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# Nuclear Actin Is Involved in the Regulation of CSF1 Gene Transcription in a Chromatin Required, BRG1 Independent Manner

**Zhaoxia Song,[1,2] Min Wang,[1] Xiaoguang Wang,[3] Xuefang Pan,[1,3] Wenguang Liu,[1] Shui Hao,[1] and Xianlu Zeng[1]***

[1]Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China
[2]Department of Medical Genetics, Basic Medical School, Jilin University, Changchun 130021, China
[3]Biology Department, Changchun Teachers College, Changchun 130032, China

**Abstract** Actin is an important protein in nucleus and has been implicated in transcription, however, the mechanism of its function in transcription is still not clear. In this article, we studied the role of actin in the regulation of human CSF1 gene transcription. Our results showed that nuclear actin stimulates the activity of CSF1 promoter, and the role in augmenting CSF1 gene transcription requires the formation of chromatin and Z-DNA structure. The ATP binding motifs of nuclear actin are essential for its function in regulating CSF1 gene transcription, and upon actin overexpression, there is an increase in the ATPase activity of nuclear proteins. Further investigation revealed that nuclear actin regulates CSF1 gene transcription in a BRG1 independent manner. Together, these original results have provided evidence for further understanding the mechanism of nuclear actin in regulating gene transcription. J. Cell. Biochem. 102: 403–411, 2007. 

**Key words:** nuclear actin; CSF1; ATPase; BRG1; gene transcription

Actin is one of the most abundant proteins in eukaryotic cells and has been proved to be essential for many cellar functions, such as movement, morphology, growth, cytokinesis as well as other crucial events. Over the years, the presence of actin in nucleus has been well established. In contrast to its functions in cytoplasm, the knowledge concerning the role of actin in nucleus is still limited [Pederson, 1998; Rando et al., 2000; Pederson and Aebi, 2002]. It has been proposed that actin plays an important role in nuclear export [Wada et al., 1998; Hofmann et al., 2001; Shumaker et al., 2003], and actin might be involved in RNA transport and indirectly participate in mRNA processing [Percipalle et al., 2001]. It was reported recently that nuclear actin co-localized with protein 4.1, linking actin to nuclear assembly processes [krauss et al., 2003]. Furthermore, nuclear actin has been found to be associated with chromatin-remodeling and histone acetyltransferase complexes, suggesting a role for actin in chromatin remodeling [Papoulas et al., 1998; Zhao et al., 1998; Galarneau et al., 2000; Ikura et al., 2000; Shen et al., 2000].

A number of studies have suggested a role for actin in transcription. In early studies, it had been shown that injection of antibodies against actin or actin-binding proteins in amphibian oocyte nuclei could block the transcription of lampbrush chromosomes [Scheer et al., 1984]. Actin was also reported to co-purify with RNA

Abbreviations used: CSF1, colony-stimulating factor 1; BRG1, *brahma*-related gene 1; NLS, nuclear localization signal; NES, nuclear export signal; RT–PCR, reverse transcription polymerase chain reaction; ATPase, adenosine triphosphate enzyme; RLA, relative luciferase assay.

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*Correspondence to: Xianlu Zeng, Renmin Street, 5268, Changchun, Jilin, China. E-mail: zengx779@nenu.edu.cn

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polymerase II (RNAP II) and to be required for efficient in vitro transcription by RNAP II, presumably acting at the pre-initiation level [Rungger, 1979; Egly et al., 1984; Percipalle et al., 2001, 2003; Zhu et al., 2004]. Recently, it was reported that actin could be recruited to the promoter region of the MHC2TA and GIP3 genes, and anti-actin antibodies inhibited the transcription both in vivo and in vitro, suggesting that actin participates in most of the gene transcriptions mediated by RNAP II [Hofmann et al., 2004; Kukalev et al., 2005]. Furthermore, nuclear actin was demonstrated to play a direct role in regulating CSF1 gene transcription in an actin polymerization independent manner [Song et al., 2004].

In this study, we further insight into the functions of nuclear actin in regulating CSF1 gene transcription. Our results showed that nuclear actin stimulates the activity of CSF1 promoter in a chromatin dependent and Z-DNA structure involved manner. The wild nuclear actin, but not the mutants deleted in ATP binding sites, could stimulate the ATPase activity of nuclear proteins and enhance the CSF1 gene transcription. There is no synergism between actin and BRG1 in regulating CSF1 gene transcription and increasing ATPase activity of nuclear proteins.

