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SERUM RESPONSE FACTOR: DISCOVERY, BIOCHEMISTRY, BIOLOGICAL ROLES AND IMPLICATIONS FOR TISSUE INJURY HEALING

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Serum response factor (SRF) is a transcription factor, which binds to a serum response element (SRE) associated with a variety of genes including immediate early genes such as *c-fos*, *fosB*, *junB*, *egr-1* and *-2*, neuronal genes such as *nurr1* and *nur77* and muscle genes such as actins and myosins. By regulating expression of these genes, SRF controls cell growth and differentiation, neuronal transmission as well as muscle development and function. SRF can be activated by a variety of agents, including serum, lysophosphatidic acid (LPA), lipopolysaccharide (LPS), 12-O-tetradecanoylphorbol-13-acetate (TPA), cytokines, tumor necrosis factor- α (TNF α), agents that increase intracellular Ca²⁺, T-cell virus1 activator protein, hepatitis B virus activator proteins pX, activated oncogenes and protooncogenes as well as extracellular stimuli such as antioxidant and UV light. SRF itself is regulated by both cellular signal transduction pathways and interaction with other transcription factors e.g. Sp1, ATF6 and myogenic regulatory factors. Its biological function is best elucidated for myocardium. Specific cardiac SRF transgenesis demonstrated that overexpression of SRF caused hypertrophic cardiomyopathy in mouse and the mouse died of heart failure within 6 months after birth. Other transgenic data suggested that sufficient SRF was needed for embryogenesis and early development. Since SRF is important regulator of numerous genes involved in cell growth and differentiation, including muscle and neural components, SRF may also play a crucial role in tissue injury and ulcer healing, e.g. healing of gastrointestinal ulcers.

Key words: *Serum response factor; serum response element, transcription factor; c-fos gene.*

Discovery of Serum Response Factor (SRF)

In 1984, Greenberg at Harvard University in Boston discovered that serum addition to quiescent cells in culture could rapidly stimulate *c-fos* gene transcription (1). The induction reached maximum within 15 min and the *c-fos* mRNA level peaked at about 30 min after serum stimulation. This phenomenon led to a series of important discoveries in the area of gene transcription regulation. *C-fos* is a cellular homolog of the FBJ murine osteosarcoma virus transforming gene *v-fos*. Since it is rapidly activated without a need of new protein synthesis, *c-fos* was classified as an immediate early gene. Its activation is required for cells, which have exited the cell cycle, to reenter G1 phase of cell cycle and subsequently enter mitosis. Later, it was discovered that in addition to serum, growth factors and other mitogens could activate *c-fos* (2). Subsequent research has focused on the signaling upstream of *c-fos* gene and was aimed to explain why serum could activate *c-fos*. Several DNA elements have been identified in the *c-fos* promoter region (3-6), but a particular attention was given to a short sequence located about 300bp upstream of the transcription initiation start site. Treisman named this sequence Serum Response Element (SRE). It is an A/T rich core flanked by an inverted repeat, CCATATTAGG. This element was necessary and sufficient to render a heterologous promoter responsive to serum. Comparison of this element with the cytoskeletal actin gene promoter revealed a similar sequence (7). To date, about 30 genes have been identified that contain CC(A/T)₆GG sequence in their promoter regions (8, 9) (*Table 1*). These genes include many immediate early genes such as *c-fos*, *fosB*, *junB*, *egr-1*, *egr-2*, etc. neuronal genes such as *nur77*, *nurr1*, etc. and muscle genes such as skeletal α -actin, α -myosin heavy chain (α -MHC), β -myosin heavy chain (β -MHC), myosin light chain (MLC), SM22 α , telokin, troponin, tropomyosin, calponin, atrial natriuretic factor (ANF), Sarcoplasmic reticulum Ca²⁺-ATPase (SERCA), dystrophin, creatine kinase M, etc. Because of its special DNA sequence structure, SRE element is also referred to as CArG box. Treisman also identified the transcription factor, protein that binds to this element to control particular gene expression, and he named this protein Serum Response Factor (SRF).

What is SRF?

