Nuclear signalling by Rac GTPase: essential role of phospholipase A_2

Byung-Chul KIM and Jae-Hong KIM[1]

Laboratory of Molecular and Cellular Genetics, Institute of Environment and Life Science, Hallym University, Chun-Cheon, Kangwon-do, South Korea 200-702

Rac, one member of Rho family GTPases, stimulates c-*fos* serum response element (SRE)–luciferase reporter gene in Rat-2 fibroblast cells. By transient transfection analysis, we demonstrated that the activation of phospholipase A_2 (PLA_2) and the subsequent production of arachidonic acid (AA) are essential for Rac-induced c-*fos* SRE activation, implying a critical role for PLA_2 in the Rac-signalling pathway to the nucleus. Either pretreatment with mepacrine, a specific inhibitor of PLA_2, or co-transfection with the expression plasmid of lipocortin-1, a proposed inhibitory protein of PLA_2, selectively abolished RacV12-induced SRE activation. Further, we demonstrated that subsequent metabolism of AA, a major product of Rac-activated PLA_2, by lipoxygenase (LO) is essential for Rac-induced c-*fos* SRE activation. In agreement with the role of the PLA_2–AA–LO cascade as a potential mediator of Rac signalling to the nucleus, the addition of exogenous AA stimulated c-*fos* SRE-luciferase activity in an LO-dependent manner. Together, our results demonstrate that 'Rac-activated PLA_2 and subsequent AA metabolism by LO' constitute a novel and specific pathway in Rac GTPase-induced c-*fos* SRE activation.

INTRODUCTION

Rac GTPase has been implicated in the regulation of a wide range of biological processes, including actin remodelling, cell transformation and G_1 cell cycle progression [1–6]. In addition, Rac protein and other Rho family GTPases have recently been shown to have a role in the signalling route to serum response element (SRE) activation through the serum response factor (SRF)-linked pathway, which probably involves the direct activation of SRF [7]. However, the signalling pathway by which Rac GTPase activates c-*fos* SRE remains largely unknown. Recently, several groups have reported that Rac and Cdc42 activate the c-*jun* N-terminal kinase (Jnks) signalling cascade in response to environmental stress [8,9]. It has also been reported that the Jnk signalling cascade activates ternary complex factor (TCF) protein, Elk-1, to stimulate SRE [10–12]. Although the possible signalling connection between Rac and Jnk-Elk-1 might exist, it remains to be determined whether Rac activates SRE via the Jnk–Elk-1-linked pathway. At any rate, the Jnk pathway is unlikely to be the only signalling pathway by which Rac mediates c-*fos* SRE activation because Rho family proteins including Rac were previously reported to be involved in the TCF/Elk-1-independent signalling pathway to SRE activation [7]. Thus Rac might mediate both TCF-dependent and TCF-independent signalling pathways to SRE activation, depending on the agonist or cell type.

It was recently demonstrated that Rac activation leads to arachidonic acid (AA) release, suggesting that phospholipase A_2 (PLA_2) is one of the targets of Rac GTPase [13]. For example, AA release and the subsequent metabolism of AA have been implicated as one of the major biochemical pathways by which Rac can influence actin remodelling in fibroblast cells [13,14]. In addition, Rac-activated PLA_2 and subsequent AA release have been shown to be required for the stimulation of Ca^{2+} influx by epidermal growth factor [15]. Despite these connections between Rac and PLA_2 activation, it is not known whether or not Rac-mediated nuclear signalling to SRE activation requires the activation of PLA_2.

In the present study we have examined the role of PLA_2 in the Rac-induced activation of c-*fos* SRE. Our results clearly demonstrate that PLA_2 and subsequent AA metabolism by lipoxygenase (LO) are essential for Rac-induced SRE activation. Further, in accordance with the idea of PLA_2–AA–LO as potential mediator of Rac signalling to the nucleus, the exogenous addition of AA, a major product of PLA_2, stimulated c-*fos* SRE-linked luciferase activity via an LO-dependent pathway.