## MATERIALS AND METHODS

### DNA Construction

The human actin expression vectors (pBJ5-actin and pBJ5-△NES-actin), and the CSF1 promoter reporter plasmids with luciferase (pGL3-CSF1-luc, pREP4-CSF1-luc, pREP4-no TG CSF1-luc, pREP4-18GC CSF1-luc, and the pREP7-Rluc) were kindly provided by Dr. Keji Zhao (NIH, Maryland). The actin expression vectors (pcDNA3.1-actin-NLS and pcDNA3.1-△NES-actin-NLS) were constructed as described previously [Song et al., 2004]. The mutations of actin (pcDNA3.1-△N-actin, pcDNA3.1-△C-actin and pcDNA3.1-△ATP + C-actin) were constructed as follows. The deletion of N-terminal of actin (△N actin, deletion of N-terminal residues 1∼18) was conducted by PCR with the following primers: forward primer (5′-GCC ACC ATG GCC GGC TTC GCG GGC GAC G-3′) and reverse primer (5′-CTA GTC CTC GGT TTT GAT TTT TTT GGG TTT GAA GCA TTT GC GGT GG-3′). The deletion of C-terminal of actin (△C-actin, deletion of C-terminal residues 337∼375) was conducted by PCR with the following primers: forward primer (5′-GCC ACC ATG GAT GAT GAT ATC GCC G-3′) and reverse primer (5′-CTA GTC CTC GGT TTT GAT TTT TTT GGG TTT GGA GTA CTT GCG C-3′), and the deletion of ATP binding site and C-terminal of actin (△ATP + C actin, deletion of ATP binding residues, and C-terminal residues 302∼375) was performed by PCR with the following primers: forward primer (5′-GCC ACC ATG GAT GAT ATC GCC G-3′) and reverse primer (5′-CTA GTC CTC GGT TTT GAT TTT TTT GGG TTT GCC AGA CAG CAC TGT GTT G-3′). The PCR product from the template of pcDNA3.1-△NES-actin-NLS was cloned into a pUCm-T vector (Sangon, Shanghai) (a 1107 bp fragment for △N-actin, a 1060 bp fragment for △C−actin and a 939 bp fragment for △ATP + C-actin) and subsequently sequenced, then cloned into pcDNA3.1.

### Cell Culture and Transient Transfection

HeLa cells purchased from the Institute of Cell Biology (Shanghai, China) were cultured at 37°C with 5% $CO_2$ in IMDM medium (GIBCO, Invitrogen) supplemented with 10% FCS and a 1% penicillin/streptomycin mixture. Transient transfection of HeLa cells ($1 \times 10^6$ cells) was conducted by using an electroporator with the Gene Pulser X cell$^{TM}$ electroporation system (Bio-Rad). Cells at the logarithmic growth phase were trypsinized, harvested, and resuspended at a concentration of $5 \times 10^6$ cells/ml in IMDM medium without FCS. An aliquot of 0.2 ml of the cell suspension was mixed with either control vector or expression vector and report vector, then subjected to an electric pulse (15 μF, 160 V) as suggested by the manufacturer.

### Dual-Luciferase Assay

After the transfected cells were cultured for 30 h, the luciferase assay was performed with the Promega Dual-Luciferase Reporter Assay System according to the product instructions. As a control, the *Renilla* luciferase control plasmid was co-transfected in all the experiments. Transfections were normalized to *Renilla* luciferase as indicated in the figure legends. Relative luciferase activity (RLA) was calculated by using the luciferase activity of

cells transfected with the reporter DNA. All the results represent the mean ± standard deviation from at least three independent experiments. Graphic drawing and data analysis were performed using Microsoft Excel.

### RT-PCR and Quantitative Real-Time PCR Analysis

Total RNA was isolated from the transfected HeLa cells. Human CSF1 cDNA was derived from 1 μg of total RNA by reverse transcription (RT) using Promega Reverse Transcription System. PCR was performed with specific human CSF1 primers, 5′-GTC ATA TGT TGA GCC TGT GG-3′ and 5′-GGC TAC GGA GAT GAC AGA AT-3′, yielding a 214 bp product. RT-PCR products were amplified in the linear range. An equal aliquot of cDNA was amplified with human GAPDH primers 5′-AGG GGG GAG CCA AAA GGG-3′ and 5′-GAG GAG TGG GTG TCG CTG TTG-3′, yielding a 435 bp product. The PCR conditions were as follows: 94°C 30 s, 55°C 30 s, 72°C 30 s, repeated 17 × for the hGAPDH primers and 30 × for the CSF1 primers. Aliquots of PCR reactions were separated on an agarose gel and visualized with UV light after ethidium bromide staining. The semi-quantitative estimation of the RT-PCR products was accomplished by photodensitometric analysis of the bands in agarose gel after electrophoresis, and the results were expressed as the relative ratio between intensity values of CSF1 and GAPDH PCR bands.

