J Physiol 570.1 (2006) pp 59–64

SYMPOSIUM REPORT

Excitation–transcription coupling in smooth muscle

Christy A. Barlow, Patricia Rose, Renee A. Pulver-Kaste and Karen M. Lounsbury

Department of Pharmacology and Totman Center for Cerebrovascular Research, University of Vermont, Burlington, VT 05405-0068, USA

Calcium (Ca^{2+}) signals affect virtually every biological process, including both contraction and gene transcription in smooth muscle. Ca^{2+}-regulated gene transcription is known to be important for both physiological and pathological responses in smooth muscle. The aim of this review is to discuss the current understanding of gene transcription regulated by excitation through Ca^{2+} signalling using a comparison of the two most characterized Ca^{2+}-regulated transcription factors in smooth muscle, Ca^{2+}–cyclic AMP response element binding protein (CREB) and nuclear factor of activated T-cells (NFAT). Recent studies have shown commonalities and differences in the regulation of CREB and NFAT through both voltage- and non-voltage-gated Ca^{2+} channels that lead to expression of smooth muscle cell specific differentiation markers as well as markers of proliferation. New insights into the regulation of specific genes through companion elements on the promoters of Ca^{2+}-regulated genes have led to new models for transcriptional regulation by Ca^{2+} that are defined both by the source and duration of the Ca^{2+} signal and the composition of enhancer elements found within the regulatory regions of specific genes. Thus the combination of signalling pathways elicited by particular Ca^{2+} signals affect selective promoter elements that are key to the ultimate pattern of gene transcription.

(Received 11 September 2005; accepted after revision 10 October 2005; first published online 13 October 2005)
Corresponding author K. M. Lounsbury: Department of Pharmacology, University of Vermont, 89 Beaumont Ave., Burlington, VT 05405, USA. Email: karen.lounsbury@uvm.edu

Excitation–contraction coupling in smooth muscle cells (SMCs) is tightly controlled by spatio-temporal Ca^{2+} events that involve both influx of extracellular Ca^{2+} and release from selective intracellular Ca^{2+} stores (reviewed in Bolton *et al.* 2004). Recent findings have revealed that the Ca^{2+}-regulated transcription factors CREB and NFAT have selective Ca^{2+} source requirements and are influenced by transcriptional coactivators and cofactors for CREB and NFAT. Excitation–transcription coupling is thus clearly relevant to both normal physiological responses and to the pathogenesis of vascular diseases that are known to include altered Ca^{2+} handling and changes in gene expression.

Ca^{2+} regulation of CREB and gene transcription in smooth muscle

CREB (Ca^{2+}–cyclic AMP response element binding protein) regulates transcription through recognition and binding to Ca^{2+}–cyclic AMP (cAMP)-response elements (CREs) in the promoter of many genes (Shaywitz & Greenberg, 1999). Stimulus-induced CREB activation requires phosphorylation of ^{133}serine to promote recruitment of CREB binding protein (CBP300) and other cofactors to form an active transcriptional complex (Gonzalez *et al.* 1989; Mayr & Montminy, 2001). Ca^{2+} from many sources can activate CREB-induced transcription through the Ca^{2+} calmodulin-dependent protein kinase (CaMK), growth factor/MAPK, and cAMP-dependent protein kinase pathways (Shaywitz & Greenberg, 1999) (Fig. 1). Inactivation of CREB through phosphatase activity can also be regulated by Ca^{2+} through Ca^{2+}–CaM activation of the protein phosphatase calcineurin which indirectly leads to dephosphorylation of CREB (Alberts *et al.* 1994).

CREB activation elicited by membrane depolarization and Ca^{2+} entry through voltage-dependent calcium channels (VDCCs) has been confirmed in both cultured SMCs and intact cerebral arteries (Cartin *et al.* 2000; Stevenson *et al.* 2001*a*). Depolarization-mediated CREB phosphorylation correlates with an increase in transcription of the CRE-containing immediate early gene, *c-fos*, and is sensitive to inhibitors of VDCCs.

