown to reduce cell viability slightly (Ma et al., 2012). Western blot analysis of Mcl-1 protein expression showed that glutamate treatment of pcDNA3 transfected cells (control) significantly increased the level of Mcl-1 protein compared with PBS treatment ($F_{3,20} = 8.37$, $P < 0.001$; q = 6.39, p$P < 0.01$ by Newman-Keul comparison; Fig. 6). However, cells expressing either the CK2α156A or the SRF99A mutant protein had lower levels of Mcl-1 protein following glutamate treatment than the glutamate-treated transfection control (q = 3.14, $P < 0.05$, and q = 5.68, $P < 0.01$, respectively).

DISCUSSION

We previously demonstrated that, in addition to the ERK1/2 and PI3-K pathways, the CK2 pathway also

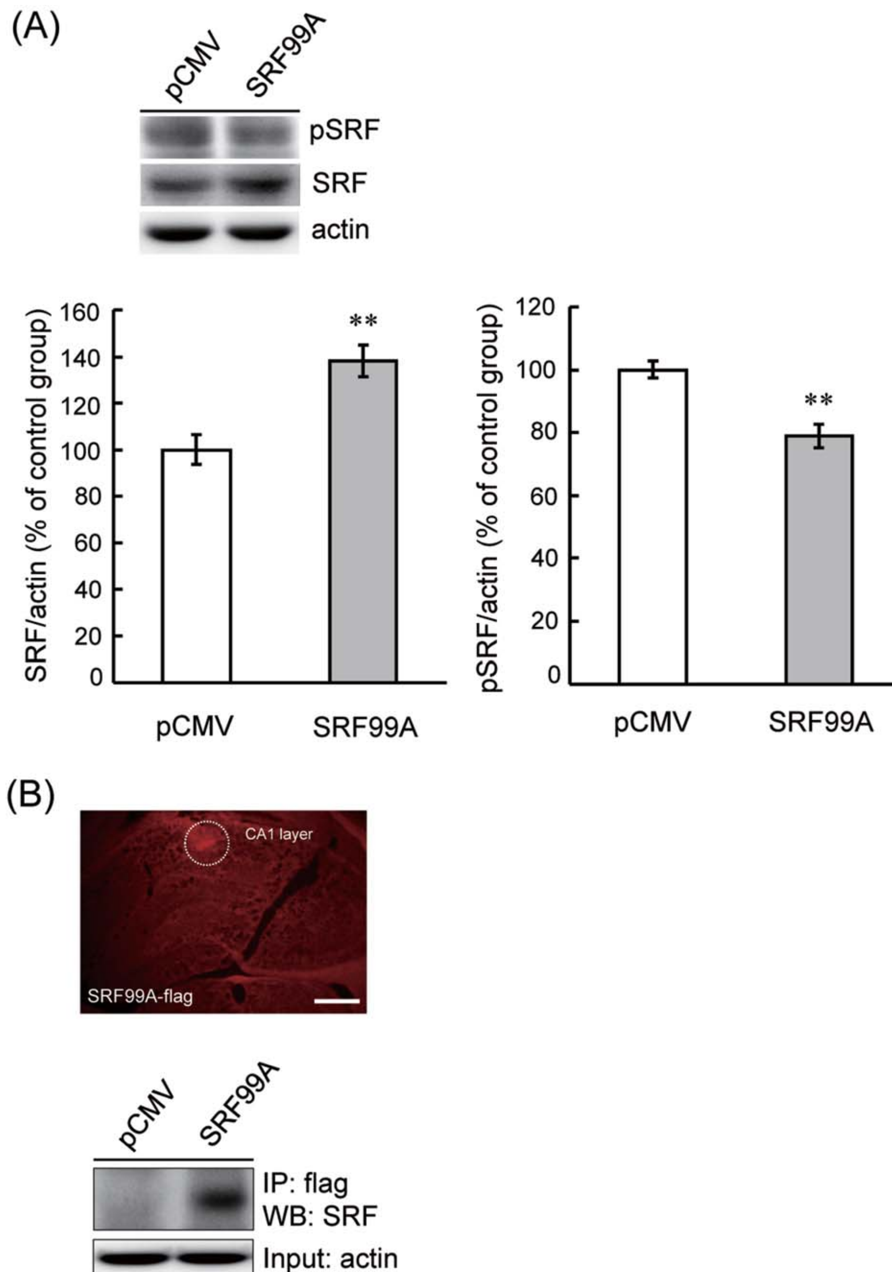

Fig. 3. Expression of transfected SRF99A protein in the hippocampal CA1 area. **A:** Effects of transfection with an SRF99A expression plasmid on the SRF protein level and phosphorylation status were examined. The CA1 areas of rats were bilaterally transfected with 1.5 μg of pCMV-control plasmid or the SRF99A-expression plasmid, and the rats were sacrificed 48 hr later (n = 6 each group). The data are expressed as mean ± SEM. Statistical significance was evaluated by the Student's *t*-test (**$P < 0.01$). **B:** Immunohistochemical staining showing flag-tagged SRF99A protein expression in the CA1 area (upper panel). The antii-flag-tag primary (IgG) antibody and Texas red-conjugated anti-IgG secondary antibody were used for visualization. The circle indicates the punch area (~1.4 mm in diameter) of the transfection. The SRF99A protein was immunoprecipitated from the CA1 tissue lysate (1 mg) using an anti-flag antibody, and the precipitate was separated by SDS-PAGE and analyzed by Western blotting using an anti-SRF antibody (lower panel). Scale bar = 300 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

mediates the antiapoptotic effect of brain-derived neurotrophic factor (BDNF) in the hippocampus of rats (Chao et al., 2011). The aim of our study was to determine whether other endogenous apoptosis-related genes may be regulated by CK2. Our results showed that manipulation of CK2 upregulates *Mcl-1* gene expression through the SRF-mediated signaling pathway. Our results also showed that CK2αWT increases the level of *Bcl-2* mRNA, but the magnitude of increase was less than that of the *Mcl-1* mRNA (Supp. Info. Fig. 2).

