

# Endothelin-1 activates *Homer 1a* expression via mitogen-activated protein kinase in cardiac myocytes

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Received January 13, 2006; Accepted February 28, 2006

**Abstract.** Homer proteins are a family of scaffolding proteins which may play an important role in calcium signaling by facilitating the assembly of signaling complexes in neuronal cells. Among the three splice variants of *Homer 1*, *Homer 1a* is rapidly up-regulated by neural stimulation and may regulate the disassembly of signaling complexes mediated by Homer proteins. In spite of its potential importance in calcium signaling, the regulation of *Homer 1a* expression in cardiac myocytes has never been investigated. In this study, we examined the regulation of *Homer 1a* expression in cardiac myocytes. *Homer 1a* was significantly up-regulated by several hypertrophic agonists, including endothelin-1 (ET-1), phenylephrine, isoproterenol and angiotensin-II, and ET-1 most strikingly induced *Homer 1a* expression. The induction of *Homer 1a* expression by ET-1 peaked at 2 h and inhibitors for mitogen-activated/extracellular signal regulated kinase (MEK) significantly suppressed the induction of *Homer 1a*. This study first clarified the regulation of *Homer 1a* expression in cardiac myocytes and demonstrated that ET-1 induced *Homer 1a* expression through the mitogen-activated protein kinase pathway.

## Introduction

Homer proteins are a family of scaffolding proteins, characterized by N-terminal Enabled/Vasp homology (EVH-1) and coiled-coil domains (1). The EVH-1 domain binds a proline-rich motif present in key components of synaptic signal transduction pathways including the metabolic glutamate receptors, mGluR1a and mGlu5a/b, inositol-1,4,5-triphosphate (IP3) receptors and the Shank family of scaffolding proteins

(2,3). Homer proteins dimerize with other Homer proteins through the coiled-coil domain and mediate the interaction between cellular proteins that directly or indirectly bind to Homer (4). For example, N-methyl-D-aspartate (NMDA) receptors are associated with Shank via other scaffolding proteins and the ability of Homer proteins to form multimers allows for a physical and functional link between mGluRs, IP3 receptors and NMDA receptors in neuronal cells (1). Thus, Homer proteins are implicated as adaptor proteins that facilitate a physical association between cell surface receptors and intracellular store calcium channels.

*Homer 1* comprises three splice variants: *Homer 1a*, *1b* and *1c* (4,5). While *Homer 1b* and *1c* are constitutively expressed, *Homer 1a* is an immediate early gene, induced by synaptic activities including administration of glutamate or brain-derived neurotrophic factor, tetanic stimuli and electroconvulsive seizure (2,5,6). *Homer 1a*, encoded by a prematurely terminated transcript and lacking the coil-coiled domain (7), may act in a dominant negative fashion by interfering with multimerization and disassembling signaling complexes (1). Indeed, *Homer 1a* overexpression in neuronal cells attenuated mGluR-evoked intracellular calcium release, suggesting that *Homer 1a* may regulate coupling between extracellular signals and calcium release from the ER (3). On the other hand, *Homer 1a* also exerts constitutive activity by disassembling signaling complexes depending on the situation. It was reported that TRPC1, a non-specific cationic channel, could bind to Homer proteins and that the disassembly of a TRPC1-Homer-IP3R complex resulted in channels that were constitutively active (8). Expression of *Homer 1a* was also shown to induce constitutive activity in mGluR1a and mGluR5 (9).

*Homer 1* is expressed in the heart as well as in the brain (4,10). Considering its potential function in neuronal cells, *Homer 1a* may be also involved in calcium signaling by mediating the interaction between cell surface receptors and ER calcium channels in cardiac myocytes, which plays an important role in cardiac hypertrophy (11,12). However, regulation of *Homer 1a* expression in the heart is totally unknown. In this study, we investigated the induction and regulation of *Homer 1a* expression by extracellular signals in the heart using cultured rat neonatal cardiac myocytes. We found that the three splice variants of *Homer 1* were all expressed in cardiac myocytes and *Homer 1a* expression was induced by several hypertrophic agonists. Endothelin-1 (ET-1)

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**Key words:** Homer 1, endothelin-1, cardiac myocyte, transcriptional activation, MAP kinase

most strikingly up-regulated *Homer 1a* expression and the induction by ET-1 was mediated by the mitogen-activated protein kinase pathway.

