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Model for regulation of non-muscle myosins

Разнообразные морфологические изменения эукариотической немышечной клетки обычно сопровождаются динамичной перестройкой в её цитоскелете. Предполагается, что конформационно-функциональное состояние молекул миозина может изменяться в значительной степени во время реорганизации цитоскелета. Хотя молекулы миозина играют немаловажную роль в различных формах клеточного движения, в т. ч. изменении конфигурации клетки, молекулярная основа для регуляции их активности сложна и малопонятна. Многочисленные экспериментальные данные показывают, что фосфорилируемая легкая цепь (20 кДа), известная как регуляторная легкая цепь (РЛЦ), является одним из ключевых модуляторов регуляторных сигналов в структуре молекулы миозина-II. Однако, "ключ", который бы координировал её состояние фосфорилирования, остаётся неразгаданным. В данной работе предлагается наиболее рациональный механизм, который мог бы регулировать состояние фосфорилирования РЛЦ и, тем самым , мог бы контролировать организацию и активность немышечных миозинов.

Introduction

The present day eukaryotic cell is a complex biological system possessing the highly dynamic structure — cytoskeleton with a complex mechanism of geometric control. The cytoskeleton reorganizes continuously as the cell changes shape, divides, and responds to its environment. All cellular motile events require a force-generating reaction and a regulatory mechanism. Virtually all eukariotic cells contain myosin molecules known as a key force-generating elements in the structure of cytoskeletal actomyosin. All the myosins consist of both heavy and light chains, and as a molecular motors, cause unidirectional movement of actin filaments using the chemical energy obtained from hydrolysis of ATP. The common feature of all myosins is a conserved motor domain (motor head). The other domains vary from myosin to myosin, and this determines the specific role of the molecule in the cell.

The myosin family of the present day vertebrate non-muscle cell is represented by a several types of myosin molecules. Among them, the muscle type myosin-II (two-headed), known as convential, is abundant. Each type of myosin has a distinct functional designation, and its regulation is thought to require a unique pathway and sequence of regulatory events. At the same time, each type of myosin can be represented by a variety of isoforms and they can differ in the intracellular distribution and dynamics. One of the most remarkable properties of non-muscle myosins, especially of myosin-II, is their ability to change sub-cellular localization during various cell motile events. Although the mechanisms that regulate these molecules remain to be clarified, virtually all non-muscle myosins are controlled by phosphorylation, and reversible and

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differential phosphorylation of the 20 kDa RLC on certain sites appears to be a pivotal in regulating their enzymatic/motor activity [4, 5, 6, 9, 13, 14, 23, 33, 34, 39].

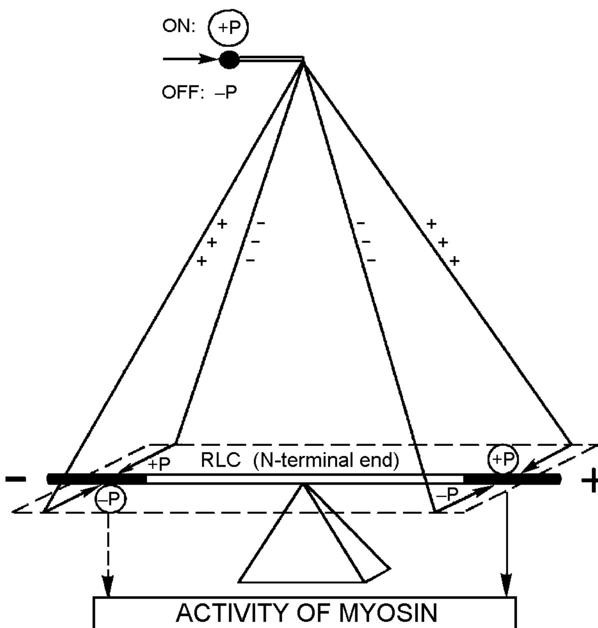
Normally, phosphorylation occurs on conserved amino acid residues near the NH_2 terminus of RLC by analogy with smooth muscle myosin (myosin-II type). There are the sites phosphorylatable by myosin light chain kinase (MLCK) – serine 19 [29] and threonine 18 [17] (MLCK sites), and the sites phosphorylatable by protein kinase C (PKC) – serine 1, serine 2, and threonine 9 [3, 18] (PKC sites), also known as the activation and the inhibitory sites, respectively. MLCK by phosphorylating its specific sites on the RLC stimulates the actin-activated MgATPase of myosin, the assembly of myosin filaments under physiological conditions, and the ability of myosin to generate force [1, 9, 14, 16, 23, 32, 37]. Although the RLC has been found to incorporate phosphate on threonine 18 at approximately 1000-fold less rate than on serine 19, the threonine residue phosphorylation markedly increases the actin-activated MgATPase activity of myosin [16, 17]. In contrast, phosphorylation by PKC inhibits the actomyosin ATPase, and two different mechanisms are known to contribute to this process [18, 19, 28]. First, when myosin is not phosphorylated by MLCK, phosphorylation at the PKC sites reduces the rate of subsequent MLCK phosphorylation, and, as a result, myosin is less likely to be activated. Second, when myosin is phosphorylated by MLCK, subsequent phosphorylation at the PKC sites is able to force myosin to reduce the affinity for actin filaments. Therefore, actin-activated myosin ATPase activity is inhibited. Moreover, the sites that have been prephosphorylated by MLCK can inhibit subsequent PKC phosphorylation [28], and thereby, these two kinases are likely to modulate one another.