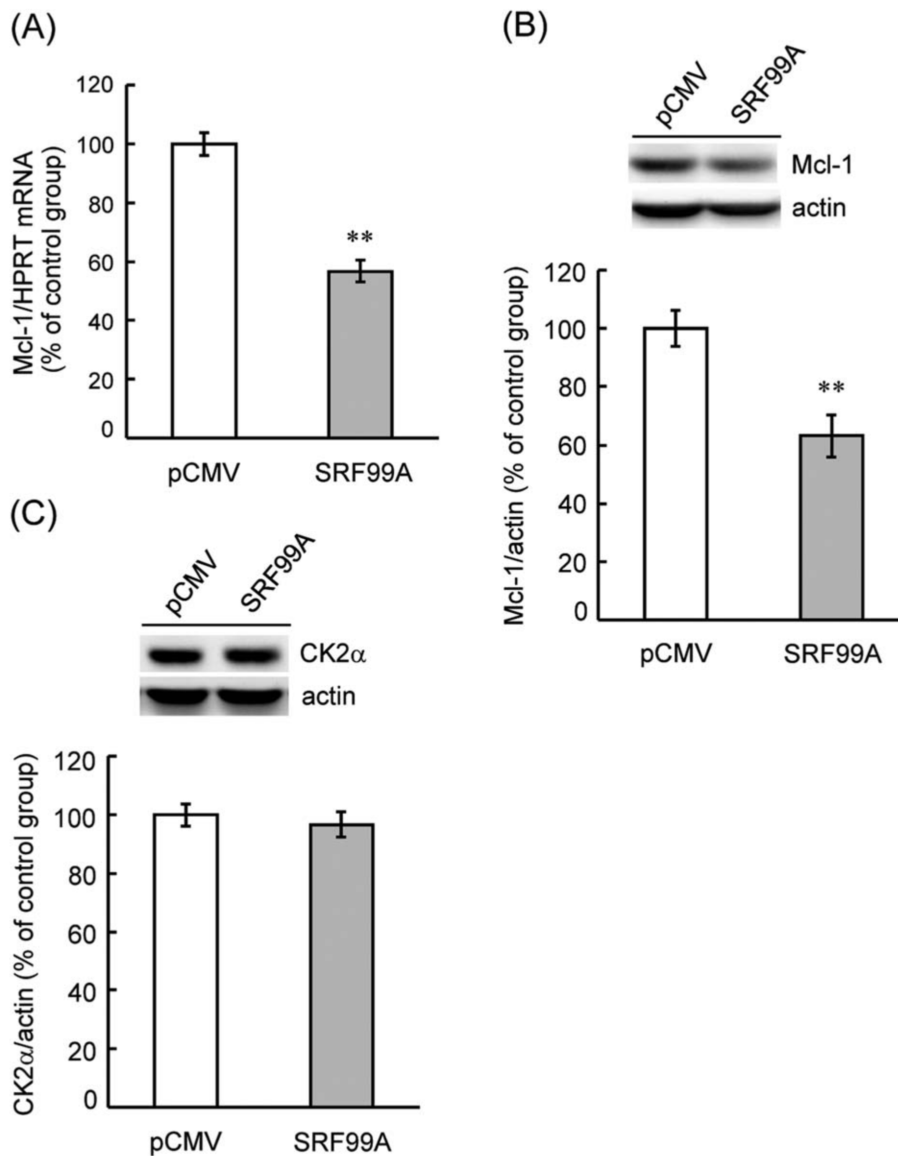

Fig. 4. SRF99A inhibits *Mcl-1* mRNA expression and protein level. Effects of SRF99A DNA transfections on *Mcl-1* mRNA level (**A**), Mcl-1 protein level (**B**), and CK2α protein level (**C**) in the CA1 area. The CA1 areas of rats were bilaterally transfected with 1.5 μg of the pCMV-control plasmid or the SRF99A expression plasmid, and the rats were sacrificed 48 hr later (n = 6). Data are mean ± SEM. Statistical significance was evaluated using the Student's *t*-test (**$P < 0.01$).

Although the CK2 holoenzyme activity is inhibited in the presence of CK2α156A in vitro (Korn et al., 1999), free forms of CK2α have been reported to express enzyme activity in vivo (Meggio et al., 1994; Dobrowolska et al., 1999). We found that transfection of the wild-type CK2α protein increased SRF phosphorylation, whereas the catalytically inactive CK2α156A protein decreased SRF phosphorylation. This result is supported by the findings of our previous study in which the expression of either CK2αWT or CK2α156A in the substantia nigra (SN) area altered CK2 activity (Chao et al., 2006). Moreover, the positive correlation between *Mcl-1* gene expression and CK2α-mediated SRF phosphorylation is consistent with our previous conclusion that CK2 enhances antiapoptotic cellular mechanisms based on our observations of the CK2-mediated upregulation of *Bcl-xL* in the rat hippocampus (Chao et al., 2011). It is also consistent with the notion that CK2 is an important enzyme in the nervous system (Blanquet, 2002). Although confocal images that were acquired in our previous study showed that CK2α was coexpressed with the neuronal nuclei protein NeuN in most of the CA1 cells examined (Chao et al., 2011), we cannot exclude the contribution of an unknown nonneuronal CK2 activity-dependent