Total RNA was isolated from the mouse YAC-1 cells. Twenty nanograms of mouse RNA was added to the transfected HeLa cells, and then total RNA was extracted. Synthesized cDNA from 1 μg of total RNA was amplified by semi-quantitative PCR using human CSF-1 or mouse GAPDH-specific primers. Mouse GAPDH primers (5′-TCG GTG TGA ACG GAT TTG GC-3′ and 5′-TGG AAG ATG GTG ATG GGC TTC-3′) were described by Michio et al. [1999]. The quantitative real-time PCR was conducted on an ABI PRISM 7000 Sequence Detection System following the manufacturer's protocol, and SYBR Green (TaKaRa, Japan) was used as a double-stranded DNA-specific fluorescent dye. Data were analyzed by calculating the $2^{-\Delta\Delta Ct}$. Sequence Detector Software was used to extract the PCR data, which were then exported to Excel for further analysis. All the results represent means ± SD of three independent experiments.

### Preparation of Nuclear Extract and BRG1 Immunoprecipitates

Transfected HeLa cells ($1 \times 10^6$ cells) were trypsinized, harvested, washed with 10 ml TBS (Tris buffered saline) and pelleted by centrifugation at 1500*g* for 5 min. The pellet was resuspended in 1 ml TBS, and pelleted again by spinning for 15 s. The cell pellet was respuspended in 400 μl of cold buffer A (10 ml HEPES, pH 7.9; 10 mM KCl; 0.1 mM EDTA; 0.1 mM EGTA; 1 mM DTT; 0.5 mM PMSF) by gentle pipetting. The cells were swelled on ice for 15 min, then 25 μl of a 10% solution of NP-40 was added and the tube was vigorously vortexed for 10 s. The homogenate was centrifuged in a microfuge for 30 s. The nuclear pellet was resuspended in 50 μl cold buffer B (20 ml HEPES, pH 7.9; 0.4 mM NaCl; 1 mM EDTA; 1 mM EGTA; 1 mM DTT; 1 mM PMSF), and the tube was vigorously rocked at 4°C on a shaking platform for 15 min. The nuclear extract was centrifuged in a microfuge at 4°C for 5 min and the supernatant was frozen in aliquots at −70°C.

Five micrograms of anti-BRG1 antibody was added to 500 μl of the nuclear extract of HeLa cells transfected with actin expression vector (wild or mutant) or control vector. Incubated at 4°C for 2 h, 50 μl of 50% protein A—Sepharose 4B (Pharmacia) was added and incubated at 4°C for 30 min to precipitate immunocomplexes. The immunoprecipitates were washed with 1 × immunoprecipitation buffer (1% Triton-100, 150 mM NaCl, 10 mM Tris, pH 7.4, 1 mM EDTA, 1 mM EGTA, pH 8.0, 0.2 mM PMSF (0.5% NP-40) for three times.

### ATPase Activity Assay

The ATPase activity of the nuclear proteins or BRG1 immunoprecipitates was assayed in the reaction mixtures (10 mM PIPES, 5 mM $MgCl_2$, 5 mM$CaCl_2$, 0.5 mM EGTA, 5 mM ATP, pH 6.7). Two hundred microliters of the reaction mixtures was preincubated at 37°C for 30 min, the reaction was started by adding 50 μl of nuclear extract and incubated at 37°C for 10 min. The enzyme activity was stopped by addition of 100 μl of 20% sodium dodecylsulfate, and then centrifuged at 4°C for 3 min. From this supernatant an aliquot was taken for Pi estimation followed by quick addition of 2 ml of reagent C (1% ammonium molybdate, 4% $FeSO_4$, 3.3% $H_2SO_4$ solution) and 1 ml of $H_2O$. Bradford

method was used to determine the Pi formation. The color developed after 2 min at 37°C was read at a wavelength of 700 nm in a Beckman spectrophotomeler DU-70.

## RESULT

### The CSF1 Promoter Can be Activated by Nuclear Actin

We have reported that CSF1 gene transcription can be augmented by β-actin [Song et al., 2004]. For further study, we constructed the actin expression vector pcDNA3.1-ΔNES-actin-NLS (ΔNES-actin-NLS), which both nuclear export signals (NES 1 and 2) were disrupted and NLS was added at the C-terminal. We co-transfected HeLa cells with CSF1 promoter-driven luciferase and the actin expression vectors (actin-NLS and ΔNES-actin-NLS) to compare the RLA of the trancfected cells. The results showed that both of the actin expression vectors could augment the activity of CSF1 promoter and the effect of ΔNES-actin-NLS was little greater (Fig. 1A).

