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Review

Actin-targeting natural compounds as tools to study the role of actin cytoskeleton in signal transduction

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ABSTRACT

Actin cytoskeleton controls a vast range of cellular processes such as motility, cytokinesis, differentiation, vesicle transport, phagocytosis, muscle contraction. A growing literature clearly demonstrated that actin cytoskeleton can play a regulating role in several signalling pathways. Cells tightly regulate actin dynamics through numerous specific proteins in order to rapidly and locally respond to various stimuli. An obvious approach to determine the involvement of actin cytoskeleton in signalling pathways is the use of actin-targeting natural compounds. These drugs modulate actin dynamics, accelerating either polymerization or depolymerization, through various mechanisms. This review focus on the use of these actin-targeting drugs as tools to demonstrate the role of actin cytoskeleton in several signal transduction pathways such as those initiated from antigen receptor in T and B cells or those involving mitogen-activated protein kinases (MAPKs) or transcription factors NF-κB and SRF (serum response factor). In this last case (SRF), the use of various actin-targeting drugs participated in the elucidation of the molecular mechanism by which actin regulates SRF-mediated transcription.

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Abbreviations: APC, antigen-presenting cells; BCR, B-cell receptor; COX-2, cyclooxygenase-2; Cyt, cytochalasin; cSMAC, central supramolecular activation cluster; ERK, extracellular-signal-regulated protein kinase; ICAM1, intercellular adhesion molecule-1; IL-1, interleukin-1; IS, immunological synapse; JNK, Jun N-terminus kinase; JP, jasplakinolide; Lat, Latrunculine; LMB, leptomycin B; LPS, lipopolysaccharides; MAPK, mitogen-activated protein kinase; MEK, MAP/ERK kinase; MHC, major histocompatibility complex; NF-AT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa B; pSMAC, peripheral supramolecular activation cluster; Shc, collagen homologous proteins; SRF, serum response factor; SwA, Swinholide A; TCR, T-cell receptor; TNFα, tumor necrosis factor α.

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1. Introduction

The cytoskeleton is a highly dynamic structure which provides mechanical support for the cell and mediates cell motility and organelles movements. It is composed of three major types of structural proteins: actin filaments also called microfilaments, intermediate filaments and microtubules. In this review, we will focus on the actin cytoskeleton.

Actin exists as a monomer called G-actin for globular-actin which is the major cellular form bound to ATP (ATP-actin) and as a linear polymer called F-actin for filamentous actin. Actin monomers bind ATP and ADP. Actin microfilaments are formed by the assembly of monomeric actin into a double-helical structure that is 8 nm in diameter. Given that the monomers are structurally polar and polymerize in a head-to-tail fashion, the filament is also polar with a barbed end (or plus end) and a pointed end (or minus end) (Fig. 1). The spontaneous polymerization of actin monomers occurs in three distinct stages: nucleation, elongation and steady state (Fig. 1) [1]. The nucleation step consists in the formation of a dimer, followed by the addition of a third subunit to yield a trimer (also called the nucleus) [1]. This step occurs very slowly because actin dimers and trimers are very unstable. Oppositely, during the elongation phase the addition of subsequent monomers is favourable and the filament can elongate rapidly, much faster at the barbed end than at the pointed end (Fig. 1) [2,3]. The actin filaments grow until the G-actin concentration reaches a critical point. At the steady state phase, there is no net filament growth. There is still a slow and constant exchange of actin subunits at filament ends with the pool of monomers (Fig. 1). However, additional levels of complexity are introduced by the enzymatic activity of the actin subunit, which hydrolyses its bound ATP to ADP and inorganic phosphate (Pi) after incorporation into a filament. ADP and Pi remain non-covalently bound to actin. The phosphate is released from the filament slowly, but the ADP does not dissociate as long as the actin subunit remains in the filament. Therefore, depending on the state of actin subunit, the elongating filaments contain three general areas: the barbed end rich in ATP-actin subunits, the center, rich in ADP-Pi-actin and the pointed end containing ADP-actin (Fig. 1). This chemical polarization of the filament explains that the critical concentration of ATP-actin at the barbed end is lower than at the pointed end. Consequently, the critical concentration for the entire filament is intermediate between the critical concentrations of the two ends. At this concentration, actin filaments reach the steady state and undergo treadmilling, in which ATP-actin monomers more rapidly associate at the barbed end and more rapidly dissociate at the pointed end without changing the length of the filament. In the cell, many actin-binding proteins regulate the dynamics of actin polymerization [4,5]. For example, profilin, an actin monomer-binding protein and the nucleating proteins Arp2/3 influence polymerization [6–9]. The capping proteins regulate the length of actin filaments and actin filament-binding proteins of the cofilin/ADF family sever and accelerate the depolymerization [1,2,10,11].

In order that actin filaments play their role in cell mechanical support and motility, they must be organized into specific complex structures. According to the crosslinking protein, actin filaments are arranged in parallel as in filopodia or organized into orthogonal, netlike meshworks as in lamellipodia [12].

As described above, actin dynamics are tightly controlled by a large number of cellular proteins allowing a quick