MATERIALS AND METHODS

Chemicals and plasmids

AA, indomethacin, nordihydroguaiaretic acid (NDGA) and mepacrine were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Fetal bovine serum (FBS), gentamycin and Dulbecco's modified Eagle's medium (DMEM) were from Gibco-BRL (Gaithersburg, MD, U.S.A.). All other chemicals were from standard sources and were molecular biology grade or higher. The reporter genes pSREwt-Luc and pSREmt-Luc have been described previously [16]. pEXV-Rac1, pEXV-RacV12 (Rac1val12) and pEXV-RhoV14 (RhoAval14) plasmids were gifts from Dr. Alan Hall, and all Rac and Rho proteins were expressed as N-terminally 9E10 epitope-tagged derivatives under simian virus 40 promoter [3]. pEXV-5LO plasmid was constructed by subcloning of 5-LO cDNA [17] into the *Eco*R1 site of pEXV plasmid. Anti-sense 5-LO (AS-5LO) and sense 5-LO (SS-5LO) plasmids were prepared by inserting the partial fragment (1 kb) of the 5-LO coding region (1–332 amino acid residues; N-terminal) into *Eco*R1/*Sma*I site of pEXV vector in anti-sense and sense orientations respectively.

Abbreviations used: AA, arachidonic acid; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; Jnk, c-*Jun* N-terminal kinase; LC-1, lipocortin 1; LO, lipoxygenase; NDGA, nordihydroguaiaretic acid; PLA_2, phospholipase A_2; SRE, serum response element; SRF, serum response factor; TCF; ternary complex factor.

[1] To whom correspondence should be addressed.

Cell culture, DNA transfections and luciferase assay

Rat-2 fibroblast cells were obtained from the American Type Culture Collection (ATCC, CRL 1764). Cells were grown in DMEM supplemented with 2 mM glutamine, 10 % (v/v) FBS and gentamycin as described previously [18,19]. Transient transfection analysis was performed by plating approx. 5×10^5 cells in 100 mm dishes. At 24 h after plating, Rat-2 cells were transfected with a total of 20 μg of DNA per 100 mm diameter dish by the calcium phosphate DNA precipitation method [20]. Cells were then incubated for 6 h at 37 °C, at which time the medium was removed. The cells were serum-starved in DMEM supplemented with 0.5 % FBS for 36 h. To control for variations in both cell numbers and transfection efficiency, all clones were co-transfected with 1 μg of pCMV-βGAL, a eukaryotic expression vector in which *Escherichia coli* β-galactosidase (lac Z) structural gene is under the transcriptional control of the CMV promoter. Lysates prepared from the harvested cells were assayed for luciferase and β-galactosidase activities; the latter was used as an internal standard to normalize the former activity directed by the test plasmid. Cells were rinsed twice with PBS and then lysed in 0.2 ml of lysis solution [0.2 M Tris (pH 7.6)/0.1 % Triton X-100] per 100 mm plate. Lysed cells were scraped and centrifuged for 1 min. Supernatants were recovered for quantification of luciferase, β-galactosidase and protein. Luciferase activity was determined as described previously [21]. Transfection experiments were performed in triplicate with two independently isolated sets and the results were averaged.

[^{3}H]AA release

Rat-2 cells in DMEM containing 10 % FBS were plated to a density of 10^5 cells per well (six-well plate). After 4 h, 2 μCi/ml [^{3}H]AA (250 μCi; Amersham) was added to each well and incubated for an additional 36 h, then washed at least three times with DMEM containing 10 % FBS. Cells were then transfected with pEXV or pEXV-RacV12 by the calcium phosphate DNA precipitation method. Cells were then incubated for 6 h at 37 °C, at which time the medium was removed and then replaced with fresh DMEM containing 0.5 % FBS for another 6 h before scintillation counting of the released [^{3}H]AA into the medium. At the end of experiments, the cells were solubilized with 0.5 ml of ethyl alcohol (EtOH) for the determination of intracellular total incorporation. Counts were corrected for intracellular pools of AA.