In addition to the mentioned above kinases, there are other candidates that are capable of regulating the myosin activity via RLC phosphorylation. Among them, such protein kinases as Rho-kinase , mitogen activated protein kinase-activated protein kinase (MAPKAPK), p21-activated kinase, and cdc2 kinase have been broadly characterized. Whereas MAPKAPK-2/4 and p21-activated kinase that phosphorylate at one of two MLCK sites (exclusively at serine 19), and Rho-kinase that can phosphorylate at both MLCK sites, stimulate MgATPase activity of myosin [2, 7, 24, 25, 36], cdc2 kinase by phosphorylating at the PKC sites seems to inhibit this activity [31, 40]. Recently, we demonstrated that cdc2 kinase from sea urchin which shows approximately 72% amino acid sequence identity to mammalian cdc2 kinase can also phosphorylate the myosin RLC at the MLCK sites in addition to the PKC sites [26], suggesting the possibility of myosin activation by this enzyme. Interestingly, an apoptotic signaling kinase, ZIP kinase [20], has been recently identified as a first non-muscle MLCK that phosphorylates the myosin RLC at both MLCK sites [27]. Thus, the continuously changing phosphorylation state of RLC is likely to constitute a basis for modulating the activity of the whole myosin pool *in vivo*, and this can play a certain physiological roles. For example, the switching of sites of myosin phosphorylation between phosphorylations at the inhibitory and activation sites during mitosis [31, 40] seems to be principal in regulating the timing of cytokinesis. In this respect, it has been reasonably proposed that

RLC phosphorylation, and thus, myosin activity, involves a dynamic balance between a number of different kinases and phosphatases [38]. Among a range of various phosphatases known to date, only one enzyme has been found may act to dephosphorylate myosin (myosin phosphatase) preferentially at the activation sites of RLC [11, 15]. However, the presence of phosphatases capable of dephosphorylating the inhibitory sites is evident. Myosin phosphatase has been demonstrated to be activated by mitosis-specific phosphorylation of its targeting subunit (MYPT) [35]. Interestingly, activated Rho-kinase can positively regulate the myosin activity by direct phosphorylation of RLC as well as by inactivation of myosin phosphatase via phosphorylation of MYPT [22].

Model

Normally, in the cell, if phosphorylation occurs, dephosphorylation is an inevitable process, and these two processes are thought to be coordinated through the common mechanism developed by evolution. Theoretically, this might be a switch-like mechanism, for example, the phosphorylation-dependent molecular switch resulting in activation of certain kinase and inhibition of corresponding phosphatase, and *vice versa.* This mechanism seems to be most rationale for “equilibrating” the function of phosphorylating-dephosphorylating system, and we, therefore, propose that the phosphorylation state of RLC might be regulated by the analogous mechanism. As shown in Figure, the only one switch can coordinate phosphorylations between the activation and inhibitory sites on the RLC. According to the proposed model, a kinase that phosphorylates the activation site(s) acts synergistically with a phosphatase that dephosphorylates the inhibitory site(s). At the same time, a kinase that phosphorylates the inhibitory site(s) jointly acts with a phosphatase that dephosphorylates the



Schematic representation of the model for regulation on non-muscle myosin activity. The N-terminal end of RLC containing activation (+) and inhibitory (-) phosphorylation sites is demonstrated as a “balancing bar” which continuously oscillates under the influence of activation and inhibitory signals (marked by three small pluses and minuses, respectively) with a subsequent effect on myosin activity. Dashed line indicates the mechanisms that can be involved in modulating the state of RLC phosphorylation at the level of RLC molecule. Further explanations are in the text.

activation site(s). Once the switching of phosphorylations between these two antagonistic groups of sites has occurred, it is extremely unlikely that the state of RLC phosphorylation would be instantly changed, and therefore, some transition period seems to be exist. During this time, phosphorylations that might occur at both groups of sites on the RLC are thought to modulate one another as it has been previously demonstrated in the *in vitro* experiments [18, 19, 28], and this might have a physiological significance. The role of this mechanism is possibly in the adapting of switched upstream signals until the new state of RLC phosphorylation to be stabilized. Thus, the model proposed here is believed to be one of the integral parts in the regulatory system of non-muscle myosins that represents the module coordinating and adapting signals necessary for the smooth and uninterrupted regulation of myosin activity.

However, the RLC which represents a unique "equilibrating basis" for modulation of myosin activity seems to be easily disbalanced by foreign signal, and this inevitably can disturb the function of whole regulation system. In light of our hypothesis, the competitive phosphorylation of myosin RLC by other protein kinases can be a serious destabilizing factor [12]. For example, protein kinases that share homology in the catalytic domain to that of MLCK can be potential competitive kinases. These are basically kinases representing the death associated protein (DAP) kinase family that can be involved in apoptotic signaling [8, 10, 20, 21, 30]. All of them are capable of phosphorylating the exogenous light chain of myosin [8, 21, 27, 30], and it is believed that through phosphorylations of the endogenous myosin RLC they can transduct death signals leading to the profound and irreversible cytoskeletal alterations.

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ABSTRACT

Diverse morphological changes of non-muscle eukaryotic cell are usually accompanied by dynamic remodeling in its cytoskeleton. The conformation/functional state of myosins is presumed to be markedly altered during cytoskeletal reorganization. Although myosins play critical roles in various forms of cellular movement and shape changes, the molecular basis for regulation of their activity is complicated and far from understood. A numerous experimental data show the phosphorylatable light chain (20 kDa), known as a regulatory light chain (RLC), as one of the key modulators of regulatory signals in the structure of myosin-II molecule. However, the clue that coordinates its phosphorylation state remains to be enigmatic. In the present work, we propose the most rationale mechanism which might regulate the state of RLC phosphorylation, and therefore, might control organization and activity of non-muscle myosins.

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