In humans, SRF gene is located on chromosome 6p21.1, and is 10607 base pair (bp) long. It contains 7 exons. The full length of its mRNA is 4201bp, exon1 (1-871), exon2 (872-1138), exon3 (1139-1400), exon4 (1401-1520), exon5 (1521-1712), exon6 (1713-1789), exon7 (1790-4201) (*Fig. 1*). The coding region is from 359 to 1885. By alternative splicing, several RNA transcripts could be generated. Conventionally, two mRNA species, 4.5kb and 2.5kb have been distinguished by Northern hybridization. In mouse, it has been reported that four RNA isoforms could be identified, depending on tissue type (10). Among these

Table 1. Genes with SRF binding sites

Gene	Function	Reference
c-fos	Proto-oncogene	Treisman, 1986 (3)
FosB	Proto-oncogene	Lazo et al., 1992 (33)
JunB	Proto-oncogene	Perez-Albuerne et al., 1993 (34)
HSP70	Heat shock protein	Wu et al., 1987 (35)
Egr-1	Early growth response	Qureshi et al., 1991 (36)
Egr-2 (Krox20)	Early growth response	Gius et al., 1990 (37)
Cyr61	Cell proliferation	Latinkic et al., 1991 (38)
Pip92	extracellular signal response	Chung et al., 1998 (39)
β -actin	Non-muscle actin	Ng et al., 1989 (40)
thrombospondin-1	angiogenic inhibitor	Framson and Bornstein, 1993 (41)
vinculin	focal adhesion protein	Moiseyeva et al., 1993 (42)
IL-2R α	T cell growth	Tan et al., 1992 (43)
Nurr1 (NR4A2)	orphan nuclear receptor	Castillo et al., 1997 (44)
Nur77 (NR4A1)	orphan nuclear receptor	Williams and Lau, 1993 (45)
Cardiac α -actin	Muscle contraction	Miwa and Kedes, 1987 (46)
Skeletal α -actin	Muscle contraction	Muscat et al., 1988 (47)
Smooth muscle γ -actin	Smooth muscle differentiation	Carson et al., 2000 (48)
smooth muscle α -actin	Muscle contraction	Kim et al., 1994 (49)
α -MHC	Muscle contraction	Molkentin et al., 1996 (50)
β -MHC	Muscle contraction	Huang et al., 1997 (51)
MLC	Muscle contraction	Henderson et al., 1989 (52)
smMHC	Muscle contraction	Katoh et al., 1994 (53)
SM22 α	Smooth muscle cell differentiation	Yamamura et al., 1997 (54)
telokin	myosin stabilization	Herring and Smith, 1997 (55)
Troponin T	actin stabilization and modulation	Wang et al., 1994 (56)
tropomyosin	actin stabilization and modulation	Toutant et al., 1994 (57)
calponin	Actin-binding protein	Miano et al., 2000 (58)
ANF	regulation of hydromineral homeostasis in atria	Sprenkle et al., 1995 (59)
SERCA	Cellular Ca ⁺⁺ level modulation	Baker et al., 1998 (60)
dystrophin	Duchenne muscular dystrophy	Klamut et al., 1990 (61)
creatine kinase M	Muscle contraction	Vincent et al., 1993 (62)

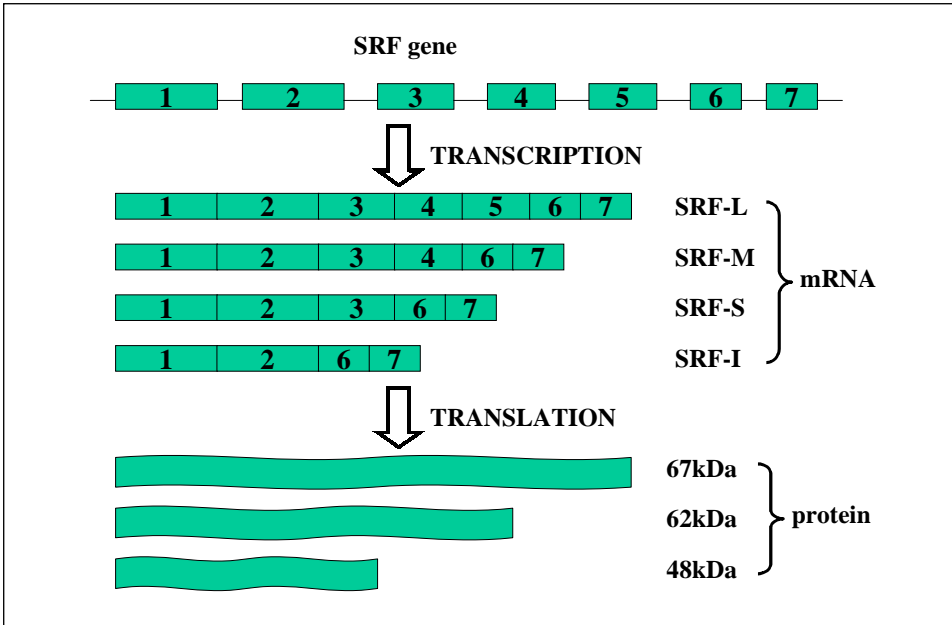


Fig. 1. Diagrammatic presentation of SRF gene structure and alternative splicing. SRF gene contains seven exons. Four mRNA isoforms have been identified by RT-PCR and three protein species have been identified by Western blotting.

