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Review

Rho GTPases: Masters of T lymphocyte migration and activation

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ABSTRACT

Rho GTPases are key signal transducer elements activated in T cells by both chemokine and antigen receptors. These two signalling pathways control the two main functions of T lymphocytes: motility and activation. Rho GTPases are thus crucial for the development of an adequate immune response. In this review, we mostly focus on the roles of RhoA, Rac1 and Cdc42 in T cells. We show their importance in phenomena such as adhesion, morphological polarization, migration and antigen recognition.

1. Introduction

1.1. T lymphocyte biology

Efficient immune surveillance requires that naive T lymphocytes circulate permanently between the blood stream, secondary lymphoid organs (SLOs) and lymphatic vessels. It has been estimated that, at a given time, the pool of T cells in the blood represents only 5% of the total T cell count, 70% of T cells being localized in lymph nodes and approximately 20% in the spleen [1]. In normal conditions, a T cell usually stays less than 30 min in the blood circulation, making repeated visits of several hours in SLOs. T lymphocytes migrate into lymph nodes by crossing the vascular endothelium at the level of specialized post-capillary vessels, termed High Endothelial Venules (HEV). Once inside the SLOs, lymphocytes scan the area in search of antigen-presenting cells (APCs). This trafficking is fundamental to increase the probability for a T cell to encounter the antigen, and thereby guarantees the immunosurveillance of the body.

If a cognate APC is encountered, a stable contact named the immunological synapse forms between the two cells and a complex signalling cascade is triggered. Eventually, lymphocytes get activated and undergo a swift proliferating period during which they differentiate into effector immune cells. The variations in structure, duration and composition of the immunological synapse crucially modulate the outcome of T cell activation and the functional features of effector cells, leading either to activation or tolerance. The fully activated lymphocytes then join back into the blood stream and the inflamed area, where, once again, they transmigrate into the targeted organ or tissue and initiate the clearance of the pathogens. The spatio-temporal regulation of T lymphocyte localization and status of activation require a faultless coordination of numerous signalling pathways. One major signalling pathway is dependent on Rho GTPases.

1.2. Rho GTPases

Rho GTPases belong to the Ras superfamily from which they differ due to the insertion of a so-called Rho insert domain. Rho GTPases are very conserved proteins present in all eukaryotic cells. They thus take part in a very large array of biological functions.

Rho GTPases cycle between an inactive GDP-bound and an active GTP-bound form. Upon GTP binding, Rho GTPases undergo a change of conformation that gives them a selective ability to bind effector proteins. This, in turn, induces a conformational change of the effectors, allowing them to achieve specific biochemical functions. Hence, Rho GTPases act in a one-to-one fashion, so they differ from

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other molecular regulators such as kinases which can rapidly phosphorylate a considerable amount of substrates.

The mammalian Rho GTPases include 23 homologous proteins that can be clustered by phylogenetic analysis into 7 subfamilies (reviewed in [2,3]). Up to now, the very large majority of studies concern the three archetypal Rho GTPases RhoA, Rac1 and Cdc42. They have therefore always been taken as a model of the biological functions of Rho GTPases, and it is easy to forget the remaining members of the family. However, it ought to be noted that some of them, namely the Rnd and RHOBTB subfamilies, RhoU, RhoV and RhoH, differ from the canonical model of the Rho GTPases (reviewed in [2,4]). Indeed, these are mainly bound to GTP and therefore are constitutively active. Their activity is regulated by other means, such as phosphorylation or localization.

Aside from the atypical Rho GTPases, a major lever of regulation is obviously the control of the GDP/GTP balance. Rho GTPases have a slow intrinsic GTP hydrolysis rate. The GAPs (GTPase Activation Proteins) accelerate this rate of hydrolysis and therefore terminate Rho GTPases signalling. They constitute a well-conserved family, with a shared GAP sequence. Conversely, the GEFs (Guanosine Exchange Factors) act as activators of Rho GTPases by promoting the replacement of GDP by GTP. The majority of GEFs contain a DH sequence, and a smaller alternative subfamily possesses instead a DHR2 sequence that allows GEFs to perform the GDP to GTP exchange reaction. One striking feature of this GAP/GEF regulatory couple is the number of identified proteins. 79 GEFs and 65 GAPs have been reported to be transcribed [3]. Compared to the 23 Rho GTPases, it implies that a given Rho GTPase can be regulated by several GAPs and GEFs. Moreover, some GEFs can activate several Rho GTPases (for instance, the GEF Vav1 can activate RhoA as well as Rac1, depending on the context). It is thus likely that the state of activity of one Rho GTPase results from the balance between the different GAPs and GEFs available nearby, as well as from the competitive presence of other Rho GTPases.

The regulation of Rho GTPases is made more complex by the existence of another regulatory family, the GDIs (Guanosine Dissociation Inhibitors). At least three have been described: the ubiquitous GDIα, the hematopoietic GDIβ and GDIγ. They were initially shown to inhibit the activation of Rho GTPases by blocking the release of GDP, a necessary step prior to the GTP loading. However, another inhibitory role of GDIs proved to be prominent: GDIs seem to sequester the Rho GTPases away from the membranes, where a large fraction of GEFs and effector proteins are thought to be localized (reviewed in [5]).