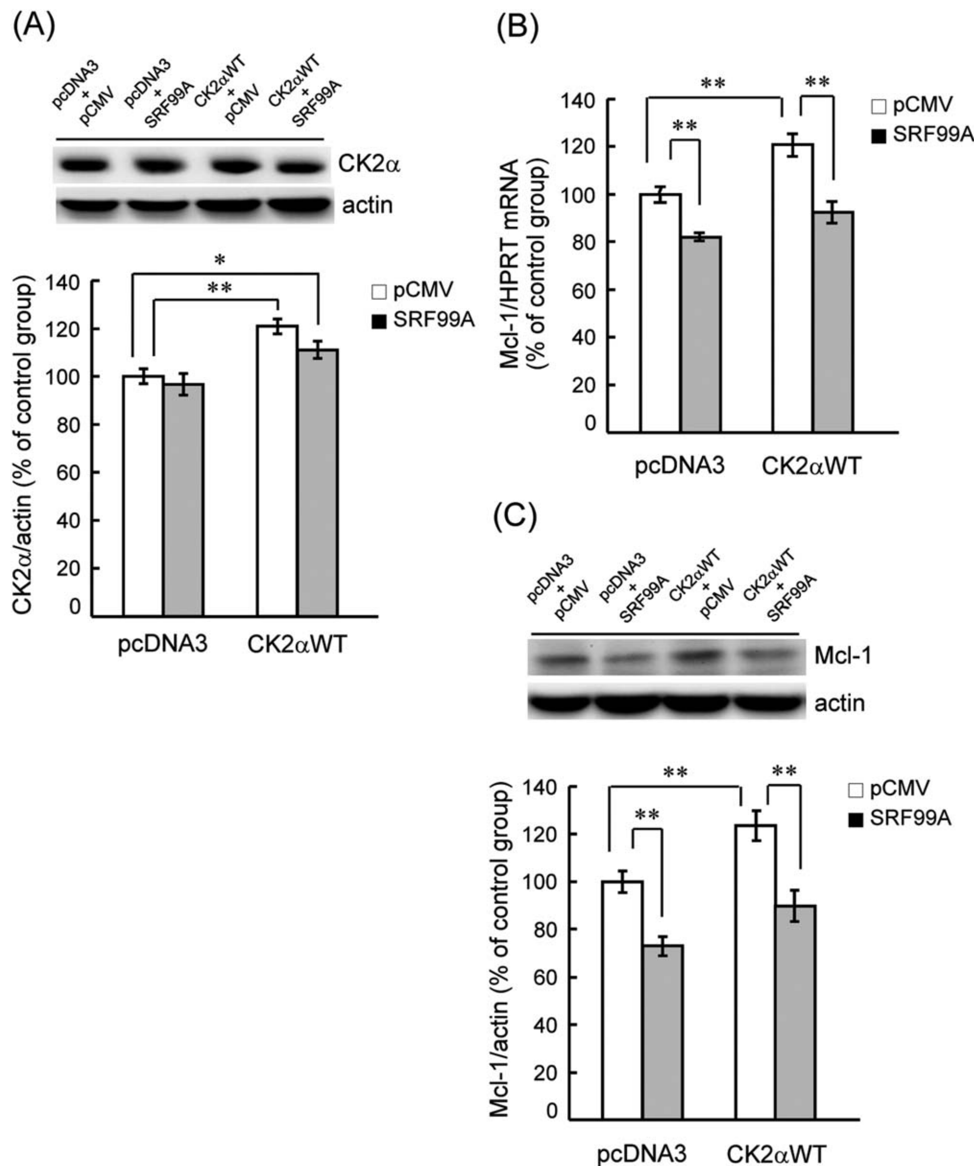

Fig. 5. SRF mediates the effect of CK2α on Mcl-1 expression. Effects of coexpression of the CK2αWT and SRF99A proteins on CK2α protein level (**A**), *Mcl-1* mRNA level (**B**), and Mcl-1 protein level (**C**) were examined by Western blotting and Q-PCR analyses. The CA1 areas of rats were cotransfected (0.75 μg each) with the pCMV and pcDNA3 control plasmids, the SRF99A expression and pcDNA3 control plasmids, the CK2αWT expression and pCMV control plasmids, or the CK2αWT and SRF99A expression plasmids, and the rats were sacrificed 48 hr later (n = 6–8). The data are mean ± SEM. Statistical significance was evaluated by one-way analysis of variance followed by the Newman-Keul comparison (**$P < 0.01$).

signaling pathway to the observed increase in *Mcl-1* gene expression.

The SRF participates in many important functions in the brain (Knöll and Nordheim, 2009) and has been shown to improve the survival of neurons and the regrowth of severed neurites following facial nerve fiber injury (Stern et al., 2012). Under oxidative stress, SRF also plays an important role in promoting the survival of SN dopamine neurons through mechanisms that likely involve Bcl-2 and BDNF (Rieker et al., 2012). We observed that both *Mcl-1* and *Bcl-2* gene expression were downregulated in CA1 cells expressing the SRF99A mutant; however, the reduction in the levels of *Bcl-2* mRNA and protein was less than that observed for *Mcl-1* (Supp. Info. Fig. 3). The different responses observed for *Mcl-1* and *Bcl-2* may reflect the different roles of these proteins in the cellular mechanisms that regulate cell death (Krajewski et al., 1995).