This report was presented at The Physiological Society Focused Meeting on Ion channels, genes and regulation in smooth muscle, at the University of Oxford, UK, 5–7 September 2005.

© 2006 The Authors. Journal compilation © 2006 The Physiological Society

DOI: 10.1113/jphysiol.2005.098426

Although CREB is predominantly nuclear, it has been shown to accumulate in the cytoplasm of vascular SMCs when nuclear import is blocked prior to membrane depolarization (Stevenson *et al.* 2001*a*). While the relevance of nuclear shuttling of CREB has not been established, it could explain CREB interactions with multiple kinases that do not cross the nuclear membrane and the perceived loss of CREB from the nucleus following ischaemia (Klemm *et al.* 2001).

A role for SR Ca^{2+} in signalling to CREB in SMCs has been demonstrated both in response to Ca^{2+} release from inositol 1,4,5-trisphosphate (IP_3) receptors and through store-operated Ca^{2+} entry. Endothelin-1, platelet-derived growth factor (PDGF), sphingosine-1-phosphate, aldosterone, low-density lipoprotein (LDL) and ischaemia have all been shown to induce CREB phosphorylation in SMCs through an IP_3-dependent mechanism (Christ *et al.* 1999; Stevenson *et al.* 2001*a*; Coussin *et al.* 2003; Egan & Nixon, 2004; Rius *et al.* 2004; Meller *et al.* 2005). The majority of these pathways are also influenced by MAPK signalling, and a link between Ca^{2+} and MAPK signalling to CREB has been observed for noradrenaline (norepinephrine)-induced CREB signalling (Hu *et al.* 1999). Store-operated Ca^{2+} entry (SOCE), in response to SR Ca^{2+} depletion, has also been shown to stimulate CREB phosphorylation in both cultured and intact vascular SMCs (Fig. 2), and a role for SOCE has been reported in CREB activation stimulated by angiotensin II (Pulver *et al.* 2004). Determinations of the relevance of SOCE activation by other IP_3 mediators and its importance in MAPK signalling are important targets of future investigations related to CREB signalling.

Not all Ca^{2+} signals lead to activation of CREB. Release of Ca^{2+} from ryanodine receptors (RyR) in the form of Ca^{2+} sparks has been shown to have an inhibitory effect on CREB activation, probably through membrane hyperpolarization (Cartin *et al.* 2000). These results stress the importance that the nature of the Ca^{2+} signal has on the downstream changes in gene transcription mediated by CREB.

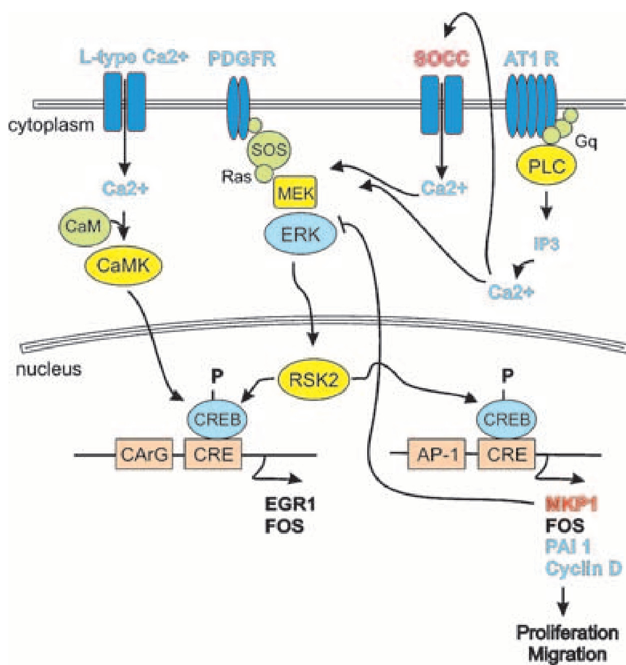

Figure 1. Regulation of CREB activation through multiple signalling cascades in SMCs.
Store-operated Ca^{2+} channel (SOCC), phospholipase C (PLC), son of sevenless (SOS), platelet-derived growth receptor (PDGFR), GTPase binding protein q (Gq), extracellular-signal regulated kinase (ERK), MAPK/ERK kinase (MEK), receptor signal kinase (RSK), angiotensin II type 1 receptor (AT1R), serum response element (CArG), plasminogen activator 1 inhibitor (PAI1).