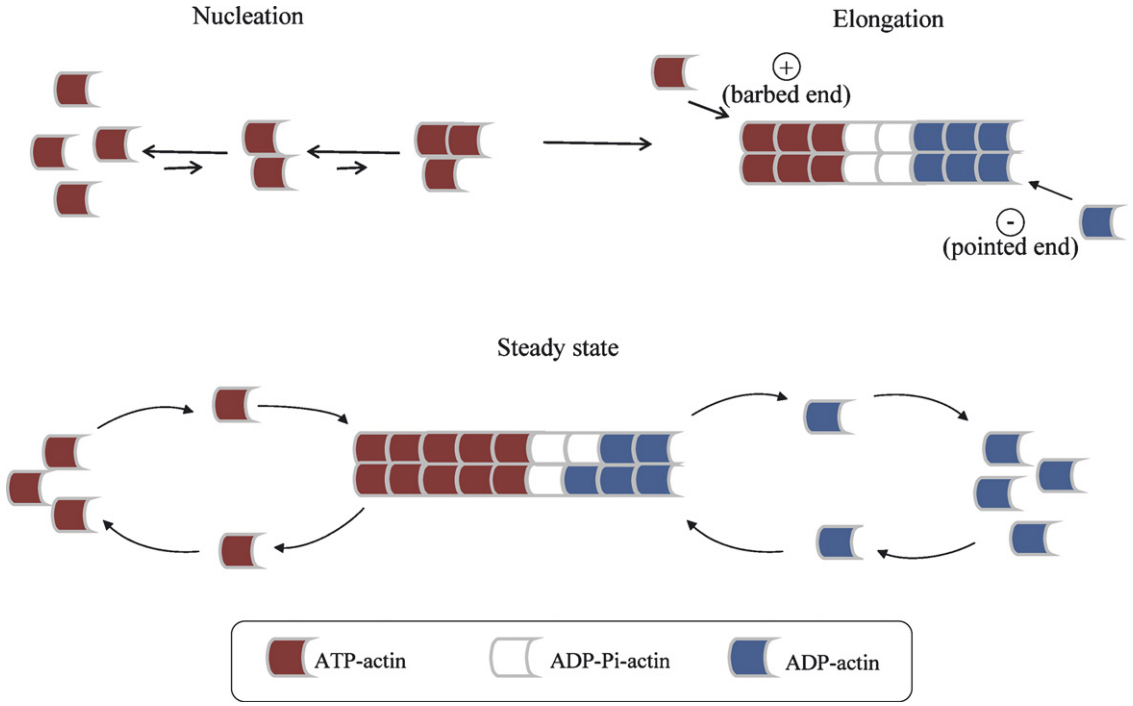


Fig. 1 – Spontaneous polymerization of actin monomers occurs in three distinct stages: nucleation, elongation and steady state.

reorganization of actin cytoskeleton in response to intracellular and extracellular signals. By this way, actin cytoskeleton plays a dominant role in many cellular functions such as cell motility [13], cytokinesis [14], phagocytosis [15,16] and muscle contraction. Beside these different roles, several other studies have suggested that actin cytoskeleton dynamics can be linked to modulations of gene expression through direct interactions with components of transduction pathways.

Two main approaches are currently used to study the involvement of actin cytoskeleton in transduction pathways. The first one consists in inducing actin cytoskeleton remodeling by modulation of the expression or the activity of endogenous actin dynamics-regulating proteins. These often targeted proteins are the small RhoGTPases (Rho, Rac and Cdc42) which mediate actin polymerization and actin cytoskeleton remodeling in response to extracellular stimuli [17,18]. The second approach uses actin-targeting natural compounds which directly interact with actin-G or actin-F and disturb actin dynamics. For several years, both methods allowed to highlight a role of actin cytoskeleton in certain transduction pathways. However, anti-actin drugs were more often used because they modulate more specifically actin dynamics than small RhoGTPases which are also involved in other signalling pathways than those leading to actin polymerization [17,19]. These compounds were used to show that the perturbation of actin dynamics modulate the activity of either specific kinases, like extracellular regulated kinase (ERK) [20], or transcription factors like nuclear factor of activated T cells (NFAT) [21], the serum response factor (SRF) [22] and the nuclear factor kappa B (NF-κB) [23].

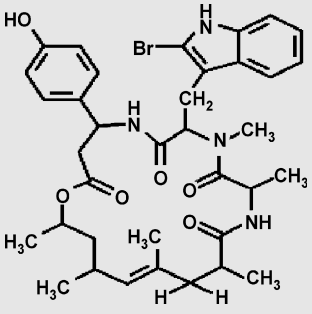
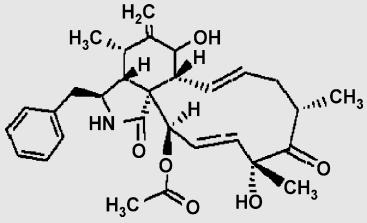
Therefore, in this review, we focused on progress in the elucidation of the role of actin cytoskeleton in different signalling pathways through the use of anti-actin drugs.

2. Actin-targeting drugs

The majority of the compounds targeting actin cytoskeleton and modulating its dynamics were isolated from terrestrial plants, fungi, bacteria, marine nudibranchs and marine-sponge-derived drugs (Table 1). These compounds are unusual macrolides that have a common structural feature involving a primarily hydrophobic core associated with stereochemically complex side groups. These natural products can be divided into two groups depending on their effect on the actin cytoskeleton: (a) those that form and stabilize actin filaments and (b) those that destabilize filaments or prevent the assembly of the microfilaments. Moreover, in each group, these compounds can be classified into several families, each one with its own biochemical and structural properties and cellular effects (Table 1).

It exists numerous filament forming/stabilizing compounds which the most used ones since few decades are the phallotoxin and jasplakinolide/jaspamide families (Table 1). The best known member of the phallotoxin family is the phalloïdin. Phalloïdin is an actin filament stabilizing compound synthesized by the poisonous ''Death cap'' mushroom Amanita phalloides [24,25]. This compound that is not able to freely enter into cells and is generally used coupled with a fluorophore to stain actin filaments. Jasplakinolide (JP) also called jaspamide is another actin filament stabilizer

Table 1 – Actin-targeting drugs families

	Major families of actin-targeting drugs	Mode(s) of action
F-actin-forming/stabilizing compounds. For example: Jasplakinolide 	Jasplakinolides (Jaspamides)	Filament stabilizers, potent inducers of actin polymerization, decrease the pool of monomeric actin
	Phallotoxins	Filament stabilizers
F-actin-destabilizing compounds. For example: Cytochalasin D 	Latrunculins	Sequester actin monomers and thereby inhibit actin polymerization
	Cytochalasins	Cap the barbed end of actin filaments, sever them, sequester actin monomers, etc
	Mycalolide B, Aplyronine A, Swinholide A	Sequester actin monomers, sequester actin dimers, sever actin filaments

isolated from the marine sponge Jaspis johnstoni [26,27], with a significantly different structure and membrane permeable unlike phalloidin (Table 1). In addition to act as filament stabilizer, JP is a potent inducer of actin polymerization [28–30].

