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Zipzap/p200 is a novel zinc finger protein contributing to cardiac gene regulation ☆

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Abstract

Serum response factor (SRF) plays an important role in the regulation of immediate-early genes and muscle-specific genes, while SRF cofactors may contribute significantly to assist in tissue-specific, development-stage related regulation of SRF-target genes. We recently cloned a novel SRF cofactor, termed zipzap/p200, which is a zinc finger protein yet to be characterized. We determined that zipzap/p200 is a 200-kDa protein with two classic C2H2 zinc fingers at the carboxyl terminus where the nucleotide sequence was highly conserved among human, mouse, and rat. The zipzap gene was expressed in multiple tissues and at multiple ages, including the fetal and adult heart. The zipzap protein interacted with SRF in vivo and was found in protein complexes containing SRF and other SRF cofactors, including p49/strap and Nkx2.5. Zipzap/p200 activated the promoter of cardiac genes and potentiated the effect of myocardin on ANF promoter activity. Therefore, zipzap may serve as a transcription co-activator for the regulation of cardiac gene expression. Our data support the notion that a number of SRF cofactors may participate in gene regulation and thereby contribute to the delicate control of gene expression in complex biological processes.

Keywords: Zinc finger protein; Cardiac; Gene expression; Cofactor

It is well appreciated that the altered patterns of gene expression are associated with physiological and morphological changes during the processes of development, maturation, and aging [1–3]. The dynamic patterns of gene expression observed in animals are likely to be the result of a delicate balance of complex transcriptional regulation [2,4]. Among the transcription factors, serum response factor (SRF) has been demonstrated to play a significant role in the regulation of cardiac genes during embryonic development, postnatal development, and adult aging [5–9]. SRF is a member of the MADS (MCM1, Agamous, Deficiens, SRF) family of transcription factors that regulates a number of immediate-early and muscle-specific genes. SRF also serves to regulate cell proliferation, cell size, and cell survival [6,10–13]. SRF dimers bind to the serum response element (SRE) in the promoter region of a number of target genes [13–15], such as atrial natriuretic factor (ANF), α-myosin heavy chain, and sarcoplasmic reticular calcium ATPase.

SRF exhibits functional interactions with a number of SRF cofactors in the regulation of SRF-target genes [16]. These interactions likely modulate SRF function and may also enable SRF to mediate tissue-specific regulation at different developmental stages [17,18]. To date, a number of SRF cofactors, including the TCF family of proteins, the SAP protein myocardin, Nkx2.5, and Hop, have been identified, and their various functions in cardiac development have been investigated [18–21]. We have recently reported the identification of a SRF cofactor, p49/Strap, which modulates cardiac gene expression and effectively represses the ANF promoter activity strongly induced by myocardin

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[4]. In our previous experiments we noticed that protein complexes from immunoprecipitation assays with either p49/strap or SRF antibody contained more protein components than we had anticipated [4]. Therefore, we hypothesized that a large protein complex might have formed in the promoter region of SRF-target genes, in which SRF and its multiple SRF cofactors, including SRF co-repressors and co-activators, would interact with each other. Some of the cofactor(s) may have strong interaction with SRF, while others may have weak interaction with SRF, as both the strong and weak interactions are important for the intracellular functions including transcription [22–24]. The interaction among those factors may determine the status of activation or repression of various SRF-target genes.

Using the same yeast two-hybrid screening strategy that we previously applied [4], we recently isolated another SRF cofactor that is likely to be a transcription co-activator, which we have named "zipzap/p200" for "zinc finger protein with activation potential". The zipzap/p200 gene encoded a 1835-residue protein with a mass of approximately 200 kDa. Zipzap/p200 was observed in the protein complex containing SRF, p49/strap, and Nkx2.5. The mammalian two-hybrid assay further indicated that zipzap/p200 had weak interactions with SRF. A high level expression of zipzap/p200 activated the promoter activity of MLC2v, cardiac actin, and ANF. Zipzap/p200 also potentiated the effect of myocardin on ANF promoter activity, suggesting that zipzap/p200 may act as a transcriptional co-activator for SRF-target genes. The zipzap/p200 mRNA expression was observed in the heart and other tissues. The zipzap/p200 protein level was elevated in myocardium of both humans and animals with advancing age. The changes in the expression of zipzap/p200, p49/strap, and other SRF cofactors during development, maturation as well as post-maturational aging further support the notion that the dynamic pattern of gene expression observed in senescence is likely not random, but rather, a well-regulated gene program that is part of the process of adult aging [2,4].

Experimental procedures

Yeast two-hybrid screening. The yeast two-hybrid screening was performed as previously described [4]. Briefly, a bait construct containing the carboxyl terminus of the SRF protein (247–499 residues) was constructed by fusing the SRF fragments to the GAL4 DNA-binding domain in the pGBT9 vector (Clontech). The construct was then used to screen an EML cDNA library [25]. The cDNA clones representing potential SRF-interacting proteins were sequenced and were compared with the GenBank database by using Blast Search.