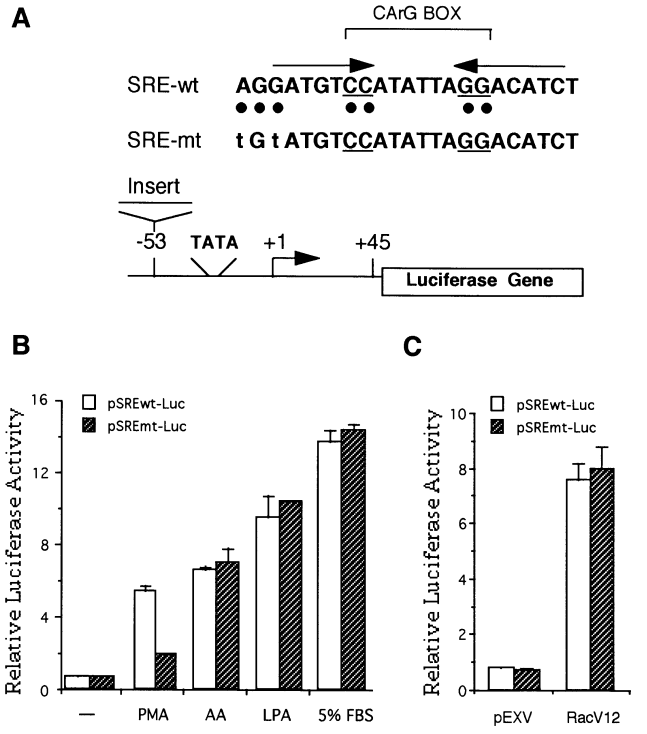

Figure 1 RacV12 activates c-*fos* SRE in a TCF/Elk-1-independent manner

(**A**) Diagram of the pSRE–luciferase reporter gene plasmids used. The structures of the constructs containing wild-type or mutant SRE oligonucleotide sequences (23-mers) inserted at the −53 position of the truncated c-*fos* promoter fused to luciferase gene are shown. The methylation interference pattern for the SRF ternary complex with TCF is indicated with filled circles. The mutant SRE has two point mutations (*AGG* to *TGT*) in the TCF-binding region that abolish the SRF/TCF ternary complex formation. (**B**) Transient transfection assay with pSREwt-Luc or pSREmt-Luc (TCF-binding-defective SRE). AA (100 μM), PMA (10 ng/ml), lysophosphatidic acid (10 μM) or 5 % (v/v) FBS was added for 2 h before harvest. The relative activation of pSREwt-Luc was calculated as described in the Materials and methods section; histograms of the results of the luciferase assay are shown. Values were representative of multiple transfections. (**C**) Transient transfection assay with RacV12 expression plasmid. A reporter gene plasmid, pSREwt-Luc (3 μg) or pSREmt-Luc (3 μg), was transiently co-transfected with 5 μg of pEXV or pEXV-RacV12. Total amounts of DNA were kept at 20 μg with calf thymus carrier DNA. Luciferase activities were measured and normalized to co-transfected β-galactosidase activities.

RESULTS AND DISCUSSION

RacV12 activates c-*fos* SRE–luciferase activity via a TCF/Elk-1-independent pathway

As a first step towards understanding the signal transduction pathway of Rac-mediated c-*fos* SRE activation, we examined whether a TCF/Elk-1-dependent signalling pathway is involved in the activation of SRE by Rac [22]. To do this, subconfluent Rat-2 fibroblast cells were transiently co-transfected with a wild-type or mutant SRE-luciferase plasmid (pSREwt-Luc or pSREmt-Luc respectively) and the plasmid pEXV-RacV12 encoding a constitutively active GTPase-deficient allele of Rac1, termed RacV12. As shown in Figure 1(A), the oligonucleotide in pSREmt-Luc plasmid contains two point mutations (*AGG* to *TGT*) that abolish TCF/Elk-1 binding [16].