Ca^{2+} regulation of NFAT in smooth muscle

Nuclear factor of activated T cells (NFAT) has recently been shown to play an important role in regulating Ca^{2+}-dependent gene transcription in SMCs.

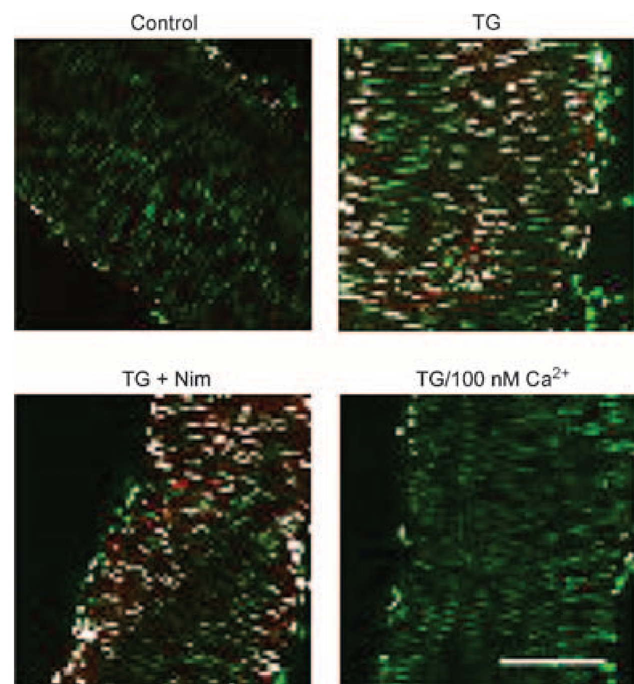

Figure 2. CREB is activated by SOCE in intact vascular smooth muscle
From Pulver *et al.* (2004), used with permission. Rat cerebral arteries were isolated and incubated in HEPES-buffered saline (HBS) with normal Ca^{2+} (2 mmol l^{-1}), 100 nmol l^{-1} Ca^{2+}, or 100 nmol l^{-1} nimodipine (Nim) for 15 min. Arteries were then exposed to 100 nmol l^{-1} thapsigargin (TG) for 15 min or 60 mM K^{+} for 10 min. CREB phosphorylation was detected by anti-P-CREB immunofluorescence. Shown are confocal images representing P-CREB (red), YOYO-1 DNA nuclear stain (green) and overlap of P-CREB and YOYO-1 DNA (white). Bar represents 100 μm.

© 2006 The Authors. Journal compilation © 2006 The Physiological Society

The activation of NFAT is regulated through its subcellular localization, which reflects the intensity of Ca^{2+}–calcineurin signalling and the activities of several nuclear protein kinases (Fig. 3) (Hogan *et al.* 2003). Upon elevation of intracellular Ca^{2+} levels, the Ca^{2+}–CaM-dependent phosphatase, calcineurin, dephosphorylates NFAT, allowing for the translocation of the NFAT–calcineurin complex into the nucleus. Because of its dependence on Ca^{2+}–calcineurin signalling, NFAT has the ability to sense dynamic changes in $[Ca^{2+}]_i$ and frequencies of Ca^{2+} oscillations (Dolmetsch *et al.* 1997; Li *et al.* 1998).