four isoforms, SRF-L contained all 7 exons, corresponding to the 4.5kb species in human. SRF-M isoform was lacking exon5 and functioned as a dominant negative mutant, which repressed SRF-dependent transcription (11). SRF-S lacking both exon5 and exon4 was only identified in the aorta. SRF-I isoform was the shortest one, contained only exons1, 2, 6 and 7, and was only detected in embryonic tissues.

SRF protein contains 508 amino acids and is visualized as a 67kDa band on Western blot. It contains three major domains: (1) a SRE DNA binding domain; (2) a transactivation domain; and (3) several phosphorylation sites. Deletion analysis identified the DNA binding and dimerization domain to the region between amino acids 133 and 222 (12). This 90-amino-acid core domain is sufficient for DNA binding, dimerization and interaction with the accessory factors. This domain is highly conserved among eukaryotes. Even in those evolutionarily divergent species such as yeasts and plants an extensive homology has been recognized. This region is designated the MADS box, standing for MCM-1 from yeasts (13), *Agamous* and *Deficiens* from plants (14), and SRF from animals. Even between fruit fly and human, there is a 93% homology in SRF (15). The transcription activation domain is located in the C-terminal region of the SRF protein, within amino acids 339 to 508 region (16).

Biological Functions of SRF

SRF is an important regulator of numerous genes associated with cell growth and differentiation. SRF also regulates transcription resulting from treatment of cells with neurotrophins (17), neurotransmitters and agents that raise intracellular calcium levels (18, 19), stress agents, and viral activators (20, 21). Therefore, identification of the mechanism by which SRF mediates the activation of genes and regulation of the SRF gene itself is important for understanding these processes.

Inhibition of SRF by microinjection of anti-SRF antibodies or the expression of antisense SRF RNA suppressed muscle marker gene expression and blocked the differentiation of myoblasts to myotubes (22, 23). Moreover, SRF gene knockout demonstrated that homozygous SRF^{-/-} mouse embryos failed to develop mesoderm (9). Mouse embryos lacking SRF developed normally until day E6.5. However, the mesodermal germ layer did not form and as a result, SRF-negative embryos died in utero between age E8.5 and E12.5. The fact that *srf*^{-/-} embryos developed normally up to E6.5 and continued to grow even in the absence of mesoderm suggests that SRF is not a condition *sine qua non* for normal cell proliferation. *In vitro* study also supports this notion (24). By specific cardiac SRF transgenesis, we found that overexpression of SRF caused hypertrophic cardiomyopathy in mouse and the mouse died of heart failure within 6 months after birth (25). Overexpression of a mutant SRF that has no DNA binding activity severely damaged either embryogenesis or early development of the mouse, depending on the transgene copy number (26). On the other hand, overexpression of antisense SRF gave the mouse a better cardiac performance (Chai et al., unpublished data). All these transgenic data suggest that sufficient SRF is needed for embryogenesis and early development. For adults, maintaining relatively low level of SRF is more beneficial (*Fig. 2*).

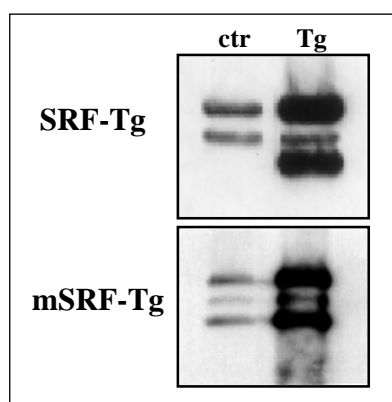


Fig. 2. Western blots showing overexpression of SRF (upper) and SRF mutant (mSRF) (lower) in transgenic mice compared to normal ones (25, 26).

Studies performed on transgenic mice with different CARG boxes with flanking sequences linked to *lacZ* (27) suggest that sequences immediately surrounding the CARG box determine the expression pattern and that CARG boxes with

muscle specificity bind SRF with reduced affinity compared with those with direct ubiquitous expression.