The second group contains various filament-blocking/destabilizing compounds that block or destabilize the actin filaments by different mechanisms notably through their binding at two distinct regions of the monomeric actin: the ATP-binding cleft and the barbed end. Most of these agents sequester monomers of actin in a conformation that prevents these subunits to participate in the process of elongation. They can also promote filament severing by directly binding to the filament and disrupting interactions between adjacent monomers [31]. The most used filament-blocking/destabilizing agents for a few decades are the Latrunculins (Lat) and the Cytochalasins (Cyt). Nowadays, eight forms of Lat have been isolated from different sponges [32,33]. These compounds prevent the assembly and the polymerization of actin monomers through their binding within the ATP-binding cleft of monomeric actin with specific efficiency. Consequently, they specifically sequester monomeric actin, by mimicking the activity of monomer sequestrating proteins [34,35]. The second family is composed of Cyt. These substances are important actin-modifying agents because they improved our initial knowledge concerning actin cytoskeleton. Indeed, Cyt is one of the first available actin-modulating agents since a decade or so ago. Nowadays, it exists numerous Cyt (natural compounds and derivatives) which can be classified into three groups depending on their structure: the 10-phenylcytochalasins (like Cytochalasins A, B and D) (Table 1), the 10-indolylcytochalasins (like Chaetoglobosin A and D) and the 10-isopropylcytochalasins (like Aspochalasin B and D) [36]. The Cyt have various effects on actin cytoskeleton whose the capping of the barbed end is the most efficient. In this way, Cyt, by capping the barbed end of actin filaments, inhibits both dissociation and association of subunits at this end [37]. This blocks the polymerization and elongation of the filament at the barbed end and can lead to the depolymerization of the filaments due to the net loss of actin monomers at the pointed end. At high concentrations, Cyt can also sever actin filaments, sequester actin monomers or perhaps dimers and stimulate the ATPase activity of actin monomers [37,38]. However, in addition of binding actin, some Cyt, such as CytA and B, can modulate actin-independent cellular events such as monosaccharide transport across the plasma membrane [38,39]. Finally, another compound, the Swinholide A (SwA) specifically sequesters actin dimers and, like some of the filament-blocking/destabilizing agents, it severs actin filaments [40].

3. Implication of actin cytoskeleton in the transduction pathways involving antigen receptors in T and B cells

Several lines of evidence have suggested that cytoskeleton and more especially actin components play essential roles in the transduction pathways from antigen receptors in T and B lymphocytes. These cells are important for adaptive immunity. Indeed, the engagement of their antigen receptor triggers signalling cascades required for their activation and the generation of functional immune responses such as the production of cytokines [41–44].

Within minutes of T-cell recognition of an APC (antigen-presenting cell), signals that emanate from TCR (T-cell receptor) lead to the formation of the mature immunological synapse [45]. It is characterized by segregation of key molecules into the central (cSMAC) and peripheral (pSMACs) supramolecular activation clusters (Fig. 2A) [46]. cSMAC is composed of clustered peptide-MHC (major histocompatibility complex) complexes bound to TCR, while outside pSMAC contains integrins for adhesion. During immunological-synapse formation, an explosive F-actin polymerization occurs in the form of a lamellipodium structure which increases the interactive T-cell-APC surface area [45,47]. Once formed, the immunological synapse mediates sustained TCR signalling and stabilized adhesion, as well as exocytosis and endocytosis allowing cytokine secretion and receptor internalization. TCR microclusters are continuously formed at the periphery of the interface, even before and after immunological synapse formation. These microclusters also recruit signalling proteins and are probably the site of antigen recognition and T-cell activation. By contrast, TCR microclusters that translocate to the cSMAC lack signalling molecules.

The use of actin-targeting compounds highlighted a role of actin cytoskeleton in T-cell activation. Indeed, treatment with inhibitors of F-actin polymerization (CytD and LatA) impairs TCR-stimulated T-cell signalling [21,48,49]. Initial interpretation of these studies was that actin cytoskeleton was solely a structural component required for tight conjugation between T-cells and their cognate target cells and for the initial movement of molecules to the contact zone. Further investigation with other actin-targeting drugs demonstrated that JP, a F-actin stabilizing compound, also inhibited T-cell signalling such as tyrosine phosphorylation, while this drug did not prevent cell spreading after immobilized anti-CD3 stimulation (Fig. 2B) [50]. These data support the notion that continuous actin remodelling is required for maintaining tyrosine phosphorylation. Another study examined the effect of anti-actin drugs on TCR microclusters formation [51]. T-cells treated with the F-actin destabilizing agent LatA failed to form TCR microclusters, exhibited impaired calcium signalling and did not show any TCR microclusters movement to the cSMAC. Altogether, these results demonstrated a crucial role for the actin cytoskeleton in TCR-microcluster formation, downregulation of TCR signalling and maintenance of T-cell activation.

Unexpectidely, other data showed that disruption of actin cytoskeleton with CytD or LatB potentiated cytokine production by T-cells in response to APCs although this treatment also prevented receptor clustering [21,48]. This upregulating effect of CytD on cytokine production resulted from a decrease of surface Ca^{2+} ATPase expression leading to prolonged increase in levels of intracellular calcium and nuclear NF-AT (nuclear factor of activated T cells) (Fig. 2B) [21]. These results highlighted a novel role for the actin cytoskeleton in modulating the duration of Ca^{2+}-NFAT signalling and indicated that actin dynamics regulate T-cell activation downstream of receptor clustering.

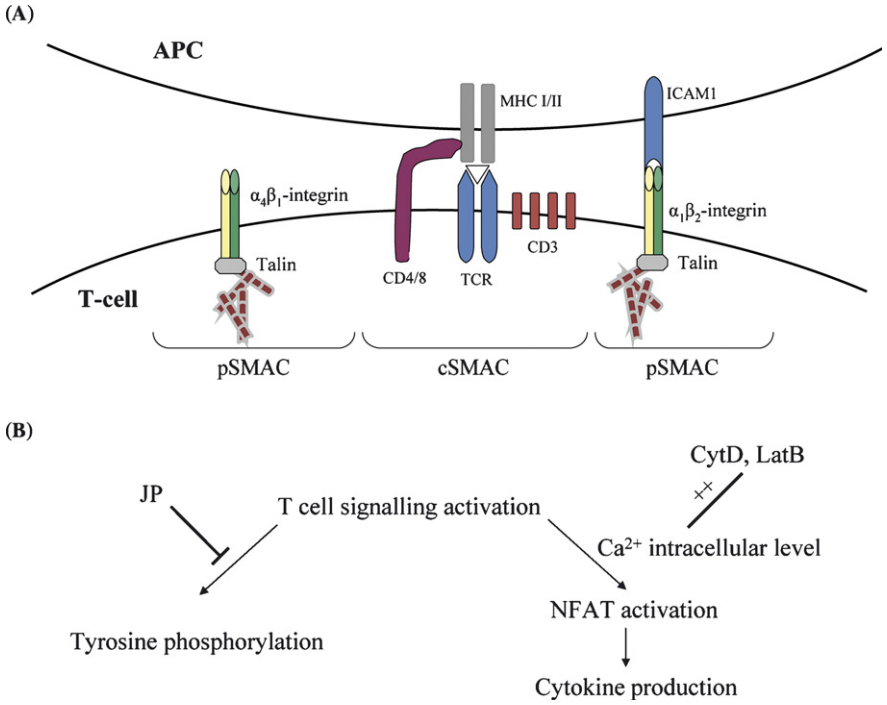


Fig. 2 – Roles of actin cytoskeleton in TCR-dependent transduction pathways. (A) The recognition of an APC signal that emanates from TCR leads to the formation of the immunological synapse. This is characterized by the segregation of key molecules into the cSMAC and the pSMAC. cSMAC is composed of clustered peptide-MHC complexes bound to TCR while pSMAC contains adhesion molecules like integrins necessary for adhesion. (B) Representation of the differential effects of actin-targeting drugs on the TCR-dependent signalling pathways.