## Materials and methods

**Materials.** Endothelin-1, angiotensin II, phenylephrine and isoproterenol were purchased from Sigma (St. Louis, MO), and all inhibitors were from WAKO (Tokyo, Japan).

**Cell culture.** Primary ventricular cardiac myocytes were prepared from 2- to 3-day-old Sprague-Dawley rats as described previously (13). Cells were plated at a density of  $2 \times 10^4$  cells/cm<sup>2</sup> on gelatin-coated dishes and incubated in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal calf serum for 24 h. All experiments were performed in serum-free conditions after 24 h of incubation in serum-free DMEM.

**Northern blot analysis.** Total RNA was purified from cultured cardiac myocytes using TRIzol reagent (Life Technologies, Gaithersburg, MD). Total RNA (20  $\mu$ g) was electrophoresed in 1.0% denaturing (formaldehyde) agarose gels and blotted onto nylon membrane. After hybridization with a [<sup>32</sup>P]-labeled probe, the membrane was washed and exposed to a BAS 5000 Imaging Plate (Fuji, Tokyo, Japan). Densitometry was performed using NIH image. A full-length cDNA for *Homer 1a* was used as a probe.

**Cell stimulation and inhibitor assay.** After incubation in serum-free DMEM for 24 h, cardiac myocytes were washed and maintained in DMEM for 20 min. The cells were stimulated by the addition of endothelin-1, angiotensin II, phenylephrine or isoproterenol. After treatment for indicated times, RNA was extracted for Northern blot analysis. Inhibitors were added 30 min before stimulation by endothelin-1.

**Statistical analysis.** Data were analyzed by one-factor ANOVA followed by Fisher's PLSD as a post-hoc test.

## Results

***Homer 1* expression in cultured neonatal cardiac myocytes.** We first examined the expression of *Homer 1* transcripts in cardiac myocytes. Northern blotting using full-length cDNA for *Homer 1a* as a probe revealed that all three splice variants of *Homer 1* were expressed in cardiac myocytes (Fig. 1A). However, the expression level of *Homer 1a* was very low at basal conditions (Fig. 1A). We next examined whether hypertrophic agonists could induce *Homer 1a* expression. Interestingly, *Homer 1a* expression was significantly up-regulated by endothelin-1 (ET-1), phenylephrine, isoproterenol and angiotensin-II (Ang II) (Fig. 1). Since ET-1 most strikingly activated *Homer 1a* expression, we examined the regulation of *Homer 1a* expression by ET-1 in cardiac myocytes in subsequent experiments.

**ET-1 regulates *Homer 1a* expression through MEK/ERK activation.** We investigated the time course of *Homer 1a* induction by ET-1 in cardiac myocytes. *Homer 1a* mRNA was