To confirm the function of actin in regulating CSF1 gene transcription, we overexpressed nuclear actin and analyzed the endogenous CSF1 gene transcription by RT-PCR (Fig. 1B, upper). Both actin-NLS and ΔNES-actin-NLS were able to stimulate the CSF1 gene transcription and the band of ΔNES-actin-NLS was brighter. Since actin is similarily involved in RNAP I, RNAP II, and RNAP III mediated gene transcription [Percipalle and Visa, 2006], we performed quantitative real-time PCR using mouse GAPDH gene as an external control. The similar results were obtained (Fig. 1B, (2)). If there is no special indication, the actin expression vector refers to ΔNES-actin-NLS in all of the following experiments.

### Activation of CSF1 Promoter by Nuclear Actin Is Chromatin Structure Dependent

Accumulating data suggested that chromatin modification is necessary for gene transcription. To address if nuclear actin involved regulation of CSF1 gene transcription is related with chromatin structure, we co-transfected actin with pGL3/CSF1 construct (which could not form proper chromatin structure), or with pREP4/CSF1 construct [which contains Epstein–Barrvirus replication origin, and transient transfected DNA could form proper chromatin structure, Liu et al., 2001] to compare the RLA

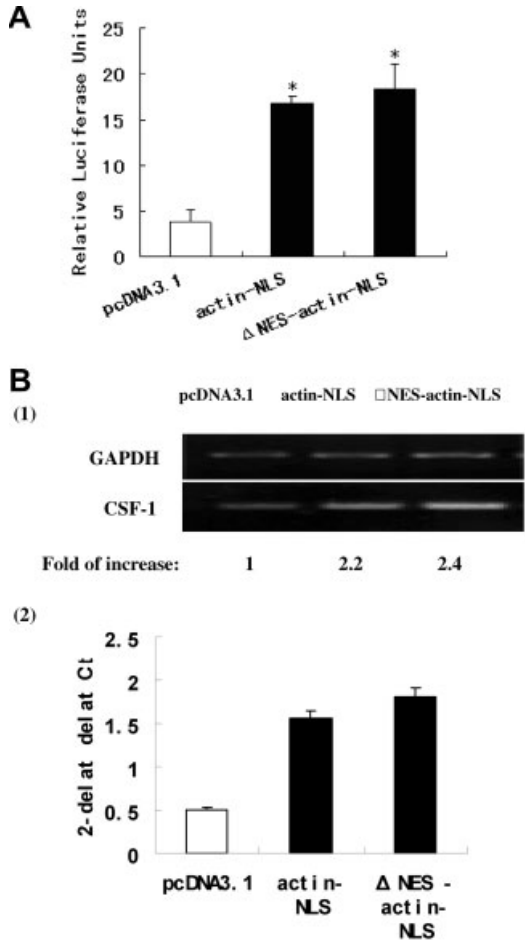


**Fig. 1.** Nuclear actin functions in the regulation of CSF1 gene transcription. **A**: HeLa cells were transiently co-transfected with actin expression vector (pcDNA3.1-actin-NLS, pcDNA3.1-ΔNES-actin-NLS) and luciferase reporter vector. For the negative control, HeLa cells were transiently co-transfected with pcDNA3.1 and luciferase reporter vector. The luciferase activity was analyzed by the dual luciferase system by using the pREP7-RL reporter as an internal control after 30 h. Results shown are the averages of three independent experiments performed in triplicate. The error bars represent the range of three experiments. * Statistically significant ($P < 0.001$) compared with the negative control. **B**: HeLa cells were transfected with actin expression vector or pcDNA3.1. RNA (1 μg) was reversely transcribed with an oligo (dT) primer, followed by PCR with human CSF1 primers, human GAPDH primers or mouse GAPDH primers. Products were analyzed by agarose gel electrophoresis followed by ethidium bromide staining and photodensitometry analyzing. (1), mRNA level of the cells transfected with actin expression vector and pcDNA3.1 empty vector. (2), Quantitative real-time PCR analysis of the endogenous CSF1 gene transcription activated by actin. Total RNA was isolated from transfected HeLa cells added with 20 ng mouse RNA, and 1 μg of total RNA was used for reverse transcription. PCR products were measured continuously with an ABI PRISM 7000 Sequence Detection System. Transcript abundance was measured in triplicate as the ratio of control or experimental sample values normalized to mouse GAPDH expression levels.

of transfected cells. The result showed a threefold increase in RLA of pREP4/CSF1 ($P < 0.001$), while no significant increase in that of pGL3/CSF1 ($P > 0.1$) (Fig. 2), suggesting that the formation of proper chromatin structure is necessary for actin to activate the CSF1 promoter.