Furthermore, the SRF is a downstream molecule common to both the ERK1/2 and the PI3K pathways, which mediate the neuroprotective mechanism of BDNF in cortical neurons (Chang et al., 2004; Almeida et al.,

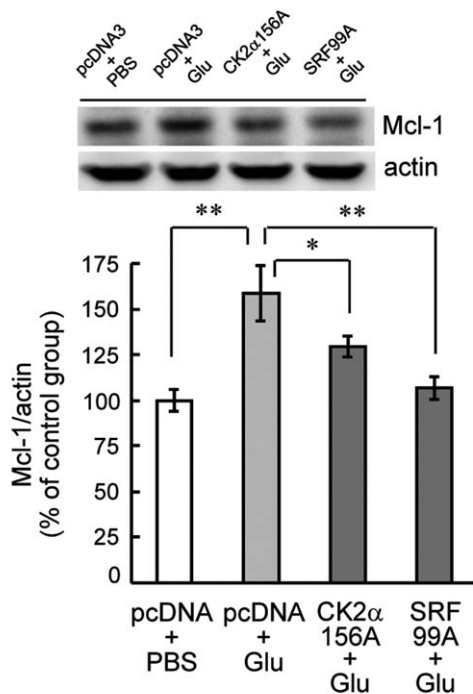

Fig. 6. Effects of CK2α blockade on *Mcl-1* expression under glutamate insult. PC12 cells were cultured at a density of 1.0×10^5 cells/well. The CK2α156A or SRF99A expression plasmid (1 μg) was transfected into the PC12 cells using the Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). After 24 hr of incubation, glutamate was added to each well to a final concentration of 5 mM, and cells were incubated for an additional 24 hr. The Mcl-1 protein levels were determined by Western blot analysis (n = 6 in two independent experiments). The data are mean ± SEM. Statistical significance was evaluated by one-way analysis of variance followed by the Newman-Keul's comparison (**$P < 0.01$).

2005). However, CK2-mediated pathways may also contribute to the antiapoptotic effects of BDNF (Chao et al., 2011). Whether the CK2 signaling cascades cross-talk with other signaling cascades to regulate SRF-mediated gene expression warrants further investigation.

The Mcl-1 protein plays a pivotal role in protecting cells against apoptosis and is overexpressed in various types of cancer (Quinn et al., 2011), and SRF knockdown in smooth muscle cells attenuates *Mcl-1* gene expression and induces apoptosis (Rodenberg et al., 2010). In the nervous system, DNA damage and caspase-mediated cell death are markedly increased in Mcl-1-deficient neurons (Mori et al., 2004; Arbour et al., 2008). In addition, the upregulation of *Mcl-1* gene expression by Purkinje cell-derived IGF-I has been shown to promote granule cell survival (Zhang and D'Ercole, 2004), and an increase in *Mcl-1* mRNA and protein levels in AICAR-preconditioned cortical neurons has been reported (Anilkumar et al., 2012). The results of our present study are consistent with such findings, showing that the blockade of CK2 and SRF reversed the subtle dosage of glutamate-induced increase in Mcl-1 protein expression. Thus, both our data and the findings of previous studies suggest that the regulation of *Mcl-1* gene expression occurs in an SRF-dependent manner.

The promoter of *Mcl-1* contains a functional SRE (Vickers et al., 2004). In addition to the downregulation of *Mcl-1* gene expression in cells expressing the mutant SRF99A, we also found that the cotransfection of CK2αWT and SRF99A expression plasmids inhibited the CK2α-induced effects on *Mcl-1* gene expression. In addition to SRF, the CK2-mediated phosphorylation of STAT1 has also been shown to promote *Mcl-1* gene expression (Timofeeva et al., 2006), and the phosphorylation of STAT1/STAT2 by the serum- and glucocorticoid-inducible protein kinase SGK also upregulates the expression of *Mcl-1*, which in turn protects against Aβ-induced apoptosis (Hsu et al., 2009). Whether other pathways synergize with the CK2-induced SRF-mediated upregulation of *Mcl-1* gene expression warrants further investigation.

In summary, our data show that CK2 induces the expression of the antiapoptotic gene *Mcl-1* through activation of the SRF-mediated pathway in hippocampal CA1 neurons. Our findings suggest an alternative cellular mechanism for the regulation of cell death, which may aid in the development of therapeutic strategies for inhibiting apoptosis in hippocampal neurons.