Co-transfection with pEXV-RacV12 activated both pSREwt-Luc and pSREmt-Luc to the same extent (approx. 7.5-fold over pEXV co-transfection), indicating that RacV12 activates c-*fos* SRE in a TCF/Elk-1-independent signalling pathway in Rat-2 fibroblasts (Figure 1C). Thus this result suggests that the Jnk pathway, which had been shown to activate SRE via a TCF/Elk-1-dependent pathway [10–12], is probably not involved in a RacV12-induced signalling pathway to c-*fos* SRE activation in Rat-2 cells. Our results seem to be in agreement with that of the earlier report by Hill et al. [7], in which it was demonstrated that Rho family GTPases including Rac activate SRE through a TCF/Elk-1-independent pathway. In a control experiment to demonstrate the specificity of the TCF mutant pSREmt-Luc, we examined the effects of various agonists. As expected, PMA (10 ng/ml), a protein kinase C-stimulating agent, did not effectively activate the mutant SRE-luciferase activity (approx. 30 % activity of wild-type SRE-luciferase stimulation; Figure 1B). In contrast, lysophosphatidic acid (10 μM), which had been shown to activate SRE by an Elk-1-independent pathway, stimulated both SRE mutant- and SRE wild-type-dependent luciferase activities to the same level. Interestingly, we observed that exogenous AA (100 μM), a major product of Rac-activated PLA_2, activates both SRE mutant and SRE wild-type luciferase to the same extent (approx. 6.5 fold), indicating that AA activates

Table 1 PLA2 is essential for RacV12-induced SRE activation

The effects of various inhibitors on RacV12- or RhoV14-induced SRE activation were analysed. A reporter gene plasmid, pSRE-Luc (3 μg), was transiently co-transfected with 5 μg of pEXV, pEXV-RacV12 or pEXV-RhoV14. Various inhibitors were added at 6 h before cell harvest. The inhibitor concentrations were 1 μM (mepacrine), 5 μM (NDGA) and 10 μM (indomethacin). Luciferase activities were measured and normalized to co-transfected β-galactosidase activities. The results are means $\pm$ S. D. for three samples.

	Relative luciferase activity		
Inhibitor added	pEXV	RacV12	RhoV14
None	1	7.2 $\pm$ 0.2	7.7 $\pm$ 0.3
Mepacrine	1.0 $\pm$ 0.1	3.1 $\pm$ 0.3	7.3 $\pm$ 0.3
NDGA	0.9 $\pm$ 0.1	3.8 $\pm$ 0.6	7.5 $\pm$ 0.4
Indomethacin	1.0 $\pm$ 0.1	6.5 $\pm$ 0.3	7.2 $\pm$ 0.3

c-*fos* SRE in a TCF/Elk-1-independent signalling pathway in Rat-2 fibroblasts (Figure 1B).

PLA_2 and subsequent AA metabolism by LO are essential for Rac signalling to SRE

To examine further the signalling pathway by which Rac mediates c-*fos* SRE activation, we examined whether or not Rac-activated PLA_2 is involved. To do this, we tested whether mepacrine, a potent PLA_2 inhibitor, could block RacV12-induced SRE activation. A reporter gene plasmid, pSRE-Luc (3 μg), was transiently co-transfected with 5 μg of pEXV, pEXV-RacV12 or pEXV-RhoV14 (encoding a constitutively activated allele of RhoA). Transfected cells were serum-deprived in 0.5% FBS/DMEM for 36 h before harvest. At 6 h before cell harvest, various inhibitors were added as shown in Table 1. Treatment with mepacrine (1 μM) selectively inhibited the RacV12-induced SRE activation by 60% but not the RhoV14-induced SRE activation (Table 1). This result indicates that PLA_2 activity is specifically required for the RacV12-induced signalling pathway to c-*fos* SRE.