### Z-DNA Formation Is Required for Nuclear Actin in Regulating CSF1 Gene Transcription

It has been reported that Z-DNA forming can open the chromatin structure to allow transcriptional factor binding, transcription machinery assembling and transcription initiating. CSF1 promoter contains long stretches of TG repeats from −125 to −76, which have a high potential to form Z-DNA [Herbert and Rich, 1999; Liu et al., 2001]. To determine if the Z-DNA forming sequence is functionally involved in the activation of CSF1 promoter stimulated by nuclear actin, we employed three CSF1 promoters for the co-transfection assay. They were pREP4-CSF1 promoter (wild CSF1 promoter containing Z-DNA forming sequence), pREP4-18 GC CSF1 promoter (TG repeats were replaced by 18 GC repeats, another Z-DNA forming sequences), and pREP4-no TG CSF1 promoter (TG repeats were replaced by a random sequence from BAF47 gene, no Z-DNA forming sequence) [Liu et al., 2001]. As shown in Figure 3, nuclear actin could enhance twofold activity of wild CSF1 promoter, and almost had no effect on no TG CSF1 promoter, indicating that TG repeats are required for the activation of CSF1 promoter by nuclear actin. It is reported that (GC)n sequence provides more stabling Z-DNA structure than (TG)n. Therefore we compared the RLA of cells transfected with 18 GC repeat CSF1 promoter and that of transfected with TG repeat CSF1 promoter, and found the activity of 18 GC CSF1 promoter increased by actin was about 10-fold. From our data we inferred that activation of CSF1 promoter by nuclear actin is correlated with the potential of Z-DNA formation.

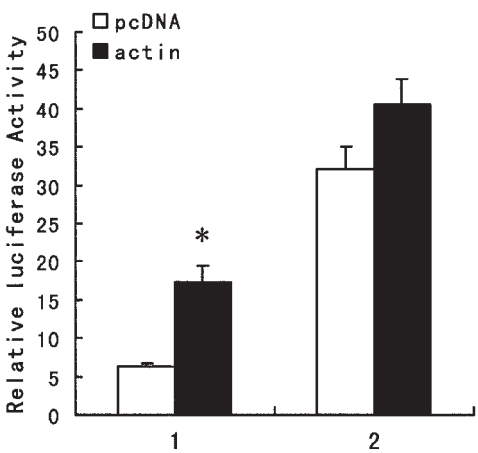


**Fig. 2.** Activation of CSF1 promoter by nuclear actin is chromatin structure dependent. (1), HeLa cells were transiently co-transfected with pcDNA3.1-ΔNES-actin-NLS and pREP4-CSF-luc. For the negative control, HeLa cells were transiently co-transfected with pcDNA3.1 and the pREP4-CSF-luc. (2), HeLa cells were transiently co-transfected with pcDNA3.1-ΔNES-actin-NLS and pGL3-CSF-luc. For the negative control, HeLa cells were transiently co-transfected with pcDNA3.1 and the pGL3-CSF-luc. The dual-luciferase assay was performed as described in Figure 1A.

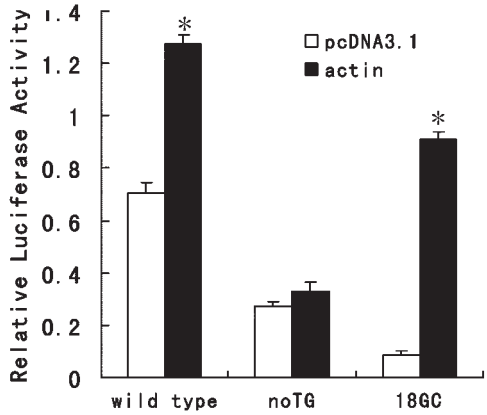


**Fig. 3.** Z-DNA formation is required for nuclear actin in augmenting the activation of CSF1 gene. HeLa cells were transiently co-transfected with pcDNA3.1-ΔNES-actin-NLS and different CSF1 reporter vectors (pREP4-CSF-luc (wild-type), pREP4-noTG-CSF-luc (no TG) and pREP4-18GC-CSF-luc (18 GC)), respectively. For the negative control, HeLa cells were transiently co-transfected with pcDNA3.1 and the corresponding CSF1 reporter vector. The dual-luciferase assay was performed as described in Figure 1A.