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REFERENCES

Abdallah B, Hassan A, Benoist C, Goula D, Behr JP, Demeneix BA. 1996. A powerful nonviral vector for in vivo gene transfer into the adult mammalian brain: polyethylenimine. Hum Gene Ther 7: 1947–1954.

Ahmad KA, Wang G, Unger G, Slaton J, Ahmed K. 2008. Protein kinase CK2α—a key suppressor of apoptosis. Adv Enzyme Regul 48:179–187.

Almeida RD, Manadas BJ, Melo CV, Gomes JR, Mendes CS, Grãos MM, et al. 2005. Neuroprotection by BDNF against glutamate-induced apoptotic cell death is mediated by ERK and PI3-kinase pathways. Cell Death Differ 12:1329–1343.

Anilkumar U, Weisová P, Düssmann H, Concannon CG, König HG, Prehn JH. 2012. AMP-activated protein kinase (AMPK)-induced preconditioning in primary cortical neurons involves activation of Mcl-1. J Neurochem doi: 10.1111/jnc.12108.

Arbour N, Vanderluit JL, Le Grand JN, Jahani-Asl A, Ruzhynsky VA, Cheung EC, Kelly MA, MacKenzie AE, Park DS, Opferman JT, Slack RS. 2008. Mcl-1 is a key regulator of apoptosis during CNS development and after DNA damage. J Neurosci 28:6068–6078.

Blanquet PR. 2002. Casein kinase 2 as a potentially important enzyme in the nervous system. Prog Neurobiol 60:211–246.

Chai J, Tarnawski AS. 2002. Serum response factor: discovery, biochemistry, biological roles and implications for tissue injury healing. J Physiol Pharmacol 53:147–157.

Chang SH, Poser S, Xia Z. 2004. A novel role for serum response factor in neuronal survival. J Neurosci 24:2277–2285.

Chao CC, Chiang CH, Ma YL, Lee EH. 2006. Molecular mechanism of the neurotrophic effect of GDNF on DA neurons: role of protein kinase CK2. Neurobiol Aging 27:105–118.

Chao CC, Ma YL, Lee EH. 2011. Brain-derived neurotrophic factor enhances Bcl-xL expression through protein kinase casein kinase 2-activated and nuclear factor kappa B-mediated pathway in rat hippocampus. Brain Pathol 21:150–162.

Desagher S, Osen-Sand A, Montessuit S, Magnenat E, Vilbois F, Hochmann A, et al. 2001. Phosphorylation of bid by casein kinases I and II regulates its cleavage by caspase 8. Mol Cell 8:601–611.

Dobrowolska G, Lozeman FJ, Li D, Krebs EG. 1999. CK2, a protein kinase of the next millennium. Mol Cell Biochem 191:3–12.

Duncan JS, Turowec JP, Duncan KE, Vilk G, Wu C, Lüscher B, Li SS, Gloor GB, Litchfield DW. 2011. A peptide-based target screen implicates the protein kinase CK2 in the global regulation of caspase signaling. Sci Signal 4:ra30.

Hanif IM, Hanif IM, Shazib MA, Ahmad KA, Pervaiz S. 2010. Casein kinase II: an attractive target for anti-cancer drug design. Int J Biochem Cell Biol 42:1602–1605.

Herdegen T, Leah JD. 1998. Inducible and constitutive transcription factors in the mammalian nervous system: control of gene expression by Jun, Fos and Krox, and CREB/ATF proteins. Brain Res Rev 28: 370–490.

Hsu WL, Chiu TH, Tai DJ, Ma YL, Lee EH. 2009. A novel defense mechanism that is activated on amyloid-beta insult to mediate cell survival: role of SGK1-STAT1/ STAT2 signaling. Cell Death Differ 16:1515–1529.

Knöll B, Nordheim A. 2009. Functional versatility of transcription factors in the nervous system: the SRF paradigm. Trends Neurosci 32: 432–442.

Korn I, Gutkind S, Srinivasan N, Blundell TL, Allende CC, Allende JE. 1999. Interactions of protein kinase CK2 subunits. Mol Cell Biochem 191:75–83.

Krajewski S, Bodrug S, Krajewska M, Shabaik A, Gascoyne R, Berean K, Reed JC. 1995. Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death in vivo. Am J Pathol 146:1309–1319.