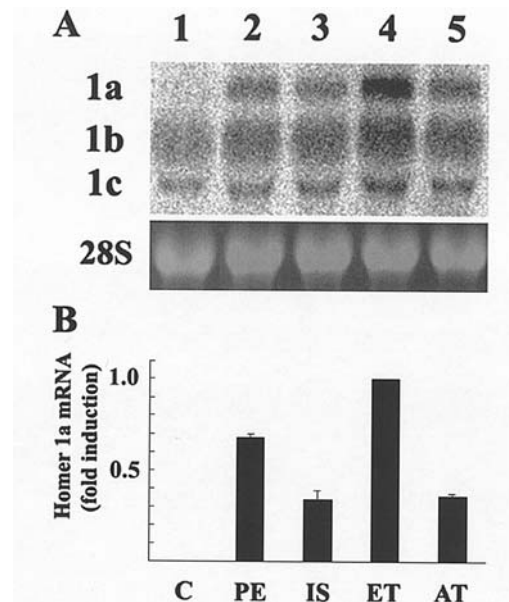


Figure 1. Expression of *Homer 1* in cardiac myocytes. Cardiac myocytes expressed all three splice variants of *Homer 1*. The immediate early gene product, *Homer 1a*, was induced by all hypertrophic agonists examined. ET-1 most strikingly up-regulated *Homer 1a* expression. (A) A representative blot showing induction of *Homer 1a* by several agonists. Cells were incubated with 50  $\mu$ M phenylephrine (lane 2, PE), 10  $\mu$ M isoproterenol (lane 3, IS), 0.1  $\mu$ M ET-1 (lane 4, ET) or 0.1  $\mu$ M Ang II (lane 5, AT) for 2 h. Lane 1, control (without any agonists). Ethidium bromide staining of 28S rRNA as a reference of RNA loading. (B) *Homer 1a* mRNA induction by several agonists. Data are shown as fold induction compared with cells incubated with ET-1. The mean  $\pm$  SD of three independent experiments is shown.

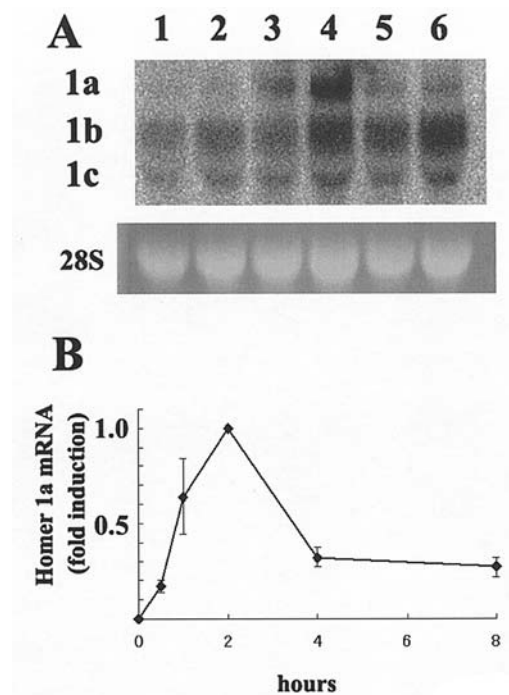


Figure 2. The time course of *Homer 1a* mRNA induction by ET-1. (A) A representative blot showing the time course of *Homer 1a* mRNA induction by ET-1. Cells were incubated with 0.1  $\mu$ M ET-1 for the indicated times. Lane 1, 0 h; lane 2, 0.5 h; lane 3, 1 h; lane 4, 2 h; lane 5, 4 h; lane 6, 8 h. Ethidium bromide staining of 28S rRNA as a reference of RNA loading. (B) Temporal induction patterns of *Homer 1a* mRNA by ET-1. Data are shown as fold induction compared with cells incubated with ET-1 for 2 h and represent the mean  $\pm$  SD of three independent experiments.