Cloning of full-length coding region sequence of zipzap/p200 gene. A cDNA clone which was isolated from yeast two-hybrid cDNA library matched in the GenBank database a gene which has not been fully characterized. It has ARID, RFX, and zinc finger domains. We named it zipzap/p200. The full-length coding region of the human zipzap/p200 gene was amplified by PCR using human heart cDNA sample (Clontech). 5′ RACE PCR was also performed to confirm the 5′ end cDNA sequence. The coding region sequence of the human zipzap/p200 gene is shown in Fig. 1, and the sequence has been submitted to GenBank with Accession No. DQ096628.

Antibodies. A peptide of 15-amino acid residue (CGR RLL KRH ENN LSV) was selected from a conserved region close to zinc finger domain, which was observed to share high degree of homology among the human, mouse, and rat zipzap genes. A polyclonal antibody against the peptide was commercially generated by standard procedures (Genemed Synthesis, Inc., South San Francisco, CA). The antibody was shown to recognize an approximately 200 kDa protein band as predicted based on the length of coding region (Fig. 6A and B). Other reagents include monoclonal antibodies HA.11 (clone 16B12, Covance), anti-Flag M2 (Sigma), SRF (Abcam), and c-myc (clone 9E10, Santa Cruz); and polyclonal antibodies anti-HA (Santa Cruz), anti-Flag (Sigma), SRF (Santa Cruz), and p49/Strap [4].

Plasmid constructs. Expression plasmid constructs pcDNA3.1/myc-His(-)-zipzap/p200 (wild-type) and pcDNA3.1/myc-His(-)-zipzap/p200-COOH (a truncated form of zipzap/p200 containing carboxyl domain from residue 853 to 1835) were generated by ligating in-frame the full-length zipzap/p200 and the carboxyl domain of the zipzap/p200 gene, respectively. The DNA constructs were verified with sequencing analysis. Other plasmid vectors includes myocardin (a generous gift of Dr. Olson, University of Texas Southwestern Medical Center at Dallas), Nkx2.5 (a generous gift of Dr. R. Harvey, Victor Chang Cardiac Research Institute), pcDNA3-HA-p49/STRAP [4]. Firefly luciferase reporter plasmids include MLC2v, cardiac actin, and ANF [4]. *Renilla* luciferase reporter plasmids include pRL-CMV (Promega), which has a *Renilla* luciferase reporter under the control of cytomegalovirus (CMV) gene promoter; pRL-SV40 (Promega, Madison, WI), which has a *Renilla* luciferase reporter under the control of simian virus 40 (SV40) gene promoter; pRL-TK (Promega, Madison, WI), which has a *Renilla* luciferase reporter under the control of herpes simplex virus thymidine kinase (HSV-TK) gene promoter.

Northern blotting and Western blotting. Healthy young adult (3-month-old) and older (20-month-old) mice were obtained from colonies maintained by the NIA, the National Institutes of Health, under contractual agreement with Harlan Sprague-Dawley, Inc. (Harlan, IN). The human heart mRNA samples were obtained from Biochain Institute (Hayward, CA). The human tissue blot and mouse tissue blot were purchased from Clontech. The Northern blotting and Western blotting were performed as previously described [4]. The studies were conducted with Institutional Review Board approval and in accordance with the NIH Guiding Principles for Research Involving Animals and Human Beings.

Co-immunoprecipitation. The expression plasmid constructs containing zipzap/p200, p49/STRAP, SRF, myocardin, and Nkx2.5 were cotransfected into NIH3T3 cells by using Lipofectamine (Invitrogen) as previously described [4]. At 48 h after the transfection, cells were harvested, and the whole-cell lysate was isolated. The lysate proteins were incubated with primary antibody diluted to 1:1000 and bound to protein A/G-Agarose beads (Santa Cruz) for 2 h at 4 °C in IP buffer (2% glycerol, 1% Nonidet P-40, 1 mM EDTA, 20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM $ZnSO_4$, and 1× protease inhibitor mixture) (Roche Applied Science). Beads were then washed four times with a cold buffer containing 0.5% glycerol, 1% Nonidet P-40, 1 mM EDTA, 20 mM Tris–HCl, pH 8.0, 100 mM NaCl, 10 mM $MgCl_2$, 0.1 mM $ZnSO_4$, and 1× protease inhibitor mixture, and bound proteins were separated via SDS-10% polyacrylamide gel and transferred to nitrocellulose membranes (Bio-Rad).

Mammalian two-hybrid assay. The interaction between zipzap/p200 and SRF was also tested by using CheckMate mammalian two-hybrid system (Promega). Briefly, a full-length cDNA of human SRF [13] was ligated into the pBind vector and formed a fusion protein with yeast GAL4 protein. The cDNA sequence of the mouse zipzap/p200 gene was released from the yeast plasmid containing zipzap/p200 insert with *Bam*HI and *Not*I, and then ligated in-frame into pACT vector. Sequencing analysis was performed to confirm the open reading frame of the genes in the vectors. The plasmid constructs were transfected into NIH3T3 cells and the cells were harvested 24 h after the transfection. The measurement of the luciferase was carried out according to the manufacturer's manual.

Dual luciferase assays. Transient transfections were carried out with the Lipofectamine and Plus reagents as previously described [4]. At 4 h after the transfection was initiated, the NIH3T3 cells were placed in