In B cells, F-actin depolymerization induced by high concentrations of LatB or CytD potentiates BCR (B cell receptor)-mediated signalling pathways leading to transcription factor activation whereas F-actin polymerization induced by high concentrations of JP abolished the BCR activation [52]. Therefore, filamentous actin could play a negative role in regulating BCR signals. Moreover, the use of actin-targeting drugs demonstrated that the actin cytoskeleton modulations could function as a modulator of the strength of BCR stimulation. Indeed, it can modulate the BCR internalization and the duration of the BCR signals notably by regulating the movement and localization of lipid rafts that are special lipid domains on the cell membrane serving as signalling platforms [53–55]. Even if it is clear that actin cytoskeleton plays an important role in the regulation of the amplitude and duration of TCR- and BCR-mediated signalling, some groups observed that a specific actin-targeting drug could upregulate or downregulate antigen receptor-mediated signals according to the amplitude of actin dynamics modulations [21,52]. For example, it has been demonstrated that low concentrations of LatB enhance BCR signalling whereas high LatB amounts inhibit BCR-mediated transcription factor activation [52].

4. Actin cytoskeleton and the mitogen-activated protein kinase (MAPK) pathways

The members of this family of serine/threonine kinases are activated through a kinase cascade in which MAPKKKs (also known as MEKK) activate MAPKKs (also called MEK), which in turn activate the MAPKs by phosphorylating threonine and tyrosine residues in response to various extracellular signals [56,57]. These MAPKs play numerous important roles in the regulation of cell proliferation, cell growth, differentiation, migration, stress response notably through the regulation of the expression of numerous genes [58,59]. The members of this family can be classified into three subfamilies: ERK/MAPK (extracellular signal-regulated protein kinase), p38 and JNK (Jun N-terminus kinase). The ERK pathway is activated by a large variety of mitogens and by phorbol esters, whereas the JNK and p38 pathways are stimulated mainly by environmental stress and inflammatory cytokines. Several studies using actin-targeting agents clearly demonstrated that actin cytoskeleton plays important roles either in the regulation of MAPKs activation or/and in their nuclear translocation allowing the phosphorylation of target transcription factors.

The ERK/MAPK subfamily is the most extensively studied one. The best known members are the isoforms p44 (Erk-1) and p42 (Erk-2) which can be activated by various mitogens and growth factors [59]. Under resting conditions, ERK is complexed with MEK (MAP/ERK kinase) in the cytoplasm. When the MAPK pathway is activated, ERK is dually phosphorylated on threonine and tyrosine by MEK in the cytoplasm leading to its activation. It dissociates from MEK and either freely diffuses as a monomer through the nuclear pore or homodimerizes and enters in the nucleus via a Ran-dependent active transport [60–62].

Perturbation of cell morphology with cytoskeleton-targeting drugs provides one important approach to the identification of shape-responsive genes and to the elucidation of involved signalling pathways. By this way, several groups have studied the role of the actin cytoskeleton in the regulation of genes encoding proteins involved in tissue remodelling processes. In a first study using pig kidney epithelial cells (LLC-PK1 cells), it has been observed that cytoskeletal reorganizations induced by CytB and CytD provoked the expression of the uPA gene coding for urokinase plasminogen activator (Fig. 3). This extracellular protease converts plasminogen into plasmin [63,64]. Further investigations concerning this pathway have proved that this cytoskeleton-dependent gene activation involves the ERK-2 pathway [65]. In the same way, the implication of actin cytoskeleton in the regulation of PAI-1 gene which is a type-1 inhibitor of uPA has been studied by different groups (Fig. 3) [66,67]. Indeed, for example, it has demonstrated that in smooth muscle cells that actin depolymerization induced by CytD led to an enhancement of the PAI-1 expression through the ERK activation [66].

Modulations of actin cytoskeleton also regulated the BCR signalling and the subsequent activation of specific transcription factors through notably the regulation of the ERK activation [52]. Indeed, actin disruption by LatB extended the BCR-induced ERK activation whereas stabilization of F-actin by JP dramatically inhibited the BCR-mediated ERK activation (Fig. 3).

Besides its effects on the activity of ERK, actin cytoskeleton regulates the cell distribution of ERK between cytoplasm and nucleus. Indeed, actin cytoskeleton influenced the expression of ERK-dependent genes by modulating positively or negatively its nuclear translocation depending on the stimulus and the expected responses [20,68–70]. Therefore, it was demonstrated that an intact actin cytoskeleton was required in ERK-dependent hypertrophic response of cardiomyocytes after mechanical stress [68]. Indeed, stimulation of cardiomyocytes by a stretch induced the two following transduction pathways: ERK phosphorylation via an integrin-dependent pathway and actin cytoskeleton rearrangement induced by activated small RhoGTPases. When RhoA and Rac1 activation in response to stretch was prevented, actin cytoskeleton reorganization did not occur and activated ERK could not translocate to the nucleus. JP, an actin polymerizer, could restore nuclear translocation of activated ERK. To confirm the involvement of actin cytoskeleton in this phenomenon, authors demonstrated that actin depolymerization by CytD did not affect ERK phosphorylation in cardiomyocytes submitted to a mechanical stress but led to retention of activated ERK in the cytoplasm (Fig. 3). These data are consistent with another report demonstrating that CytD impaired integrin-stimulated nuclear translocation of active ERK in NIH 3T3 cells [69]. Stretch-induced ERK activation and nuclear translocation were also reported to be disrupted via destabilization of actin cytoskeleton in mesangial cells [70].