NFAT translocation and transcriptional activity has been clearly demonstrated in both cultured and native SMCs (Boss *et al.* 1998; Stevenson *et al.* 2001*b*). In cerebral SMCs, the vasoconstrictor UTP, and other $G_{q/11}$-coupled receptor agonists induce NFAT4 nuclear accumulation. This induction is dependent on release of Ca^{2+} from intracellular stores and requires function of VDCCs (Stevenson *et al.* 2001*b*; Gomez *et al.* 2002). However, counter to expectations, a sustained increase in $[Ca^{2+}]_i$ induced by membrane depolarization is not sufficient to achieve NFAT4 nuclear accumulation in vascular SMCs (Stevenson *et al.* 2001*b*).

One possibility for this finding is that NFAT nuclear accumulation is further regulated by serine/threonine protein kinases, which promote the nuclear export of NFAT. Recent work examining the role of c-Jun amino-terminal kinase (JNK) using JNK knockout animals suggest that although elevated Ca^{2+} levels are sufficient to promote NFAT nuclear import, suppression of NFAT nuclear export activity is also required. Specifically, nuclear JNK2 has been shown to selectively promote the nuclear export of NFAT4 in both transfection studies (Chow *et al.* 1997) and native isolated cerebral arteries (Gomez *et al.* 2003). These data suggest that although Ca^{2+} elevation is necessary, it may not be the rate-limiting step in NFAT nuclear accumulation in SMCs.

In intact arteries, NFAT translocates to the nucleus in response to physiological intraluminal pressure. Translocation is dependent on Ca^{2+} influx through VDCCs, requires the nitric oxide/protein kinase G pathway, and correlates with an inhibition of JNK-dependent nuclear export (Gonzalez Bosc *et al.* 2004). These results implicate a potential role for endothelial-derived nitric oxide in the regulation of NFAT activity and suggest that physiological pressure changes are sufficient to induce transcriptional activity through NFAT.

Ca^{2+} and smooth muscle-specific gene expression

Many vascular disease states are characterized by changes in gene expression that inhibit differentiation and promote the proliferative phenotype. The serum response factor (SRF) DNA binding site or CArG box ($CC[A/T]_6GG$) plays an important role in regulating SMC-specific genes (Kumar & Owens, 2003). Evidence is now emerging that suggests a complex interaction between Ca^{2+} signalling, regulatory elements and cofactors that affect SRF binding and/or transcriptional activity. Wamhoff *et al.* (2004) found that Ca^{2+} influx through VDCCs stimulates expression of SRF-dependent SMC differentiation markers through a mechanism requiring Rho kinase and the SRF coactivator myocardin. In addition, Ca^{2+}-dependent CRE elements have been found adjacent to CArG elements in several SRF-regulated SMC genes (Sekiguchi *et al.* 2001; Najwer & Lilly, 2005). Furthermore, Ca^{2+}-dependent NFAT activation has been shown to cooperatively regulate the activity of an intronic CArG enhancer of smooth muscle α-actin (Gonzalez Bosc *et al.* 2005). Thus, co-activators as well as CREB and NFAT have the capacity to direct expression of SRF-regulated genes in response to Ca^{2+} signalling in SMCs.

The paradox that remains is the existence of CArG elements in genes that promote de-differentiation as well as differentiation. What mechanisms underlie this distinction? New evidence supports the hypothesis that growth and developmental signals modulate SRF-dependent gene expression by regulating repressive cofactors that compete for SRF binding (Wamhoff *et al.* 2004; Wang *et al.* 2004). In light of the complexities regulating SRF function, future experiments are warranted