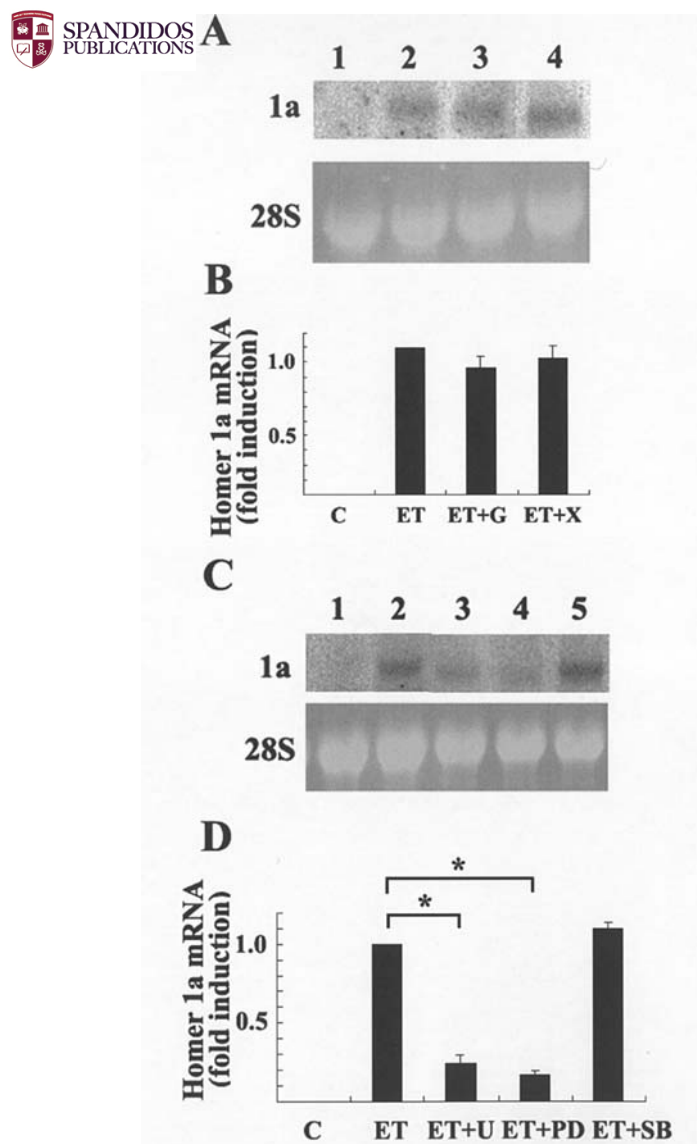


Figure 3. *Homer 1a* mRNA induction by ET-1 was blocked by inhibition of MEK. (A and B) Inhibition of PKC or IP3 did not affect *Homer 1a* induction by ET-1. Cells were preincubated with 0.1  $\mu$ M GFX (lane 3, ET+G) or 2  $\mu$ M xestospongine C (lane 4, ET+X) for 30 min and further incubated with 0.1  $\mu$ M ET-1 for 2 h. (A) A representative blot. Lane 1, control (C); lane 2, ET-1 alone (ET); lane 3, pretreatment with GFX (ET+G); lane 4, pretreatment with xestospongine C (ET+X). Ethidium bromide staining of 28S rRNA as a reference of RNA loading. (B) Data are shown as fold induction compared with cells incubated with ET-1 alone. The mean  $\pm$  SD of three independent experiments is shown. (C and D) *Homer 1a* induction by ET-1 was blocked by inhibition of MEK. Cells were preincubated with 5  $\mu$ M U0126, 30  $\mu$ M PD98059 or 5  $\mu$ M SB203580 for 30 min and further incubated with 0.1  $\mu$ M ET-1 for 2 h. Lane 1, control (C); lane 2, ET-1 alone (ET); lane 3, pretreatment with U0126 (ET+U); lane 4, pretreatment with PD98059 (ET+PD); lane 5, pretreatment with SB203580 (ET+SB). Ethidium bromide staining of 28S rRNA as a reference of RNA loading. (C) A representative blot. (D) Data are shown as fold induction compared with cells incubated with ET-1 alone. The mean  $\pm$  SD of three independent experiments is shown. \*Significantly different from cells treated with ET-1 alone (ET) by Fisher's PSD test.

induced as early as 1 h after stimulation with ET-1 (Fig. 2). The induction peaked by 2 h and persisted up to 8 h (Fig. 2).

In the heart, the ET<sub>A</sub> receptor is the principal receptor for ET-1 and is coupled to the Gq group of heterotrimeric G proteins (14,15). Binding of ET-1 to the ET<sub>A</sub> receptor results in the activation of protein kinase C (PKC) by diacylglycerol

and release of Ca<sup>2+</sup> from the ER by IP3 (14). We then examined the roles of PKC and IP3 in *Homer 1a* induction. However, inhibition of PKC (0.1  $\mu$ M GFX) or the IP3 receptor (2  $\mu$ M xestospongine C) had no effect on *Homer 1a* induction, suggesting that the activation of PKC or Ca<sup>2+</sup> release from the IP3 receptor was not involved in the induction of *Homer 1a* by ET-1 (Fig. 3A and B).