In a further study to examine the role of PLA_2 in mediating the Rac signal to *c-fos* SRE, we tested whether the subsequent metabolism of AA, a major product of Rac-activated PLA_2 [4], is also involved in Rac-induced signalling to SRE activation. In mammalian cells, AA has been known to be oxidized via two major metabolic pathways: the LO and the cyclo-oxygenase pathways [23,24]. To analyse whether AA metabolism is involved in Rac signalling to SRE, LO and cyclo-oxygenase activity were selectively inhibited by specific inhibitors and the resulting promoter activities were analysed in transient transfection experiments. The inhibition of LO, by a 10 min preincubation with NDGA (5 μM), abolished SRE activation by RacV12, whereas indomethacin (10 μM), an inhibitor of cyclo-oxygenase, did not show any inhibition (Table 1). In contrast, RhoV14-induced SRE activation was not inhibited by any those inhibitors, suggesting that the action of NDGA is specific to RacV12 (Table 1). Together, these results suggest that PLA_2 activation and subsequent AA metabolism by LO are critical for the RacV12-induced signalling cascade to SRE and thus the 'PLA_2–AA–LO cascade' seems to be at least one of the major signalling routes of Rac to c-*fos* SRE.

To gain evidence for the signalling connection between Rac and PLA_2 activation, we determined whether RacV12 transfection could induce the release of AA. To do this we measured the release of [^{3}H]AA from RacV12-transfected cells that had been prelabelled with [^{3}H]AA (2 μCi/ml) for 36 h. As shown in Figure 2, RacV12 transfection to Rat-2 cells stimulated the release of [^{3}H]AA significantly in a dose-dependent manner. By transfection with 5 μg of pEXV-RacV12, AA was released by 3.5-fold over control (pEXV vector transfection). In contrast, neither pEXV nor pEXV-RhoV14 transfection showed any stimulation of [^{3}H]AA release (Figure 2). The release of AA by RacV12 was, however, completely abolished by 1 μM of mepacrine (Figure 2). These results suggest that PLA_2 activation is functionally linked to Rac in Rat-2 fibroblast cells.

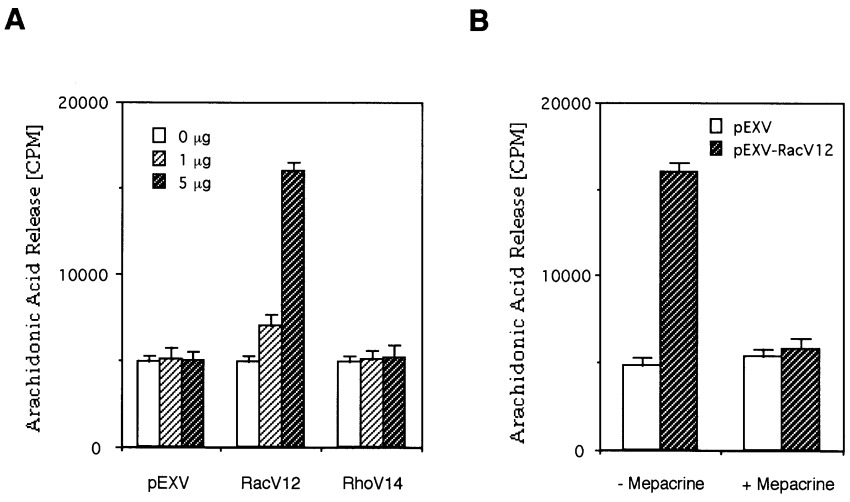

Figure 2 PLA_2 mediates RacV12-induced [^{3}H]AA release

(**A**) RacV12 induces the release of [^{3}H]AA. The release of [^{3}H]AA was measured from pEXV, pEXV-RhoV14 or pEXV-RacV12 (0, 1 or 5 μg)-transfected Rat-2 cells that had been prelabelled with [^{3}H]AA (0.5 μCi/ml; 36 h). The [^{3}H]AA released into the medium was quantified by scintillation counting and normalized to the total counts as described in the Materials and methods section. (**B**) RacV12-induced AA release is inhibited by mepacrine. The prelabelled Rat-2 cells were transiently transfected with 5 μg of pEXV or pEXV-RacV12 plasmid. After transient transfection, the medium was replaced by DMEM/0.5% (v/v) FBS with or without 1 μM of mepacrine (a PLA_2 inhibitor), and incubated for 6 h before harvest. The [^{3}H]AA released into the medium was quantified by scintillation counting and normalized to the total counts as described in the Materials and methods section.