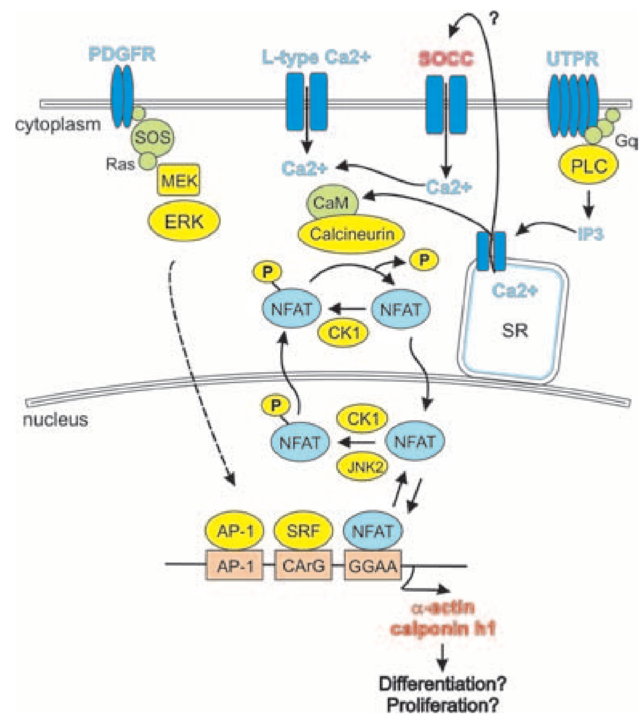

Figure 3. Regulation of NFAT via nuclear translocation in SMCs

© 2006 The Authors. Journal compilation © 2006 The Physiological Society

to explore these and other potential requirements for SRF-dependent progression to distinct SMC phenotypes.

CREB and NFAT in smooth muscle pathologies

The pathogenesis of vascular diseases such as hypertension includes altered Ca^{2+} handling that triggers changes in gene expression, and these changes are probably attributed to the ability of mature smooth muscle cells to de-differentiate and proliferate (Somlyo & Somlyo, 1994). Although proliferation is important in recovering from vascular injury, arterial intervention procedures such as angioplasty result in abnormal proliferation and restenosis.

Cerebral arteries from hypertensive animals exhibit elevated SMC Ca^{2+}, phospho-CREB and c-fos mRNA (Wellman *et al.* 2001). Interestingly, these effects are readily reversed by *in situ* inhibition of VDCCs, suggesting a defect in the 'off' mechanism of CREB activation. These findings provide an important link between altered gene regulation through CREB and de-regulation of Ca^{2+} signalling in a disease state. In pulmonary artery SMCs CREB is necessary and sufficient for induction of transient receptor potential cation channel 4 (TRPC4), that has been linked to the development of pulmonary hypertension (Landsberg & Yuan, 2004; Zhang *et al.* 2004).

Increased levels of phospho-CREB have also been correlated with the proliferative response associated with arteriolar injury including angiotensin II-induced hypertension, chronic nicotine administration and oxidative endothelial injury (Gerzanich *et al.* 2003). In addition, expression of dominant negative CREB constructs suppresses neointimal formation and increases apoptosis following balloon injury (Tokunou *et al.* 2003). Transient ischaemia also leads to CREB phosphorylation and increased CRE-mediated Bcl-2 expression (Meller *et al.* 2005). Together, these results suggest a role for CREB in both survival and proliferation of SMCs following injury. The role of CREB in the proliferative response to disease is clearly complex, however, in that SMC proliferation induced by *in vivo* hypoxia correlates with a reduction in CREB content (Klemm *et al.* 2001).

Although NFAT has also been shown to regulate genes related to cell cycle progression and cell differentiation in T cells and neurones, the functions of NFAT in SMCs are largely unknown. In aortic SMCs, NFAT2 nuclear translocation has been correlated with differentiation and found to be important for driving transcription of the smooth muscle-specific Sm-myosin heavy chain (MHC) promoter in a Ca^{2+}- and calcineurin-dependent manner (Wada *et al.* 2002). However, NFAT also plays a role in vascular SMC (VSMC) proliferation and motility induced by both receptor tyrosine kinase (RTK) and G protein-coupled receptor agonists (GPCR), PDGF and thrombin, respectively (Yellaturu *et al.* 2002; Liu *et al.* 2004). Taken together these findings reveal that NFAT, like CREB, is a likely candidate for mediating both differentiation and mitogenic effects in SMCs.