In addition to the activation of the Gq class of G proteins, ET-1 rapidly and transiently stimulates the extracellular signal-regulated kinase (ERK) cascade in cardiac myocytes (14). For this cascade, mitogen-activated protein kinase kinase (MAPKKK) is Raf and MAPKK is mitogen-activated/extracellular signal regulated kinase (MEK) (14). We thus investigated the effects of MEK inhibition on *Homer 1a* induction by ET-1. Although inhibition of p38 (5  $\mu$ M SB203580) showed no effects, two kinds of MEK inhibitors (5  $\mu$ M U0126 or 30  $\mu$ M PD98059) significantly suppressed the induction of *Homer 1a*, demonstrating that ET-1 induced *Homer 1a* expression through activation of the MEK-ERK pathway (Fig. 3C and D).

## Discussion

Calcium signaling in response to extracellular signals plays an important role in cardiac hypertrophy (11,12,16). Because of their potential importance in calcium signaling in neuronal cells, we hypothesized that Homer proteins may be also involved in signal transduction mediating cardiac hypertrophy. However, the expression pattern of *Homer* in cardiac myocytes has never been examined. In this study, we focused on the expression pattern of the immediate early gene (IEG) product, *Homer 1a*, because we anticipated that induction of *Homer 1a* may be involved in feedback regulation of hypertrophic signals. Interestingly, all hypertrophic agonists examined induced expression of *Homer 1a*, suggesting that *Homer 1a* induction may be a common phenomenon associated with hypertrophic signal activation.

The mitogen-activated protein kinase (MAPK) pathway regulates cellular proliferation and hypertrophy (14,17). Interestingly, ET-1 activated *Homer 1a* expression via the MEK-ERK cascade, which also mediates hypertrophic signals (11,18,19), suggesting that *Homer 1a* may be involved in the modulation of hypertrophic signals. Westhoff *et al* reported that *Homer 1c* decreased the ryanodine type 2 receptor (RyR2)-mediated Ca<sup>2+</sup> release from microsomes prepared from the sarcoplasmic reticulum (SR) of rat cardiac myocytes but that *Homer 1a* reversed the effect of *Homer 1c*, indicating that aggregation of a part of RyR2 population into signaling complexes by Homer proteins may regulate the release of Ca<sup>2+</sup> from intracellular stores in cardiac myocytes (20). Therefore, *Homer 1a*, induced via the MEK-ERK cascade, may regulate disassembly of signaling complexes including SR channels and modulate Ca<sup>2+</sup> homeostasis, thus mediating crosstalk between the MAP kinase and Ca<sup>2+</sup> signaling pathways during cardiac hypertrophy.

In summary, *Homer 1a* was induced by several hypertrophic signals and ET-1 most strikingly up-regulated *Homer 1a* expression in cardiac myocytes. *Homer 1a* induction by ET-1 was mediated through the MEK-ERK cascade. *Homer 1a* induction may modulate hypertrophic signals in



cardiac myocytes and Homer proteins may represent a potential therapeutic target in cardiac hypertrophy.

### Acknowledgements

We gratefully acknowledge Keiko Kobayashi for technical assistance. This work was supported by research grants from the Ministry of Education, Science, Sports, and Culture of Japan (15590738, 12CE2006 and 13307034), research grants from the Ministry of Health, Labor, and Welfare of Japan (Comprehensive Research on Aging and Health Grant H14-choju-012), and grants provided by the Ichiro Kanehara Foundation, the Takeda Science Foundation and the Suzuken Memorial Foundation.