Another study also demonstrated a role of cytoskeleton in regulation of ERK nuclear entry in endoderm differentiation of embryonic carcinoma and stem cells [20]. It was shown that the impaired response to growth factors in differentiated F9 cells resulted from the cytoplasmic retention of active ERK which could no longer activate *c-fos* transcription and cell proliferation. Disruption of actin cytoskeleton by CytD abolished suppression of c-Fos expression in differentiated F9 cells meaning that an intact cytoskeleton is required to restrict ERK nuclear entry in differentiated cells (Fig. 3). Since cytoskeleton is not involved in ERK nuclear translocation in non-differentiated F9 cells and because the differentiation of

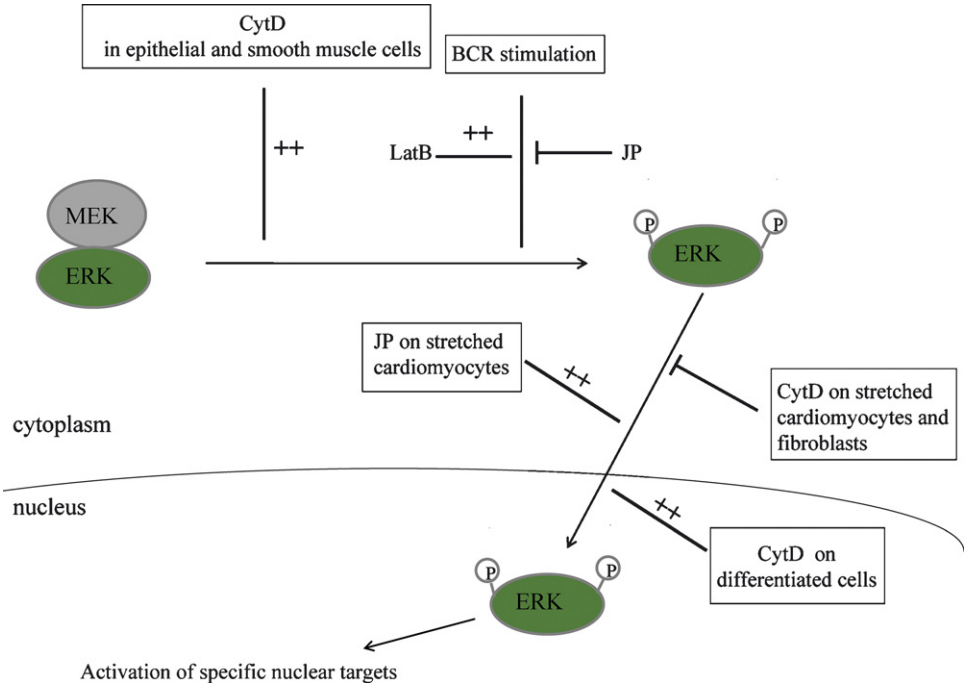


Fig. 3 – Actin cytoskeleton-mediated ERK regulation. Upon stimulation, cytoplasmic ERK is phosphorylated and released from MEK. Some ERK-dependent signalling pathways require its nuclear translocation. Actin cytoskeleton is involved in ERK-dependent transduction pathways in stimulus- and/or cell type-dependent manner.

F9 cells is characterized by the rearrangement of the cytoskeleton, the authors favour the idea that in differentiated cells, ERK could be sequesterer by cytoskeleton, restricting its nuclear translocation.

Actin-targeting compounds used in these different studies clearly showed that actin cytoskeleton can modulate the ERK-dependent responses either by influencing its phosphorylation or by promoting or restricting its nuclear translocation according to the cell context.

The p38 subfamily contains four isoforms whose the p38α is the best well characterized [59,71]. p38 is expressed in many cell types and its activity is stimulated by many growth factors, cytokines and chemotactic substances. This subfamily is obviously more involved in inflammation, differentiation and apoptosis [71]. The activity of this MAPK can also be regulated by actin cytoskeleton, either positively or negatively, according to the cellular context. Indeed, in articular chondrocytes, actin cytoskeleton disruption by CytD was reported to inhibit NO-induced dedifferentiation, apoptosis, cyclo-oxygenase-2 (COX-2) expression partially via inhibition of p38 activation [72]. Another group demonstrated that actin cytoskeleton alterations induced by CytD in intestinal epithelial cell lines induced an enhancement of the p38 activity leading to an increased IL-8 (interleukin-8) mRNA stability [73]. Several reports support the notion that p38 activation during adverse environmental conditions that destabilizes actin dynamics is involved in homeostasis. Indeed, the disruption of actin filaments by CytD in differentiated skeletal muscle cells led to an increase in p38 association with actin and to a higher p38 activity [74]. Interestingly, pretreatment with anisomycin, a potent activator of p38, protects actin filaments from depolymerization, suggesting a role of p38 in actin filament reorganization. An increase in JNK activity after CytD treatment was also observed in differentiated skeletal muscle cells but JNK did not seem to play a role in actin remodeling. Therefore, it is possible that, by disrupting actin, CytD generates a stress that stimulates p38 to save the actin filaments and to protect cells from the multifaceted effects of stress. This hypothesis is supported by a previous report demonstrating that actin dynamics can be regulated by p38-mediated phosphorylation of hsp27 (heat shock protein) [75].

In another context, it has been demonstrated that an intact actin cytoskeleton is required for the correct propagation of insulin-induced signalling [76]. The signalling pathways initiated by insulin are complex and involve a cascade of adaptor molecules such as Src and Shc (collagen homologous proteins) proteins, as well as protein- and lipid-regulated kinases and phosphatases that mediate the nuclear effects of the hormone such as DNA synthesis and expression of immediate early genes (c-fos and c-jun) [77–79]. Using actin-disrupting compounds such as CytD and LatB, a specific inhibition of the insulin-induced MAPK (ERK and p38) pathway leading to c-fos expression in muscle cells was observed. However, other growth factors such as PDGF (platelet-derived growth factor) can induce ERK activation under conditions where a complete actin disassembly is detected. This demonstrates again the dependence regarding cell type and stimulus in MAPK activation by actin cytoskeleton remodeling [80].