Few studies have directly compared NFAT and CREB signalling in SMCs. In a model of VLDL-induced SMC proliferation, NFAT activation correlates with a decrease in phospho-CREB, suggesting that the proliferative effect of VLDL increases NFAT activity while reducing CREB activity (Lipskaia *et al.* 2003). One caveat to these experiments is that in this model CREB is phosphorylated under basal conditions, an observation that has not been detected in other SMC culture cell systems or in intact arteries (Cartin *et al.* 2000; Pulver *et al.* 2004; Meller *et al.* 2005).

Overall, the evidence suggests that physiological or pathological alterations in Ca^{2+} signalling pathways are likely to have effects on both CREB and NFAT function and have the potential to disrupt normal patterns of gene transcription in SMCs. Future studies focused on CREB- and/or NFAT-dependent transcription patterns hold the promise of better understanding the role of excitation–transcription coupling as it relates to genomic effects on smooth muscle phenotype.

References

Alberts AS, Montminy M, Shenolikar S & Feramisco JR (1994). Expression of a peptide inhibitor of protein phosphatase 1 increases phosphorylation and activity of CREB in NIH 3T3 fibroblasts. *Mol Cell Biol* **14**, 4398–4407.

Bolton TB, Gordienko DV, Povstyan OV, Harhun MI & Pucovsky V (2004). Smooth muscle cells and interstitial cells of blood vessels. *Cell Calcium* **35**, 643–657.

Boss V, Abbott KL, Wang XF, Pavlath GK & Murphy TJ (1998). The cyclosporin A-sensitive nuclear factor of activated T cells (NFAT) proteins are expressed in vascular smooth muscle cells. Differential localization of NFAT isoforms and induction of NFAT-mediated transcription by phospholipase C-coupled cell surface receptors. *J Biol Chem* **273**, 19664–19671.

Cartin L, Lounsbury KM & Nelson MT (2000). Coupling of Ca^{2+} to CREB activation and gene expression in intact cerebral arteries from mouse: roles of ryanodine receptors and voltage-dependent Ca^{2+} channels. *Circ Res* **86**, 760–767.

Chow CW, Rincon M, Cavanagh J, Dickens M & Davis RJ (1997). Nuclear accumulation of NFAT4 opposed by the JNK signal transduction pathway. *Science* **278**, 1638–1641.

Christ M, Gunther A, Heck M, Schmidt BMW, Falkenstein E & Wehling M (1999). Aldosterone, not estradiol, is the physiological agonist for rapid increases in camp in vascular smooth muscle cells. *Circulation* **99**, 1485–1491.

Coussin F, Scott RH & Nixon GF (2003). Sphingosine 1-phosphate induces CREB activation in rat cerebral artery via a protein kinase C-mediated inhibition of voltage-gated K^+ channels. *Biochem Pharmacol* **66**, 1861–1870.

© 2006 The Authors. Journal compilation © 2006 The Physiological Society

Dolmetsch RE, Lewis RS, Goodnow CC & Healy JI (1997). Differential activation of transcription factors induced by Ca^{2+} response amplitude and duration. *Nature* **386**, 855–858.

Egan CG & Nixon GF (2004). Endothelin-1- and depolarization-induced differential regulation of cAMP response element-binding protein in proliferating and developed vascular smooth muscle. *Cellular Signalling* **16**, 1387–1396.

Gerzanich V, Ivanova S & Simard JM (2003). Early pathophysiological changes in cerebral vessels predisposing to stroke. *Clin Hemorheol Microcirc* **29**, 291–294.

Gomez MF, Bosc LV, Stevenson AS, Wilkerson MK, Hill-Eubanks DC & Nelson MT (2003). Constitutively elevated nuclear export activity opposes Ca^{2+}-dependent NFATc3 nuclear accumulation in vascular smooth muscle: role of JNK2 and Crm-1. *J Biol Chem* **278**, 46847–46853.