Table 2 Inhibition of RacV12-induced SRE activation by co-transfection with pLC-1 or pAS-5LO

A reporter gene plasmid, pSRE-Luc (3 μg) and 5 μg of pEXV, pEXV-RacV12 or pEXV-RhoV14 were transiently co-transfected with indicated amounts of lipocortin-1 expression plasmid, pLC-1, or the anti-sense 5-LO expression plasmid pAS-5LO. Total amounts of DNA were kept at 20 μg with calf thymus carrier DNA. Transfected cells were serum-deprived in DMEM/0.5% (v/v) FBS for 36 h before assays for luciferase activities. The results are means $\pm$ S.D. for three samples.

			Relative luciferase activity			
	Co-transfected DNA	Amount of co-transfected DNA (μg) ...	0	1.0	2.5	5.0
pEXV	pLC-1		1	1.1 $\pm$ 0.1	0.9 $\pm$ 0.2	1.1 $\pm$ 0.2
pEXV-RacV12	pLC-1		7.2 $\pm$ 0.2	4.8 $\pm$ 0.2	3.6 $\pm$ 0.4	3.3 $\pm$ 0.3
pEXV-RhoV14	pLC-1		7.9 $\pm$ 0.3	8.3 $\pm$ 0.7	7.6 $\pm$ 0.6	7.9 $\pm$ 0.3
pEXV	pAS-5LO		1	0.9 $\pm$ 0.2	1.0 $\pm$ 0.1	1.1 $\pm$ 0.2
pEXV-RacV12	pAS-5LO		7.2 $\pm$ 0.2	5.5 $\pm$ 0.2	4.3 $\pm$ 0.3	3.9 $\pm$ 0.2
pEXV-RhoV14	pAS-5LO		7.8 $\pm$ 0.3	7.6 $\pm$ 0.3	8.0 $\pm$ 0.3	7.6 $\pm$ 0.4

Inhibition of RacV12-induced SRE activation by co-transfection with lipocortin 1 (LC-1) or anti-sense 5-LO

To obtain further insight into the role of PLA_2 and subsequent AA metabolism by LO in RacV12-signalling to SRE, we examined whether RacV12-induced SRE activation is sensitive to other types of inhibitors of PLA_2 or LO. For the inhibition of PLA_2, lipocortin-1 [25], also called annexin-1, expression was used. Lipocortin-1 has been suggested to inhibit PLA_2 specifically via a still-unknown mechanism in various cases [25,26]. Cells were transiently co-transfected with various amounts (1, 2.5 or 5 μg) of pcDNA3 (vector) or pcDNA3–LC-1 to determine whether LC-1 co-transfection blocks RacV12- or RhoV14-induced SRE activation. We observed that LC-1 specifically blocked RacV12-induced SRE activation in a dose-dependent manner without affecting RhoV14-induced SRE activation (Table 2). In a control test, co-transfection with the same amounts of pcDNA3 had no effect on RacV12- or RhoV14-induced SRE activation (results not shown). These results again suggest that the signalling pathway of RacV12 to SRE is dependent on functional PLA_2 activity.

Next we tested another type of LO inhibitor on RacV12-induced SRE activation. For the inhibition of LO, we prepared an anti-sense expression plasmid of 5-LO (5LO) which was shown to catalyse leucotriene production as one major type of LO in fibroblasts [5]. Cells were co-transfected with an anti-sense 5-LO cDNA expression plasmid (AS-5LO) that is transcribed, within the cell, to produce complementary RNA directed to sequences (1–332 amino acid residues; N-terminal) of the 5-LO coding region. As shown in Table 2, co-transfection with AS-5LO plasmid (1, 2.5 or 5 μg) resulted in a specific and dose-dependent inhibition of the RacV12-induced SRE stimulation (approx. 40–45% decrease in luciferase activity), whereas RhoV14-induced SRE stimulation was not affected. This finding indicates that the metabolism by LO, especially 5-LO, plays a role in RacV12-induced activation of c-*fos* SRE. Together, these results again point to a critical role of 'PLA_2 and AA metabolism by LO' in the Rac-signalling cascade to c-*fos* SRE.