### ATP Binding Motif Is Important for Nuclear Actin in Regulating CSF1 Gene Transcription

Actin binds one molecule of ATP (or ADP) in a hydrophobic pocket between subdomains 3 and 4, and the floor of this pocket comprises one turn of a $3_{10}$ helix 302∼308 [Kabsch and Vandekerckhove, 1992; Morita, 2003]. In order to address the mechanism of nuclear actin in the regulation of CSF1 gene transcription, we constructed three actin mutants (△N actin, △C-actin, and △ATP + C actin, Figure 4A). Then we co-transfected HeLa cells with wild actin or the mutants and compared the RLA of transfected cells. As shown in Figure 4B, wild actin

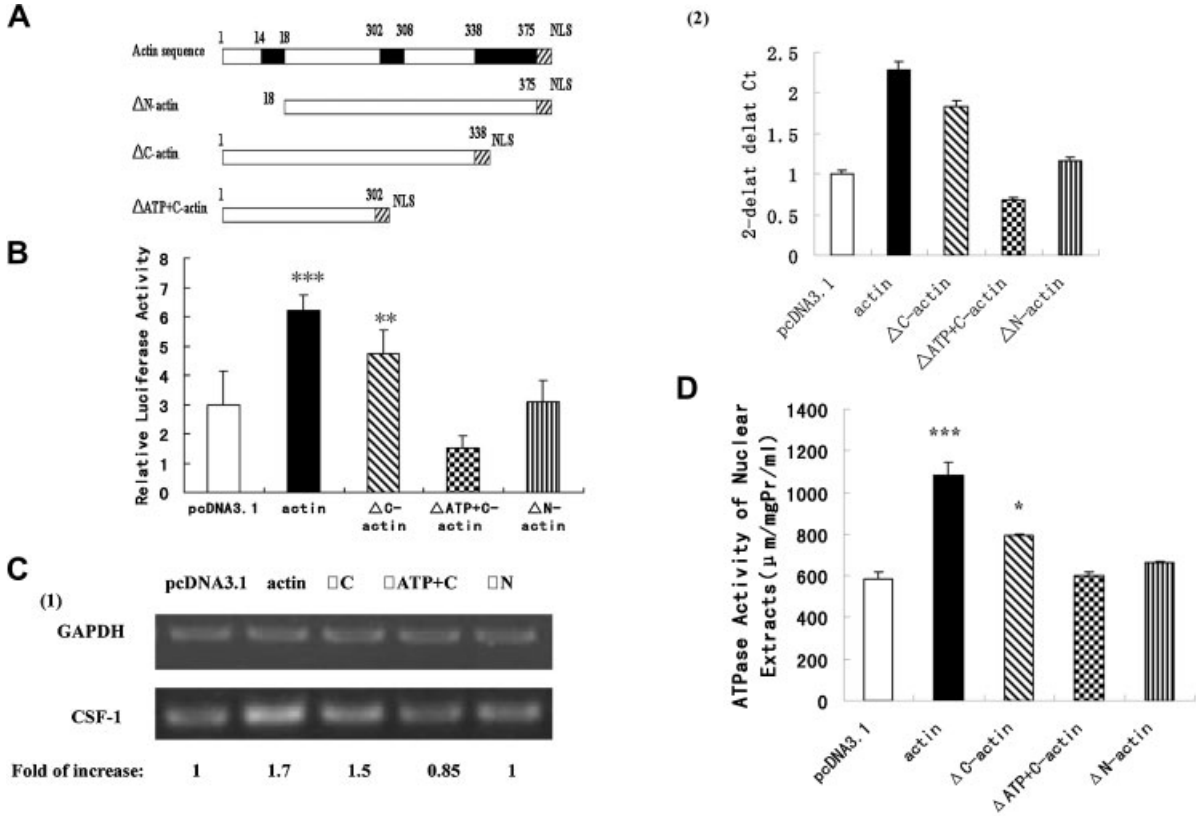


**Fig. 4.** ATP binding motif is important for nuclear actin in regulating CSF1 gene transcription. **A**: The structure of actin and its mutants. **B**: HeLa cells were transiently co-transfected with different actin expression vectors and luciferase reporter vector. For the negative control, HeLa cells were transiently co-transfected with pcDNA3.1 and the luciferase reporter vector. The luciferase activity was analyzed by the dual luciferase system by using the pREP7-RL reporter as an internal control after 30 h. **C**: HeLa cells were transfected with pcDNA3.1 and different actin expression vectors, RT-PCR and quantitative real-time PCR analysis were performed as described in Figure 1B. **D**: The nuclear extract was prepared from the transfected HeLa cells and then added to the ATPase reaction mixtures to incubate at 37°C for 10 min. The reaction was stopped by adding 20% sodium dodecylsulfate and the supernatant was collected for the ATPase activity analysis by Bradford method. Results shown are the averages of three independent experiments performed in triplicate. The error bars represent the range of three experiments. *** Statistically significant ($P < 0.001$), ** statistically significant ($P < 0.01$), * statistically significant ($P < 0.05$) compared with the negative control.

caused about twofold RLA increase, and △C-actin induced a 1.5-fold increase in the promoter activation, while △N actin and △ATP + C actin lost the capability of activating CSF1 promoter. To test whether the mutants will affect CSF1 mRNA level, we transfected HeLa cells with wt (wild-type) or mutant actin and performed RT-PCR and quantitative real-time PCR. We found the results of RT-PCR and quantitative real-time PCR responded to that of double luciferase assay (Fig. 4C, (1) and (2)). Our results suggested that ATP binding motif is essential for nuclear actin to regulate the CSF1 gene transcription.