Gomez MF, Stevenson AS, Bonev AD, Hill-Eubanks DC & Nelson MT (2002). Opposing actions of inositol 1,4,5-trisphosphate and ryanodine receptors on nuclear factor of activated T-cells regulation in smooth muscle. *J Biol Chem* **277**, 37756–37764.

Gonzalez GA, Yamamoto KK, Fischer WH, Karr D, Menzel P, Biggs W III, Vale WW & Montminy MR (1989). A cluster of phosphorylation sites on the cyclic AMP-regulated nuclear factor CREB predicted by its sequence. *Nature* **337**, 749–752.

Gonzalez Bosc LV, Layne JJ, Nelson MT & Hill-Eubanks DC (2005). Nuclear factor of activated T cells and serum response factor cooperatively regulate the activity of an alpha-actin intronic enhancer. *J Biol Chem* **280**, 26113–26120.

Gonzalez Bosc LV, Wilkerson MK, Bradley KN, Eckman DM, Hill-Eubanks DC & Nelson MT (2004). Intraluminal pressure is a stimulus for NFATc3 nuclear accumulation: role of calcium, endothelium-derived nitric oxide, and cGMP-dependent protein kinase. *J Biol Chem* **279**, 10702–10709.

Hogan PG, Chen L, Nardone J & Rao A (2003). Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* **17**, 2205–2232.

Hu Z-W, Shi X-Y, Lin RZ, Chen J & Hoffman BB (1999). α_1-Adrenergic receptor stimulation of mitogenesis in human vascular smooth muscle cells: role of tyrosine protein kinases and calcium in activation of mitogen-activated protein kinase. *J Pharmacol Exp Ther* **290**, 28–37.

Klemm DJ, Watson PA, Frid MG, Dempsey EC, Schaack J, Colton LA, Nesterova A, Stenmark KR & Reusch JE-B (2001). cAMP response element-binding protein content is a molecular determinant of smooth muscle cell proliferation and migration. *J Biol Chem* **276**, 46132–46141.

Kumar MS & Owens GK (2003). Combinatorial control of smooth muscle-specific gene expression. *Arterioscler Thromb Vasc Biol* **23**, 737–747.

Landsberg JW & Yuan JX-J (2004). Calcium and TRP channels in pulmonary vascular smooth muscle cell proliferation. *News Physiol Sci* **19**, 44–50.

Li W, Llopis J, Whitney M, Zlokarnik G & Tsien RY (1998). Cell-permeant caged $InsP_3$ ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* **392**, 936–941.

Lipskaia L, Pourci M-L, Delomenie C, Combettes L, Goudouneche D, Paul J-L, Capiod T & Lompre A-M (2003). Phosphatidylinositol 3-kinase and calcium-activated transcription pathways are required for VLDL-induced smooth muscle cell proliferation. *Circ Res* **92**, 1115–1122.

Liu Z, Dronadula N & Rao GN (2004). A novel role for nuclear factor of activated T cells in receptor tyrosine kinase and G protein-coupled receptor agonist-induced vascular smooth muscle cell motility. *J Biol Chem* **279**, 41218–41226.

Mayr B & Montminy M (2001). Transcriptional regulation by the phosphorylation-dependent factor CREB. *Nat Rev Mol Cell Biol* **2**, 599–609.

Meller R, Minami M, Cameron JA, Impey S, Chen D, Lan J-Q, Henshall DC & Simon RP (2005). CREB-mediated Bcl-2 protein expression after ischemic preconditioning. *J Cereb Blood Flow Metab* **25**, 234–246.

Najwer I & Lilly BJ (2005). Calcium/calmodulin-dependent protein kinase IV activates cysteine-rich protein 1 through adjacent CRE and CArG elements. *Am J Physiol Cell Physiol* **289**, C785–93.

Pulver RA, Rose-Curtis P, Roe MW, Wellman GC & Lounsbury KM (2004). Store-operated Ca^{2+} entry activates the CREB transcription factor in vascular smooth muscle. *Circ Res* **94**, 1351–1358.