Exogenous AA stimulates SRE via LO-dependent pathway

Next, encouraged by the above and earlier results, we examined more directly whether exogenous AA, a principal product of PLA_2, could activate c-*fos* SRE via an LO-dependent pathway. After transient transfections with pSRE-Luc (3 μg) plasmid, Rat-2 cells were serum-starved in DMEM containing 0.5% FBS for 36 h before adding AA. At the same time, we tested whether NDGA could block AA-induced SRE stimulation. As shown in Table 3, there was a marked (approx. 65%) inhibition of AA-induced SRE activation by NDGA, not by indomethacin. In addition, we observed approx. 50% inhibition of AA-induced SRE activation by co-transfection with anti-sense 5-LO (AS-5LO) but not with sense 5-LO (SS-5LO; results not shown). Thus LO is clearly involved in mediating SRE activation by RacV12 and also by AA, providing further evidence for the existence of the signalling cascade of Rac–PLA_2–AA–LO. In accordance with the idea of PLA_2–AA as one of the potential mediators of the RacV12-induced signal to SRE, exogenous AA was shown to stimulate c-*fos* SRE in a TCF/Elk-1-independent manner, resembling the signalling mode of RacV12 (Figure 1B).

Table 3 AA stimulates *c-fos* SRE in a lipoxygenase-dependent manner

Effects of NDGA pretreatment or AS-5LO (anti-sense 5-LO) co-transfection on AA- or lysophosphatidic acid (LPA)-induced SRE activation were analysed. The SRE-stimulating activities were analysed either by co-transfection with 5 μg of pEXV or pEXV-AS-5LO, or by pretreatment with inhibitors. The inhibitor concentrations were 5 μM (NDGA) and 10 μM (indomethacin). Luciferase activities were measured and normalized to co-transfected β-galactosidase activities. The results are means $\pm$ S.D. for three samples.

		Relative luciferase activity		
Inhibitor	Co-transfected DNA	Buffer	AA	LPA
None	None	1	6.5 $\pm$ 0.3	11.9 $\pm$ 0.7
NDGA	None	0.9 $\pm$ 0.1	2.1 $\pm$ 0.4	11.2 $\pm$ 0.4
Indomethacin	None	1.0 $\pm$ 0.1	6.2 $\pm$ 0.5	11.5 $\pm$ 0.6
None	pEXV	1	6.3 $\pm$ 0.3	11.4 $\pm$ 0.5
None	pEXV-AS-5LO	1.1 $\pm$ 0.1	3.2 $\pm$ 0.2	11.3 $\pm$ 0.6

Together, our results clearly suggest that 'PLA_2–AA–LO' mediates, at least partly, the signalling of RacV12 to c-*fos* SRE stimulation. Our proposed mode of a 'Rac–PLA_2–AA–LO' signalling cascade to SRE activation seems very similar to that of the actin remodelling pathway suggested by Peppelenbosch et al. [4]. In the actin remodelling system, Rac was shown to stimulate growth factor-dependent actin stress fibre formation via PLA_2 activation and subsequent AA metabolism by LO in fibroblast cells [5]. Thus the PLA_2–AA–LO signalling cascade seems to be commonly involved in the Rac-mediated signalling pathways to either actin remodelling or SRE regulation. Further studies aimed at understanding the signalling link between AA and c-*fos* SRE activation in the control of Rac-mediated c-*fos* SRE

activation will lead to additional insights into the regulation of Rac-mediated nuclear signalling.

We thank Dr. D. S. Na (Ulsan Medical School, Seoul, Korea) for lipocortin-1 expression plasmid and discussion throughout this study, and Dr. Allan Hall (University College London, U.K.) for providing us with pEXV, pEXV-RacV12 and pEXV-RhoV14 plasmids. This study was supported by the Korean Ministry of Education through Research Fund (to J.-H. K.).