This brings about another layer of complexity in the regulation of Rho GTPases. Although a lot of grey areas remain, it is generally admitted that efficient Rho GTPase signalling requires an association with the membranes as mentioned above. As an example, it has been biochemically shown in several cellular models that active Rho GTPases associate with the lipophilic fraction upon stimulation (in neutrophils [6], Swiss 3T3 [7], cardiac myocytes [8], smooth muscle cells [9], endothelial cells [10] and hairy cells lymphoma [11]). A microscopy-based observation also supports those results [12]. However, the precise mechanism and dynamics of these recruitments still remain somewhat unclear. Precise membrane localization of Rho GTPases is not only required for their efficient activation, but also to spatially organize the activation of effectors, for example during the polarization of migrating leukocytes (see below). The subcellular localization of Rho GTPases is influenced by several factors. Rho GTPases contain a CAAX sequence at their C-terminal end. This CAAX sequence is post-translationally processed by cleavage of the AAX amino acids, and methylation of the now terminal Cysteine. Eventually, the Cysteine is prenylated by a geranylgeranyl or a farnesyl lipidic compound (reviewed in [2]). This prenylation is important for increasing the association of Rho GTPases with membranes [13]. GDIs mediate the sequestration in the cytosol by burying the prenyl moiety within a hydrophobic pocket and thus preventing it from interacting with membranes. Some Rho GTPases, such as RhoB, possess an upstream additional palmitoylation which prevents the GDI from binding [14]. Thus, palmitoylated proteins would be insensitive to GDI-mediated cytosolic sequestration, as opposed to palmitoyl-free proteins, like RhoA.

The subcellular localization is further regulated by other domains. Several Rho GTPases have a polybasic region upstream of the C-terminal Cysteine. This domain, depending on its composition, appears to favour a localization to the plasma membrane (reviewed in [15]). However, the combination of the CAAX sequence, the palmitoylation site and the diverse polybasic domains might yield even more complex patterns of localization. Thus, RhoB, containing a CXXC palmitoylation site, is targeted to the plasma membrane and the Golgi apparatus in MDCK cells, whereas TC10, containing a polybasic domain in addition to its palmitoylation site, is localized at the plasma membrane and endosomes [14]. As a conclusion, the precise localization of Rho GTPases is crucial for the proper achievement of the biological processes they govern and is tightly regulated by multiple intrinsic and extrinsic factors.

Finally, several proteins have been reported to regulate the state of activity of Rho GTPases whilst lacking any sign of identity with GAPs, GEFs or GDIs. Memo seems to favour the activation of RhoA by targeting it into a membrane-associated complex formed with Memo, RhoA and its effector mDia1, which ultimately results in alterations of microtubule dynamics in SKBR3 cells [16]. Another protein, F11L is expressed by the vaccinia virus and inhibits RhoA activity [17]. Its mechanism of action is not very well known. However, it has been shown that F11L decreases the amount of GTP-bound RhoA. F11L similarly binds the GDP- and GTP-bound form of RhoA. It also shares some key amino acids with the RhoA effector ROCK and competes with it for binding to RhoA. It is therefore likely that F11L is a competitive inhibitor of RhoA, and that the observed decrease in RhoA-GTP upon F11L expression is due to the disruption of a ROCK-mediated positive feedback loop. Possibly, several atypical regulators of Rho GTPases are yet to be discovered, and would improve our understanding of Rho GTPases mechanisms of control.

Most of what we know about Rho GTPases signalling comes from a set of "classic" Rho GTPases experimental tools. These include the use of dominant-negative (DN) or constitutively active (CA) mutants, which allowed functional identification of the cellular processes in which they are involved. However, one should keep in mind that DN mutants act by sequestering GEFs that are specific for a particular Rho DN form. Because several GEF can usually bind the same Rho GTPase DN mutant, it means that the effect observed with DN mutants results from the concomitant paralysis of several GEF proteins. Moreover, as one particular GEF can very often interact with several Rho GTPases members, all the Rho DN mutants of these members will be able to interfere with the function of the same GEF. Therefore, the conclusions drawn from studies using DN mutants must be interpreted cautiously. For example, Rac1 had been described as being necessary for macrophage migration using a DN mutant [18]. When this question was investigated again using Rac1-deficient macrophages, the same authors failed to find a role for this Rho GTPase in macrophage migration [19], although the possibility that such Rac1-deficient cells have adapted and switched to another mode of migration which would be Rac1-independent, cannot be entirely ruled out. It is thus likely that with the generation of specific inhibitors and the generalization of knock-down and knock-out strategies in Rho studies, information gained from experiments using DN mutants of Rho GTPases might lead to different interpretations.

CA mutants are usually expressed at high levels in cells so that some discrete changes in the subcellular localization of active Rho