Rius J, Martinez-Gonzalez J, Crespo J & Badimon L (2004). Involvement of neuron-derived orphan receptor-1 (NOR-1) in LDL-induced mitogenic stimulus in vascular smooth muscle cells: role of CREB. *Arterioscler Thromb Vasc Biol* **24**, 697–702.

Sekiguchi K, Kurabayashi M, Oyama Y, Aihara Y, Tanaka T, Sakamoto H, Hoshino Y, Kanda T, Yokoyama T, Shimomura Y, Iijima H, Ohyama Y & Nagai R (2001). Homeobox protein Hex induces SMemb/nonmuscle myosin heavy chain-B gene expression through the cAMP-responsive element. *Circ Res* **88**, 52–58.

Shaywitz AJ & Greenberg ME (1999). CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu Rev Biochem* **68**, 821–861.

Somlyo AP & Somlyo AV (1994). Smooth muscle: excitation-contraction coupling, contractile regulation, and the cross-bridge cycle. *Alcohol Clin Exp Res* **18**, 138–143.

Stevenson AS, Cartin L, Wellman TL, Dick MH, Nelson MT & Lounsbury KM (2001*a*). Membrane depolarization mediates phosphorylation and nuclear translocation of CREB in vascular smooth muscle cells. *Exp Cell Res* **263**, 118–130.

Stevenson AS, Gomez MF, Hill-Eubanks DC & Nelson MT (2001*b*). NFAT4 movement in native smooth muscle. A role for differential Ca^{2+} signaling. *J Biol Chem* **276**, 15018–15024.

Tokunou T, Shibata R, Kai H, Ichiki T, Morisaki T, Fukuyama K, Ono H, Iino N, Masuda S, Shimokawa H, Egashira K, Imaizumi T & Takeshita A (2003). Apoptosis induced by inhibition of cyclic AMP response element-binding protein in vascular smooth muscle cells. *Circulation* **108**, 1246–1252.

© 2006 The Authors. Journal compilation © 2006 The Physiological Society

Wada H, Hasegawa K, Morimoto T, Kakita T, Yanazume T, Abe M & Sasayama S (2002). Calcineurin-GATA-6 pathway is involved in smooth muscle-specific transcription. *J Cell Biol* **156**, 983–991.

Wamhoff BR, Bowles DK, McDonald OG, Sinha S, Somlyo AP, Somlyo AV & Owens GK (2004). L-type voltage-gated Ca^{2+} channels modulate expression of smooth muscle differentiation marker genes via a rho kinase/myocardin/SRF-dependent mechanism. *Circ Res* **95**, 406–414.

Wang Z, Wang D-Z, Hockemeyer D, McAnally J, Nordheim A & Olson EN (2004). Myocardin and ternary complex factors compete for SRF to control smooth muscle gene expression. *Nature* **428**, 185–189.

Wellman GC, Cartin L, Eckman DM, Stevenson AS, Saundry CM, Lederer WJ & Nelson MT (2001). Membrane depolarization, elevated Ca^{2+} entry, and gene expression in cerebral arteries of hypertensive rats. *Am J Physiol Heart Circ Physiol* **281**, H2559–H2567.

Yellaturu CR, Ghosh SK, Rao RK, Jennings LK, Hassid A & Rao GN (2002). A potential role for nuclear factor of activated T-cells in receptor tyrosine kinase and G-protein-coupled receptor agonist-induced cell proliferation. *Biochem J* **368**, 183–190.

Zhang S, Remillard CV, Fantozzi I & Yuan JX-J (2004). ATP-induced mitogenesis is mediated by cyclic AMP response element-binding protein-enhanced TRPC4 expression and activity in human pulmonary artery smooth muscle cells. *Am J Physiol Cell Physiol* **287**, C1192–C1201.

© 2006 The Authors. Journal compilation © 2006 The Physiological Society