5. Relationship between the actin cytoskeleton and the NF-κB pathway

The transcription factor NF-κB regulates the expression of an exceptionally large array of genes, particularly those involved in immune and inflammatory responses, the control of apoptosis and cell proliferation [for review, 81]. NF-κB binds specific DNA sequences as dimers of the Rel/NF-κB family [reviewed in 82,83]. NF-κB complexes are sequestered in the cytoplasm of most resting cells by inhibitory proteins belonging to the IκB family [84]. Upon stimulation with numerous proinflammatory agents such as cytokines (tumor necrosis factor α (TNFα), interleukin-1 (IL-1)) or bacterial and viral products, IκBα is phosphorylated, ubiquitinated, then degraded by the 26S proteasome, allowing NF-κB to translocate to the nucleus and transactivate its target genes [reviewed in 82,83]. The canonical pathway induced by proinflammatory stimuli involves a specific IκB kinase (IKK) which phosphorylates IκBα on serines 32 and 36. IKK is a complex composed of two catalytic subunits, IKKα and IKKβ, and a regulatory subunit, IKKγ/NEMO [83,85]. Activation of the IKK complex by all proinflammatory stimuli depends on phosphorylation of either the IKKβ or IKKα catalytic subunits at two conserved serines [83,86].

Since more than 10 years, microtubules-depolymerizing agents are known to activate the transcription factor NF-κB in many different cell types [87–90]. The first line of evidence suggesting a link between actin cytoskeleton and NF-κB comes from a more recent work [91]. In this study, immunofluorescence assays revealed that the p65/RelA subunit of NF-κB interacted with F-actin-containing structures (focal contact, stress fiber or lamellipodia) in rat normal or transformed fibroblasts. Disassembly of actin filaments caused by CytD was accompanied by redistribution of RelA into actin aggregates. Finally, RelA bound directly or indirectly to immobilized filamentous actin in vitro. However, in this study, the functional importance of the interaction between NF-κB and actin cytoskeleton was not investigated.The first data establishing a role of the actin network in controlling NF-kappaB activation came from the work of Nemeth and colleagues [73]. In this study, the treatment of human intestinal epithelial cells with CytD or LatB was shown to cause an increase of the NF-κB DNA binding and transcriptional activity (Fig. 4). This NF-κB activation by CytD was secondary to an effect on the inhibitory protein IκB because its degradation and the NF-κB-mediated transcription were prevented by a dominant negative IκB mutant. Exposure of the cells to CytD or LatB also increased gene expression and the release of IL-8 in cell supernatant.

Similarly, our group studied the effect of actin dynamics perturbations on NF-κB activation in several cell types [23]. F-actin-depolymerizing agents (CytD and LatB) as well as F-actin-stabilizing compounds such as JP were shown to induce NF-κB activation specifically in myelomonocytic cell lines and in human monocytes (Fig. 4). The transduction pathway involved the IKK complex and a degradation of IκBα. Since both F-actin-depolymerizing and -stabilizing agents led to NF-κB activation, this suggested that there was no correlation between a specific state of actin polymerization and NF-κB activation. The signal seems to be a perturbation of actin dynamics sensed by myelomonocytic cells and converted into

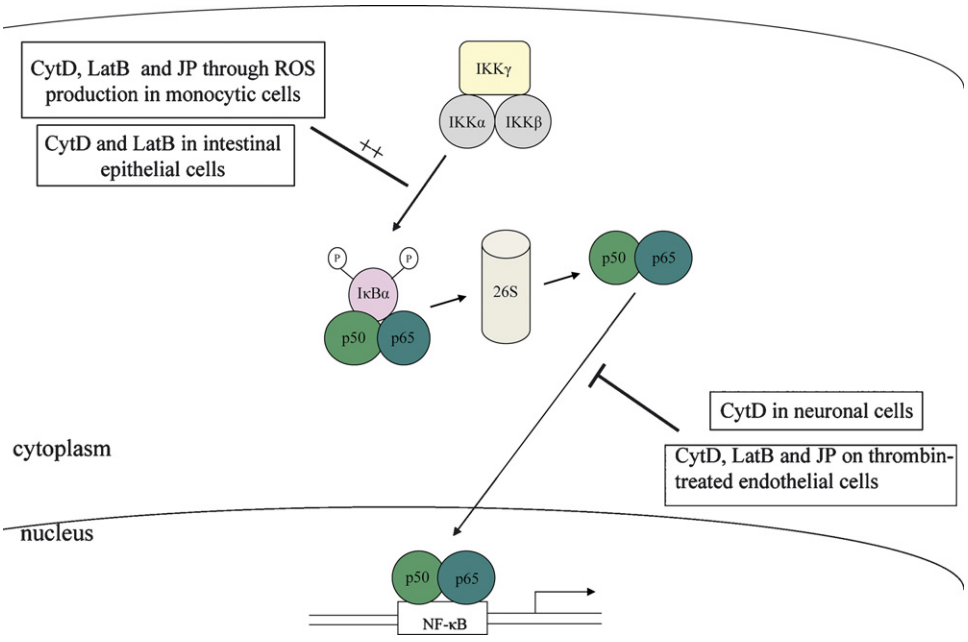


Fig. 4 – Actin cytoskeleton-mediated NF-κB regulation. Anti-actin drugs induce differential effects on the NF-κB pathway. Depending on the stimulus and/or the cell type, the modulations of NF-κB activation are either on IκBα phosphorylation or NF-κB transport from cytoplasm to nucleus.

NF-κB activation. Further investigations demonstrated that the CytD-induced NF-κB activation pathway in myelomonocytic cells required ROS (reactive oxygen species) as second messengers. These ROS are released from CytD-treated human monocytes, through the activation of the NADPH oxidase. Therefore, NF-κB activation observed in myelomonocytic cells after actin dynamics perturbations could be physiologically relevant during monocyte activation and/or recruitment into injured tissues, where cellular attachment, migration and phagocytosis result in cyclic shifts in cytoskeletal organization and disorganization [23].

The connective tissue growth factor, CCN2, an inducible and profibrotic molecule, is overexpressed in mechanical overload-bearing tissues, which probably initiates the fibrotic reactions observed in mechanical overload-associated pathologies. The study of underlying molecular mechanism allowed highlighting a new actin dynamics-dependent NF-κB activation pathway [92]. In cyclically stretched bladder smooth muscle cells (SMCs) in vitro, the activity of CCN2 promoter constructs was increased through an NF-κB binding element. Mechanical constrain induced nuclear translocation of NF-κB and its binding to the CCN2 promoter within intact chromatin. Simultaneously, mechanical forces induced actin polymerization either in vitro in stretched cells or in vivo in mechanically overload bladder tissue. The treatment with LatB inhibited mechanical stretch-induced CCN2 promoter activity. Collectively, these data indicated that mechanical stretch-dependent effects on actin dynamics regulate NF-κB activation in smooth muscle cells.