REFERENCES

1 Hall, A. (1994) Annu. Rev. Cell Biol. **10**, 31–54
2 Nobes, C. D. and Hall, A. (1995) Cell **81**, 53–62
3 Ridley, A. J., Paterson, H. F., Johnston, C. L., Diekmann, D. and Hall, A. (1992) Cell **70**, 401–410
4 Peppelenbosch, M. P., Qiu, R. G., de Vries-Smits, A. M. M., Tertoolen, L. G. J., de Laat, S. W., McCormick, F., Hall, A., Symons, M. H. and Bos, J. L. (1995) Cell **81**, 849–856
5 Olson, M. F., Ashworth, A. and Hall, A. (1995) Science **269**, 1270–1272
6 Qiu, R.-G., Chen, J., Kirn, D., McCormick, F. and Symons, M. (1995) Nature (London) **374**, 457–459
7 Hill, C. S., Wynne, J. and Treisman, R. (1995) Cell **81**, 1159–1170
8 Cosa, O. A., Chiariello, M., Yu, J. C., Teramoto, H., Crespo, P., Xu, N., Miki, T. and Gutkind, J. S. (1995) Cell **81**, 1137–1146
9 Minden, A., Lin, A., Claret, F. X., Abo, A. and Karin, M. (1995) Cell **81**, 1147–1157
10 Whitmarsh, A. J., Shore, P., Sharrocks, A. D. and Davis, R. J. (1995) Science **269**, 403–407
11 Cavigelli, M., Dolfi, F., Claret, F.-X. and Karin, M. (1995) EMBO J. **14**, 5957–5964
12 Gille, H., Strahl, T. and Shaw, P. E. (1995) Curr. Biol. **5**, 1191–1200
13 Peppelenbosch, M. P., Tertoolen, L. G. J., Hage, W. J. and de Laat, S. W. (1993) Cell **74**, 565–575
14 Peppelenbosch, M. P., Tertoolen, L. G. J., Hertog, J. D. and de Laat, S. W. (1992) Cell **69**, 295–303
15 Peppelenbosch, M. P., Tertoolen, L. G. J., de Vries-Smits, A. M. M., Qiu, R. G., M'Rabet, L., Symons, M. H., de Laat, S. W. and Bos, J. L. (1995) J. Biol. Chem. **271**, 7883–7886
16 Kim, J. H., Cho, Y. S., Kim, B. C., Kim, Y. S. and Lee, G. S. (1997) Biochem. Biophys. Res. Commun. **232**, 223–226
17 Dixon, R. A., Jones, R. E., Diehl, R. E., Bennett, C. D., Kargman, S. and Rouzer, C. A. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 416–420
18 Bushel, P., Kim, J. H., Johansen, F. E., Catino, J. J., Prywes, R. and Kumar, C. C. (1995) Oncogene **10**, 421–430
19 Kim, J. H., Bushel, P. and Kumar, C. C. (1993) Biochem. Biophys. Res. Commun. **190**, 1115–1122
20 Kim, J. H., Johansen, F. E., Robertson, N., Catino, J. J., Prywes, R. and Kumar, C. C. (1994) J. Biol. Chem. **269**, 13740–13743
21 Croissant, J. D., Kim, J. H., Eichele, G., Goering, L., Lough, J., Prywes, R. and Schwartz, R. J. (1996) Dev. Biol. **177**, 250–264
22 Treisman, R. (1990) Semin. Cancer Biol. **1**, 47–58
23 Ridley, A. J. and Hall, A. (1992) Cell **70**, 389–399
24 Sellmayer, A., Uedelhoven, W. M., Weber, P. C. and Bonventre, J. V. (1991) J. Biol. Chem. **266**, 3800–3807
25 Croxtall, J. D., Choudhury, Q., Newman, S. and Flower, R. J. (1996) Biochem. Pharm. **52**, 351–356
26 Samamoto, T., Repasky, W. T., Uchida, K., Hirata, A. and Hirata, F. (1996) Biochem. Biophys. Res. Commun. **220**, 643–647

Received 27 January 1997/14 April 1997; accepted 25 April 1997