Then we tested the ATPase activity of nuclear proteins from HeLa cells transfected with wt or mutant actin. As shown in Figure 4D, the ATPase activity of nuclear proteins could be increased by transfecting wt ($P < 0.001$) or △C-actin ($P < 0.05$), while it could not be effected by transfecting △N or △ATP + C actin. The above results implicated that the augmentation of CSF1 gene transcrption by nuclear actin may relate to its function on the ATPase activity of nuclear proteins.

### The Function of Nuclear Actin and BRG1 on Activating CSF1 Gene Transcription Is not Co-Related

Since it is reported that actin is tightly bound to BRG1 (a core protein with ATPase activity in BAF complex) and enhances BRG1 ATPase activity, we next assayed the ATPase activity of BRG1 immunoprecipitates obtained by using

anti-BRG1 antibody. Surprisingly, there was no any difference in the ATPase activity of BRG1 immunoprecipitates in the HeLa cells transfected with wt or mutant actin (Fig. 5A). Then we co-transfected SW-13 cells, which contains no detectable BRG1 proteins, with nuclear actin, BRG1 or both of them, respectively, to measure ATPase activity of the nuclear proteins. The results showed that there was no difference among the transfectants (Fig. 5B), suggesting the ATPase activity of nuclear proteins could be activated by both nuclear actin and BRG1, and the function was independent. Next we co-transfected SW-13 cells with nuclear actin, BRG1 or both of them, respectively, and compared the RLA of the transfected SW-13 cells. As shown in Figure 5 C, nuclear actin, without BRG1, could activate the CSF1 promoter and there was no difference in the presence or absence of BRG1.

## DISCUSSION

With the presence of actin in nucleus is well established, its function becomes a major issue that attracts many researchers. Recently a number of studies have suggested that nuclear actin is connected with transcriptional events [Percipalle and Visa, 2006]. In this study, we showed that nuclear actin is involved in the regulation of CSF1 gene transcription in a chromatin required, BRG1 independent manner.

It has been proposed that chromatin structure presents a barrier for gene transcription, and chromatin remodeling is a pre-requisite for the pre-initiation complex (PIC) formation, moreover, actin is believed to have a direct involvement in PIC formation [Hofmann et al., 2004]. Actin is a component of the human chromatin remodeling complex (BAF), which interacts with chromatin during gene activation processes [Zhao et al., 1998; Rando et al., 2002]. Furthermore, actin superfamily proteins provide histone chaperone activity to regulate gene expression through the organization of chromatin structure [Harata et al., 2002; Blessing et al., 2004]. In our present article, we demonstrated that the CSF1 gene transcription could be augmented by nuclear actin, and the regulation requires chromatin structure formation.

Our data also showed an interaction between actin and Z-DNA structure. Actin could only promote the activity of promoter in which