On the other hand, other studies have demonstrated the involvement of the actin cytoskeleton in the NF-κB transport from the cytoplasm to the nucleus. Indeed, while NF-κB movement through the cytoplasm to the nucleus in TNFα-stimulated non-neuronal cells is not affected by actin-depolymerizing drugs [93], the constitutive p65 nuclear translocation is significantly downregulated in neuronal cells by CytD treatment (Fig. 4) [94]. Further investigations demonstrated that NF-κB transport is mediated by motor proteins moving along the cytoskeleton in neuronal cells where the distances between sites of NF-κB activation and nucleus can be very large [94].

More recently, one group addressed the role of actin cytoskeleton in the mechanism of thrombin-induced NF-κB activation in endothelial cells (HUVECs) [95]. They observed by confocal microscopy that thrombin induced the formation of stress fibers in these cells. Interfering with actin dynamic either by destabilizing or stabilizing actin filaments by CytD and LatB or JP respectively, prevented the NF-κB nuclear translocation and NF-κB-mediated transcription while these actin-targeting drugs did not perturbate the degradation of IκBα in response to thrombin treatment (Fig. 4). Importantly, thrombin induced association of RelA/p65 with actin and this interaction was disrupted by stabilization or destabilization of the actin filaments. The inhibitory effect of these actin-targeting drugs was specifically observed on thrombin-induced NF-κB activation while no effect was detected on the TNFα response in HUVECs cells. Actin cytoskeleton could play important role notably during inflammatory responses associated with intravascular coagulation in thrombin-induced endothelial cells and the use of actin-modulators could be a strategy to prevent these cellular processes.

Altogether, these studies revealed the existence of actin cytoskeleton-dependent and -independent NF-κB activation pathways that may be engaged in a stimulus- and cell type-specific manner.

6. Involvement of the actin cytoskeleton in the regulation of SRF

The serum response factor (SRF) is a MADS-box transcription factor that is conserved in most eukaryotic cells. It controls growth factor-inducible genes like the transcription factor *c-fos*, components of actin cytoskeleton and numerous genes specifically expressed in skeletal, smooth and cardiac muscles through the mutually exclusive association of different cofactors on specific promoter sites [96,97]. The SRF target genes have a single copy or multicopies of the SRF binding consensus element (CArG box) which is common to all SRF target genes [98]. Additionally to this CArG box, target genes can have an adjacent cis element, termed ETS site that binds the Elk family of SRF cofactors [99]. Nowadays, two main families of SRF cofactors can specifically modulate the activity of SRF. The first family consists of the MRTFs (myocardin-related transcription factors) that are essentially controlled by the Rho GTPases and by the concentration of monomeric actin [100,22]. The members of this family are notably: the myocardin, MAL (also called BSAC, MRTF-A or MLK1) and MRTF-B [101]. The second family is activated by the MAPK (mitogen-activated protein kinase) pathways. This second family is the TCF (ternary complex factor) family of Ets domain proteins that includes Elk-1, SAP-1 and Net (also called SAP-2) [102].

The first data establishing a link between SRF regulation and actin dynamics were published by Sotiropoulos and colleagues in 1999 [103]. At this time, RhoA was already known to be involved in serum-induced SRF activation in fibroblasts. Since RhoA can promote F-actin stabilization and de novo F-actin assembly, the effect of several actin dynamics-modulating drugs on SRF activation were studied. LatB, which sequesters G-actin monomers and inhibits actin polymerization, completely blocked serum-induced SRF activation while JP, which binds and stabilizes F-actin, strongly activated transcription of the SRF reporter gene. To address whether SRF sensed increased F-actin level itself, an increased F-/G-actin ratio or a decreased G-actin level, the effect of two other drugs, swinholide A and CytochalasinD were investigated. Swinholide A (SwA) sequesters G-actin as dimers and CytD caps actin filaments and stimulates ATP hydrolysis on G-actin. Interestingly, both of these compounds strongly activated SRF reporter genes. Since both agents did not induce actin polymerization, authors proposed that it was depletion of the G-actin pool that induced SRF activation. Such a model predicts that a coactivator of SRF could be sequestered in the cytoplasm by direct physical interaction with G-actin. A decrease in G-actin levels in response to actin de novo polymerization (serum, JP) or the treatment with CytD or SwA could induce the release of this coactivator and its nuclear translocation leading to SRF-mediated transcription.

Indeed, as reported in a more recent article, such a coactivator has been identified [22,104,105]. This protein, called MAL, is encoded by a ubiquitously expressed gene implicated in human leukemia. Miralles and colleagues observed that MAL is predominantly localized to the cytoplasm in serum-starved fibroblasts but is rapidly redistributed to the nucleus upon serum stimulation. By using a panel of actin dynamics-modulating drugs, CytD and swinholide A as well as JP were shown to efficiently induce MAL nuclear translocation, which suggests that MAL nuclear accumulation corroborated with SRF activation (Fig. 5A). Consistent with the predicted G-actin sensing ability of SRF, MAL binds to unpolymerized actin. Inhibiting MAL function through the use of dominant interfering forms of MAL blocks SRF activation. Chromatin immunoprecipitation experiments revealed that MAL is associated with endogenous promoters of genes known to be sensitive to Rho signalling. Accordingly, Miralles and colleagues proposed the following model (Fig. 5A). MAL may be prevented from entering the nucleus due to a steric occlusion of its nuclear import domain. Upon Rho activation, the consequent accumulation of F-actin leads to a depletion of G-actin and the release of MAL, which then translocates to the nucleus and associates with SRF to activate a subset of SRE-containing genes.

A muscle-specific actin-binding protein named striated muscle activator of Rho signalling (STARS) was shown to induce the nuclear translocation of MAL and SRF activation through a Rho-dependent mechanism providing a potential muscle-specific mechanism for linking changes in actin dynamics and sarcomere structure with striated muscle gene expression [106].