Activation and Regulation of SRF

SRF can be activated by a variety of agents, including serum, lysophosphatidic acid (LPA), anisomycin, mitogens, lipopolysaccharide (LPS), 12-O-tetradecanoylphorbol-13-acetate (TPA), redox, cytokines, tumor necrosis factor- α (TNF α); agents that elevate intracellular calcium levels; viral activator proteins such as the human T-cell lymphotropic virus type-1 activator protein Tax-1 and the hepatitis B virus activator protein pX; activated oncogenes including *v-src*, *v-fps*, *v-ras*, and the activated proto-oncogene *c-raf* as well as extracellular stimuli such as antioxidants, UV light, and microgravity. SRF is regulated both by cellular signal transduction pathways and by its interaction with other transcription factors including Sp1, ATF6, GATA4, Nkx2.5, and myogenic regulatory factors. Several mechanisms have been shown to regulate SRF activity, including (a) association with positive and negative cofactors (28), (b) phosphorylation-dependent changes in DNA binding (29), (c) alternative RNA splicing (10, 11), and regulated nuclear translocation (30).

There are two general classes of signaling mechanisms involving the SRF regulating SRE activity. A ternary complex factors (TCF)-dependent pathway involves the ras-raf-MAPK-ERK cascade (31). Both phosphorylation of TCFs and the binding of TCFs to SRF are required for activation of the SRE by this pathway. A TCF-independent pathway involves the Rho family of GTPases (32). Several MAPK pathways have been found to activate TCF. Among them, the extracellular-signal regulated kinase (ERK) can be activated by the extracellular stimulations mentioned above to phosphorylate TCF via Ras-Raf-MEK1/MEK2. The Jun N-terminal kinases (JNKs), also known as stress-activated protein kinases (SAPKs), can be turned on by cellular stress such as heat or UV light through small GTPases such as Rac and Cdc42 to phosphorylate TCF. Another subfamily of MAPK, p38/Mpk2/RK, could be also involved in the TCF activation. On the other hand, the biochemistry of SRF activation and the signaling pathways still remain unclear. It was recognized that all the factors that stimulate SRF expression have ability to activate the small GTPase RhoA. Therefore, it has been proposed that this might be the path how SRF is triggered. Ca²⁺/calmodulin-dependent kinase (Cam) might be involved in the process of SRF activation by cytoplasmic Ca²⁺ elevation (*Fig.3*).

Potential Implications for Tissue Injury Healing

As described above, genes under SRF regulation are mainly classified into three groups: immediate early genes, muscle genes and neuronal genes. Many of them are involved in cellular response to growth factor stimulation and tissue injury. Since SRF is an important regulator of these genes involved in cell growth

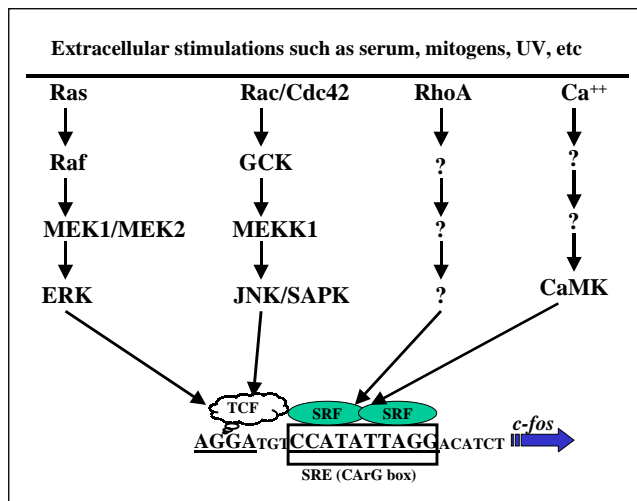


Fig. 3. Diagrammatic presentation of possible pathways involved in *c-fos* gene activation. Question marks indicate unknown pathways.

and differentiation one of its important functions may be involvement in tissue injury and gastrointestinal ulcer healing. Since gastrointestinal injury is usually associated with the destruction of epithelium, smooth muscle structures and neural network and the healing process requires activation of numerous growth factors, it is reasonable to speculate that SRF might play an important role in gastrointestinal recovery from various injuries. Healing of these injuries requires regeneration of epithelial, muscle and neural structures and the neural/brain – gut interactions play important role in gastrointestinal mucosal and pancreatic function, defense repair and healing (63-68).

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