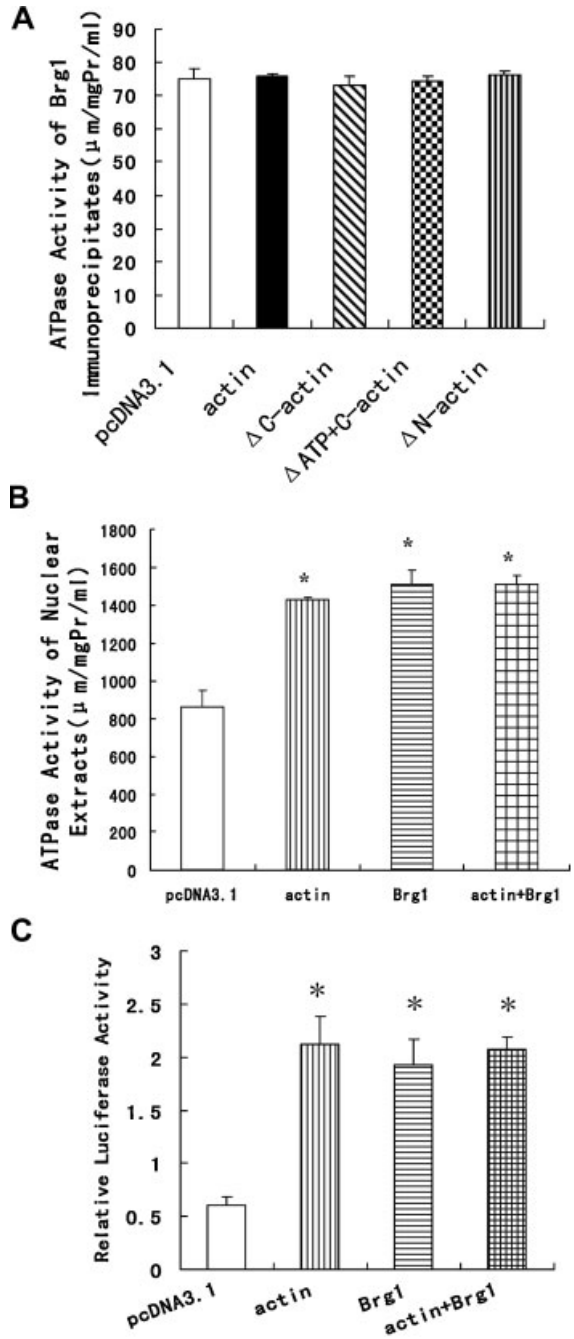


**Fig. 5.** The function of nuclear actin on the regulation of CSF1 gene transcription is independent of BRG1. **A**: Nuclear extracts were prepared from the transfected HeLa cells and then incubated with anti-BRG1 antibody. After incubation, protein A Sepharose 4B was added to precipitate the immunocomplexes. The ATPase activity was analyzed by Bradford method. **B**: The nuclear extract was prepared from the transfected SW-13 cells and then incubated with ATPase reaction mixtures. The supernatant was collected for the ATPase activity analysis. **C**: SW-13 cells were transiently co-transfected with CSF1 reporter vector and actin expression vector or BRG1 expression vector (or both of them), respectively. For the negative control, SW-13 cells were transfected with the CSF1 reporter vector and an empty vector. The dual-luciferase assay was performed as described in Figure 1A.

Z-DNA structure formed. Z-DNA structure is a left-handed double helix with 12 residues within each turn and has higher free energy than B-DNA. Even though the eukaryotic genome has tens of thousands of potential Z-DNA forming units, no function has been identified yet. A number of experiments in prokaryotes have been used to demonstrate that Z-DNA forms in vivo as a result of transcription and Z-DNA forming sequences are concentrated in the regulatory regions of genes [Schroth et al., 1992]. Z-DNA forming further opens the chromatin structure that allows transcriptional factor binding and transcription machinery assembling, as well as transcription initiation [Liu et al., 2001]. In our experiment, we showed that nuclear actin requires Z-DNA forming sequences to enhance the CSF1 gene transcription.

To explore the association between the ATPase activity and the role of actin in regulating CSF1 gene transcription, we deleted ATP binding residues, and found that the actin mutants lost the capability of stimulating CSF1 gene transcription. While deleting C-terminal residues but not the ATP binding sites, actin could still remain its function. Next we tested the ATPase activity of nuclear proteins in the cells transfected with wt or mutant actin, and the data indicated that the ATPase activity of nuclear proteins would increase by wt and $\triangle$C actin, but wouldn't by $\triangle$N or $\triangle$ATP + C actin. Our results demonstrated the ATPase activity of nuclear proteins are important for actin in regulating CSF1 gene transcription. To test if actin may function as a co-factor to regulate CSF1 gene transcription by enhancing the ATPase activity of BRG1, we transfected HeLa cells with wt or mutants, then assayed the ATPase activity of BRG1 immunoprecipitates. To our surprise, all of the ATPase activity of BRG1 immunoprecipitates were at the similar level, suggesting that the ATPase activity of BRG1 is not affected by actin. To confirm our results, we co-transfected SW-13 cells with actin expression vector and CSF1 reporter vector to compare the RLA of co-transfected cells. The data showed that the CSF1 promoter was significantly activated by actin, even though SW-13 cells have only a partial BAF complex lacking the essential BRG1 subunit. This experiment further suggested that the activity of CSF1 promoter augmented by nuclear actin is independent of BRG1.

Hofmann had demonstrated that antibody to β-actin inhibited the transcription in an in vitro transcription assay on naked DNA that does not require chromatin remodeling. They believed that actin functions as a bridge between the polymerase and the other constituents of the PIC, and actin is required at a step involving the initiation of transcription that is independent of chromatin remodeling by BRG1 [Hofmann et al., 2004]. Since actin is tightly associated with the BRG1 even after treatment by urea and actin is required for the maximal ATPase activity of BRG1, we asked if there is synergism between actin and BRG1 on CSF1 gene transcription. Unfortunately our data excluded the possibility. The capacity of actin in regulating CSF1 gene transcription was not enhanced in the presence of BRG1. We presumed that actin exists in many chromatin remodeling complex participating in gene transcription and there may be redundant mechanisms meaning that Brg1 is necessary but not sufficient and its role can be taken up by another complex. In conclusion, our results have provided evidence for further understanding the mechanism of nuclear actin in regulating CSF1 gene transcription.

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