Li PF, Li J, Muller EC, Otto A, Dietz R, von Harsdorf R. 2002. Phosphorylation by protein kinase CK2: a signaling switch for the caspase-inhibiting protein ARC. Mol Cell 10:247–258.

Litchfield DW. 2003. Protein kinase CK2: structure, regulation and role in cellular decisions of life and death. Biochem J 369:1–15.

Ma S, Liu H, Jiao H, Wang L, Chen L, Liang J, Zhao M, Zhang X. 2012. Neuroprotective effect of ginkgolide K on glutamate-induced cytotoxicity in PC 12 cells via inhibition of ROS generation and Ca^{2+} influx. Neurotoxicology 33:59–69.

Manak JR, Prywes R. 1991. Mutation of serum response factor phosphorylation sites and the mechanism by which its DNA-binding activity is increased by casein kinase II. Mol Cell Biol 11:3652–3659.

Manna SK, Manna P, Sarkar A. 2007. Inhibition of RelA phosphorylation sensitizes apoptosis in constitutive NF-kappaB-expressing and chemoresistant cells. Cell Death Differ 14:158–170.

Marais RM, Hsuan JJ, McGuigan C, Wynne J, Treisman R. 1992. Casein kinase II phosphorylation increases the rate of serum response factor-binding site exchange. EMBO J 11:97–105.

Meggio F, Pinna LA. 2003. One-thousand-and one-substrates of protein kinase CK2. FASEB J 17:349–368.

Meggio F, Boldyreff B, Issinger OG, Pinna LA. 1994. Casein kinase 2 down-regulation and activation by polybasic peptides are mediated by acidic residues in the 55–64 region of the beta-subunit. A study with calmodulin as phosphorylatable substrate. Biochemistry 33:4336–4342.

Mori M, Burgess DL, Gefrides LA, Foreman PJ, Opferman JT, Korsmeyer SJ, Cavalheiro EA, Naffah-Mazzacoratti MG, Noebels JL. 2004. Expression of apoptosis inhibitor protein Mcl1 linked to neuroprotection in CNS neurons. Cell Death Differ 11:1223–1233.

Quinn BA, Dash R, Azab B, Sarkar S, Das SK, Kumar S, et al. 2011. Targeting Mcl-1 for the therapy of cancer. Expert Opin Invest Drugs 20:1397–1411.

Rieker C, Schober A, Bilbao A, Schütz G, Parkitna JR. 2012. Ablation of serum response factor in dopaminergic neurons exacerbates susceptibility towards MPTP-induced oxidative stress. Eur J Neurosci 35: 735–741.

Rodenberg JM, Hoggatt AM, Chen M, Touw K, Jones R, Herring BP. 2010. Regulation of serum response factor activity and smooth muscle cell apoptosis by chromodomain helicase DNA-binding protein 8. Am J Physiol Cell Physiol 299:C1058–C1067.

Schratt G, Philippar U, Hockemeyer D, Schwarz H, Alberti S, Nordheim A. 2004. SRF regulates Bcl-2 expression and promotes cell survival during murine embryonic development. EMBO J 23:1834–1844.

Stern S, Sinske D, Knöll B. 2012. Serum response factor modulates neuron survival during peripheral axon injury. J Neuroinflamm 26:78.

Timofeeva OA, Plisov S, Evseev AA, Peng S, Jose-Kampfner M, Lovvorn HN, Dome JS, Perantoni AO. 2006. Serine-phosphorylated STAT1 is a prosurvival factor in Wilms' tumor pathogenesis. Oncogene 25:7555–7564.

Tyan SW, Tsai MC, Lin CL, Ma YL, Lee EH. 2008. Serum- and glucocorticoid-inducible kinase 1 enhances zif268 expression through the mediation of SRF and CREB1 associated with spatial memory formation. J Neurochem 105:820–832.

Vickers ER, Kasza A, Kurnaz IA, Seifert A, Zeef LA, O'Donnell A, Hayes A, Sharrocks AD. 2004. Ternary complex factor-serum response factor complex-regulated gene activity is required for cellular proliferation and inhibition of apoptotic cell death. Mol Cell Biol 24:10340–10351.

Zhang J, D'Ercole AJ. 2004. Expression of Mcl-1 in cerebellar granule neurons is regulated by IGF-I in a developmentally specific fashion. Brain Res Dev Brain Res 152:255–263.