More recently, the mechanism by which actin regulates MAL activity and SRF-mediated transcription was re-examined [107]. It appeared that MAL shuttled between cytoplasm and nucleus in unstimulated cells since leptomycin B (LMB) induced nuclear accumulation of MAL. They showed that the increase in the nuclear import was not the major mechanism of MAL relocalization after serum-stimulation. Indeed, MAL nuclear export was dramatically reduced after serum stimulation and almost completely inhibited by drugs that induce MAL nuclear accumulation and SRF activation including CytD, SwA and JP (Fig. 5B). They next studied the interaction between recombinant MAL and purified actin. MAL mutant forms exhibiting a greatly reduced affinity for actin were nuclear in unstimulated cells. The export rate of these mutants was low and identical to that of the wild-type protein in the presence of drugs that disrupt actin-MAL interaction (CytD, SwA). These data suggested that interaction with actin was required for Crm1-dependent MAL nuclear export. Although LMB treatment induced MAL nuclear accumulation, this treatment did not activate transcription of the MAL-dependent SRF target genes in the absence of serum or CytD stimulation, suggesting that disruption of actin-MAL interaction was required for nuclear MAL to activate SRF (Fig. 5B). Finally, using FRET (fluorescence resonance energy transfer) it was shown that actin interacted with MAL in both nucleus and cytoplasm. Serum-induced signals and actin-binding drugs (CytD, SwA) decreased this interaction in a way consistent with the functional data. Accordingly, the following model was proposed (Fig. 5B). In unstimulated cells, high export rates ensure a cytoplasmic localization of MAL whereas nuclear actin prevents SRF activation. Upon stimulation by growth factors, actin polymerization induces a depletion of nuclear and cytoplasmic G-actin pools and a decrease of actin-dependent MAL export. Nuclear MAL accumulation and diminished interaction with actin allows SRF activation.

Another report demonstrated that actin dynamics can directly modulate the intracellular distribution of SRF. Indeed,

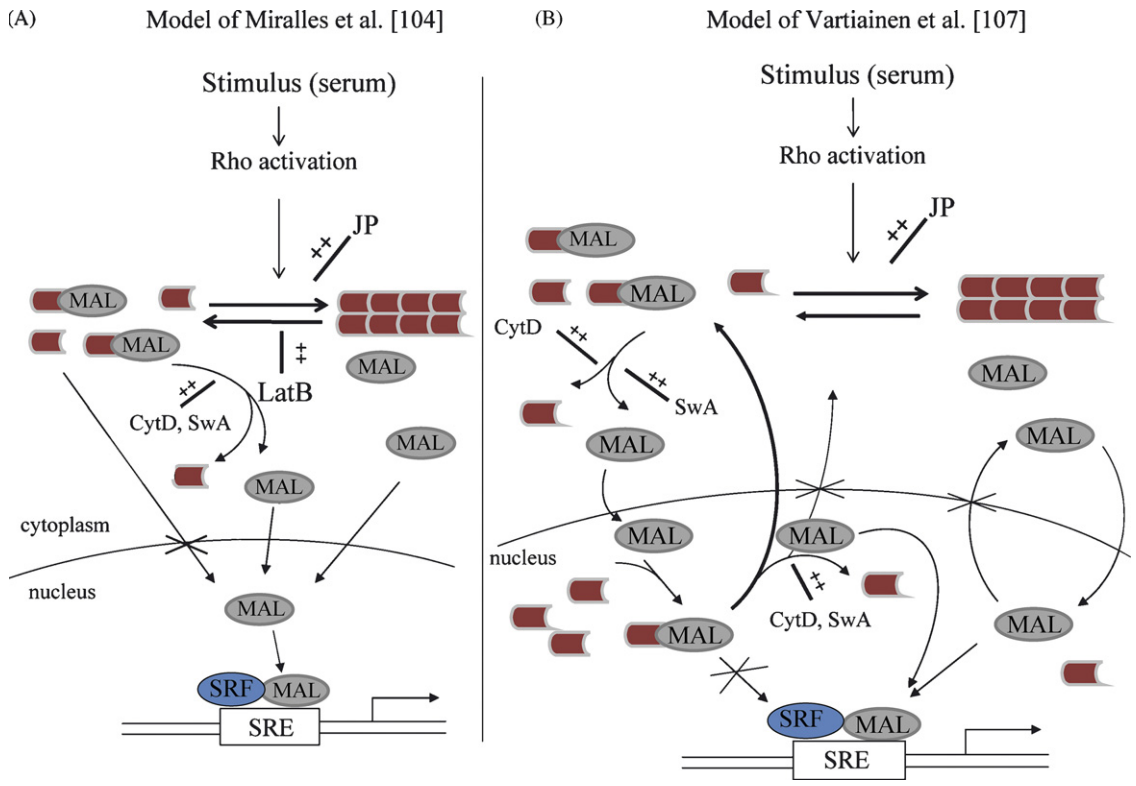


Fig. 5 – Models of actin-dependent SRF activation. (A) In the model proposed by Miralles and co-workers [104], the cofactor MAL is sequestered by monomeric actin in cytoplasm of unstimulated cells. When the Rho GTPase pathway is activated, the pool of monomeric actin decreases and consequently, MAL is released and then can translocate into nucleus where it promotes the activation of SRF target genes. In the presence of LatB, G-actin pools increase and MAL is not released whereas JP, by inducing a decrease of G-actin pools, allows MAL translocation into the nucleus. (B) In the model proposed by the group of Vartiainen [107], actin regulates MAL activity at three levels: nuclear import, nuclear export and activation of target gene transcription. In unstimulated cells, high export rates ensure MAL cytoplasmic localization, whereas nuclear actin prevents SRF activation. Upon stimulation by growth factors, actin polymerization induces a depletion of nuclear and cytoplasmic G-actin pools and a decrease of actin-dependent MAL export. Nuclear MAL accumulation and diminished interaction with actin allows SRF activation. JP, by inducing actin polymerization, and CytD and SwA, by disrupting interaction between G-actin and MAL, lead to MAL-dependent SRF activation.

the inhibition of RhoA signalling or treatment with LatB selectively reduced nuclear SRF protein content and partially redistributed SRF from nucleus to cytoplasm in smooth muscle cells, as revealed by quantitative immunocytochemistry [108]. Together, these results demonstrated for the first time that the RhoA pathway controls smooth muscle gene transcription in part by regulating the subcellular localization of SRF. It is conceivable that the RhoA/Rho kinase pathway influences SRF localization through its effect on actin polymerization dynamics.

7. Conclusions

Altogether, these data clearly demonstrated that actin-targeting natural compounds can be used as tools to study the role of actin cytoskeleton in signal transduction. These actin modulating drugs demonstrated the involvement of actin cytoskeleton in various signalling pathways such as those triggered by TCR and BCR and those leading to MAPK, NF-κB and SRF activation. In the case of SRF, a vast panel of drugs modulating actin dynamics through different mechanisms have been used, allowing elucidation of the molecular mechanisms by which actin cytoskeleton tightly regulates this signalling pathway.

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