### References

1. Thomas U: Modulation of synaptic signalling complexes by Homer proteins. *J Neurochem* 81: 407-413, 2002.
2. Brakeman PR, Lanahan AA, O'Brien R, Roche K, Barnes CA, Huganir RL and Worley PF: Homer: a protein that selectively binds metabotropic glutamate receptors. *Nature* 386: 284-288, 1997.
3. Tu JC, Xiao B, Yuan JP, Lanahan AA, Leoffert K, Li M, Linden DJ and Worley PF: Homer binds a novel proline-rich motif and links group 1 metabotropic glutamate receptors with IP3 receptors. *Neuron* 21: 717-726, 1998.
4. Xiao B, Tu JC, Petralia RS, Yuan JP, Doan A, Breder CD, Ruggiero A, Lanahan AA, Wenthold RJ and Worley PF: Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* 21: 707-716, 1998.
5. Sato M, Suzuki K and Nakanishi S: NMDA receptor stimulation and brain-derived neurotrophic factor upregulate homer 1a mRNA via the mitogen-activated protein kinase cascade in cultured cerebellar granule cells. *J Neurosci* 21: 3797-3805, 2001.
6. Kato A, Ozawa F, Saitoh Y, Hirai K and Inokuchi K: Vesl, a gene encoding VASP/Ena family related protein, is upregulated during seizure, long-term potentiation and synaptogenesis. *FEBS Lett* 412: 183-189, 1997.
7. Bottai D, Guzowski JF, Schwarz MK, Kang SH, Xiao B, Lanahan A, Worley PF and Seeburg PH: Synaptic activity-induced conversion of intronic to exonic sequence in Homer 1 immediate early gene expression. *J Neurosci* 22: 167-175, 2002.
8. Yuan JP, Kiselyov K, Shin DM, Chen J, Shcheynikov N, Kang SH, Dehoff MH, Schwarz MK, Seeburg PH, Muallem S, *et al*: Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors. *Cell* 114: 777-789, 2003.
9. Ango F, Prezeau L, Muller T, Tu JC, Xiao B, Worley PF, Pin JP, Bockaert J and Fagni L: Agonist-independent activation of metabotropic glutamate receptors by the intracellular protein Homer. *Nature* 411: 962-965, 2001.
10. Sandona D, Tibaldo E and Volpe P: Evidence for the presence of two homer 1 transcripts in skeletal and cardiac muscles. *Biochem Biophys Res Commun* 279: 348-353, 2000.
11. McKinsey TA and Olson EN: Cardiac hypertrophy: sorting out the circuitry. *Curr Opin Genet Dev* 9: 267-274, 1999.
12. Frey N, McKinsey TA and Olson EN: Decoding calcium signals involved in cardiac growth and function. *Nat Med* 6: 1221-1227, 2000.
13. Sadoshima J, Jahn L, Takahashi T, Kulik TJ and Izumo S: Molecular characterization of the stretch-induced adaptation of cultured cardiac cells. An *in vitro* model of load-induced cardiac hypertrophy. *J Biol Chem* 267: 10551-10560, 1992.
14. Sugden P and Bogoyevitch M: Endothelin-1-dependent signaling pathways in the myocardium. *Trends Cardiovasc Med* 6: 87-94, 1996.
15. Belloni AS, Guidolin D, Ceretta S, Bova S and Nussdorfer GG: Acute effect of ischemia on adrenomedullin immunoreactivity in the rat heart: an immunocytochemical study. *Int J Mol Med* 14: 71-73, 2004.
16. Molkentin JD, Lu JR, Antos CL, Markham B, Richardson J, Robbins J, Grant SR and Olson EN: A calcineurin-dependent transcriptional pathway for cardiac hypertrophy. *Cell* 93: 215-228, 1998.
17. Seger R and Krebs EG: The MAPK signaling cascade. *FASEB J* 9: 726-735, 1995.
18. Clerk A and Sugden PH: Activation of protein kinase cascades in the heart by hypertrophic G protein-coupled receptor agonists. *Am J Cardiol* 83: 64H-69H, 1999.
19. Bogoyevitch MA: Signalling via stress-activated mitogen-activated protein kinases in the cardiovascular system. *Cardiovasc Res* 45: 826-842, 2000.
20. Westhoff JH, Hwang SY, Duncan RS, Ozawa F, Volpe P, Inokuchi K and Koulen P: Vesl/Homer proteins regulate ryanodine receptor type 2 function and intracellular calcium signaling. *Cell Calcium* 34: 261-269, 2003.