Distinct roles of Rho-kinase pathway and myosin light chain kinase pathway in phosphorylation of myosin light chain: kinetic simulation study.

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Cytoskeleton plays a crucial role in regulation of cell various cellular processes such as cell movement, cell morphology and cell contraction (1-4). The contractile force necessary for these processes is provided by actin-myosin interaction (1-4). In non-muscle cells including smooth muscle cells, phosphorylation of myosin light chain (MLC) has been shown to be a key reaction for regulation of actin-myosin interaction (5). The MLC phosphorylation has been shown to be regulated by an increase of intracellular Ca^{2+} followed by activation of Ca^{2+}/calmodlulin-dependent MLC kinase (MLCK) (5-8). Recent progress also revealed that Rho-kinase, one of the effecters for Rho small GTPase, regulates the MLC phosphorylation (9-11). Rho-kinase phosphorylates MLC (10, 12). Rho-kinase also phosphorylates myosin-binding subunit (MBS), a regulatory subunit of myosin phosphatase, and consequently inhibits the myosin phosphatase activity, resulting in elevation of phosphorylation level of MLC (9, 12). Thus, the MLC phosphorylation is dually regulated by two linear pathways such as MLCK and Rho-kinase pathways.

However, it still remains unclear whether agonist-dependent MLC phosphorylation can be reproduced by both the MLCK and Rho-kinase pathways. To address this issue, it is important to utilize the computational framework of kinetic simulation. We here built the computational simulation model of the MLC phosphorylation (Fig. 1) based on the kinetics parameters available in the literature or determined

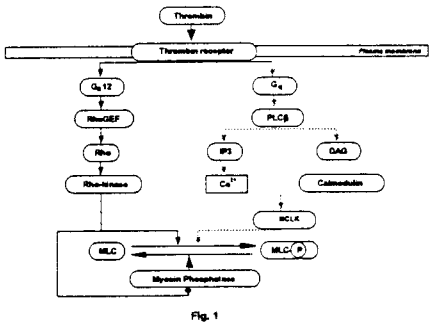

Fig. 1

by the experiments by taking advantage of the recently developed program, GENESIS/kinetikit (13). In this study, the MLC phosphorylation was simulated based on the following two biochemical reactions; the protein-protein (molecule-molecule) interactions and enzymatic reactions.

The reaction of protein-protein interactions is given by the following formulation.

$$[A]+[B]\underset{Kb}{\overset{Kf}{\rightleftarrows}}[AB]$$

Enzymatic reactions involve the phosphorylation and dephosphorylation. This reaction is given by the following formulation of Michaelis-Menten.

$$[E]+[S]\underset{K2}{\overset{K1}{\rightleftarrows}}[ES]\xrightarrow{K3}[E]+[P]$$

where E, S and P denote enzyme, substrate and product, respectively.

Phosphorylation of MLC induced by thrombin has been experimentally shown to consist of two phases: the initial and the prolonged phases (14). The simulation reproduced the initial phase of the phosphorylation of MLC, whereas the simulation could not reproduce the prolonged phase (Fig. 2). The simulation reproduced the time course of the activation of two linear pathways such as the MLCK and Rho-kinase pathways, suggesting that the reason why the simulation failed to reproduce the prolonged phase is due to an existence of an unidentified pathway.

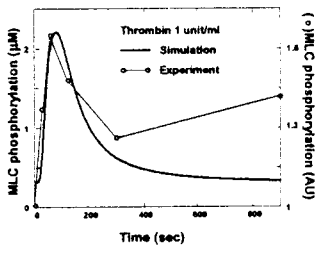

Fig. 2

The MLC phosphorylation is dually regulated by the MLCK and

Rho-kinase pathways, however, it is intuitively difficult to understand how much their connections to MLC contribute to the whole process of the MLC phosphorylation (Fig. 3). To access the role of each connection, we took advantage of the kinetic simulation by deleting each connection (Fig. 3A). We found that the direct MLC phosphorylation is mainly regulated by MLCK, but not by Rho-kinase, and that the inhibition of myosin phosphatase by the MBS phosphorylation by Rho-kinase is essential for the sufficient MLC phosphorylation (Fig. 3B). Thus, the simulation is also useful to predict the role of molecule in a diverse pathway.

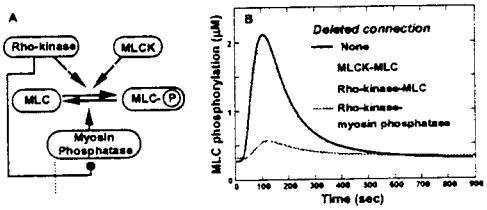

Fig. 3

In addition, we attempted a re-interpretation of the effect of dominant active form of Rho (DA Rho), thought to be constitutively active in the cells, in the MLC phosphorylation. MLCK activation is essential for the sufficient MLC phosphorylation in the kinetic simulation. On the other hand, the introduction of DA Rho results in the sufficient MLC phosphorylation (9, 15-17), although no evidence has been reported that the introduction of DA Rho leads to the elevation of Ca^{2+}, necessary for the activation of MLCK. If Ca^{2+} concentration is not affected by Rho, then how can Rho induces the MLC phosphorylation without MLCK activation? To address this issue, we

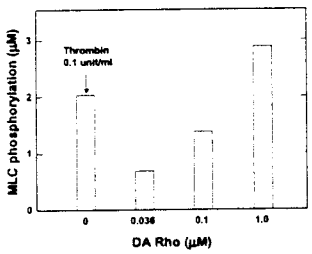

Fig. 4

examined the effect of DA Rho on the MLC phosphorylation without Ca^{2+} elevation by holding the concentration of DA Rho in the kinetic simulation (Fig. 4). The introduction of DA Rho induced the MLC phosphorylation in a dose-dependent manner (Fig. 4). When the concentration of DA Rho was the same as that induced by the stimulation of thrombin (0.1 unit/ml), MLC was not sufficiently phosphorylated. However, when the concentration of DA Rho was increased to 1 μM, MLC was phosphorylated to the similar extent to that induced by thrombin. When the concentration of DA Rho was held at 0.036 μM, about 10% of Rho-kinase was activated, whereas the concentration of the activated Rho was held at 1 μM, about 90% of Rho-kinase was activated (data not shown). Thus, it is likely that the overexpression of DA Rho induces the excess activation of Rho-kinase which is unlikely to occur under the physiological conditions.

Thus, the kinetics simulation is a novel tool to access the roles of signaling molecules and to predict a missing pathway(s) necessary to reproduce the whole process of phosphorylation of MLC.

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