Rac1 and Rac3 in cell morphology and adhesion: brothers and foes
Hajdo-Milašinović, A.

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The true sign of intelligence is not knowledge but imagination.

Albert Einstein
Preface

Background
In order to mechanistically and functionally take part in the complex structures of tissues and organs, mammalian cells require a dynamic cytoskeleton and the capacity to adhere. The ability to rapidly change morphology and sophisticated adhesions, either to the neighboring cells (cell-cell adhesion) or to the surface (cell-matrix adhesion), are prerogative for both static and migratory aspects of the cell function. Concomitantly, failure of cytoskeletal rearrangements and dysfunctional cell adhesions lead often to various pathological processes, and are on many levels involved in tumor development and metastasis.

Considering their complexity and importance, it is not surprising that these aspects of cell biology are strictly regulated in time and space by a multitude of signaling cascades. Substantial contribution to this regulation is provided by small Rho-like GTPases (extensively described in chapter 1). These proteins belong to the superfamily of small GTPases, sometimes called the ‘Ras superfamily’ with reference to their founding member, that are divided into five major subfamilies according to their sequence and function: Ras, Rho, Rab, Ran and Arf. In addition to the Ras proteins, the Ras subfamily includes Rap, Ral, and Rheb GTPases, whereas the Rho subfamily is further divided into three subgroups: Rho, Rac and Cdc42.

Small GTPases are nucleotide binding molecules that switch between an active and inactive state by binding either GTP or GDP, respectively. In an active, GTP-bound state, Rho-like proteins interact with a variety of downstream effectors, regulating in this way not only cytoskeletal dynamics and cell adhesion, but also cell polarity, cell migration, cell division, apoptosis, gene transcription and many other cellular processes.

The regulation of nucleotide binding is a crucial element of small GTPase function and is brought about by three different sets of proteins. Guanine nucleotide exchange factors (GEFs) activate small GTPases by catalyzing GTP exchange, and are antagonized by GTPase activating proteins (GAPs), which accelerate GTP hydrolysis
and guanosine nucleotide dissociation inhibitors (GDIs), which maintain GTPase in GDP-bound (inactive) state.

Small GTPases share a significant degree of structural homology, especially within the subfamilies, yet they are functionally very different, interacting with specific pools of downstream effectors and affecting different signaling pathways. Therefore it is highly important to understand the issue of their specificity: which parameters enable these highly homologous small proteins to efficiently affect so many different processes in the cell?

To begin with, membrane association is crucial for the function of small GTPases. These proteins are subjected to a post-translational modification, where an isoprenoid lipid moiety is added to their carboxy terminus, which subsequently facilitates insertion of the GTPase into the membrane. Additional value of this lipid tail is the fact that it contributes to some extent to the specificity: while Ras subfamily proteins are farnesilated or palmitoylated, Rho and Rab GTPases are predominantly geranylgeranylated (with minor exceptions).

Secondly, the GEFs and GAPs that regulate GTPases activity are fairly specific and often capable of binding and positioning downstream effectors in the close proximity of the GTPase, being therefore an important tool in determining the outcome of GTPase activity. However, they cannot be accounted for all aspects of specificity, since often highly similar GTPases are capable of in vitro binding to the same GEFs and same downstream effectors, yet their expression in the cell affects for example the cell morphology in a very different way.

The third and so far final feature that contributes to the specificity of the GTPase function is their localization in the cell. The majority of Rho GTPases localizes in their active state to the plasma membrane, most likely at the specialized lipid or protein-defined signaling platforms like calveolae and rafts, but also to specific structures like focal adhesions. Ras GTPases are targeted to endosomes, Golgi, the plasma membrane and mitochondria, Ral and Rab GTPases to specific vesicle compartments, whereas Arf GTPases localize to either plasma membrane or specific
vesicle compartments. Recent findings revealed that not only the lipid moiety and anchoring in specific membranes, but foremost protein-protein interactions are of importance for the correct subcellular localization of small GTPases. There are some indications that a hypervariable sequence, which is in RhoGTPases situated just upstream of the CAAX box at the C terminus, is a vital element that ensures targeting to the proper compartment.

The issue of specificity is particularly intriguing in the case of the Rac subfamily of RhoGTPases. Next to the well-studied, ubiquitously expressed Rac1, this subfamily harbors the hematopoietic-cell specific Rac2 and the recently found and poorly characterized Rac3, whose transcript is enriched in brain. Their overall homology is more then 90%, with effector loop identity of 100%. Rac proteins show different pattern of expression, both during the development as in adult organism. However often they are also simultaneously expressed. A few studies showed that in hematopoietic cells, Rac1 and Rac2 have partially redundant but also different effects on the cell morphology and behavior. As for Rac3, the scarce investigations often utilize over-expression of constitutively active mutants that are often non-specific in binding (the) downstream effectors. Thus far, the specific contribution of individual Rac proteins in Rac-mediated cell signaling has been poorly understood, and the mechanisms that lay behind the proposed differences remain largely illusive.

Scope of the thesis
In order to gain insight to the issue of small GTPases specificity, we explored the contribution of particular Rac GTPases to cytoskeletal rearrangements and cell adhesions in cultured epithelial and neuronal cells. We have addressed the issues of subcellular localization, structural differences that contribute to this specificity and explored the signaling pathways that underlay them. Our findings are presented in this thesis as follows:

Chapter 1 offers an elaborate overview on Rho GTPases classification, function and regulation with respect to cell motility, cell-matrix and cell-cell adhesion and tumorigenesis.
Chapter 2 shows that small GTPase Rap, a bona fide regulator of integrin-based cell-matrix adhesions, also plays an important stimulatory role in cadherin-based cell-cell adhesions in epithelial cells. Expression of active Rap1A restored epithelial morphology in Ras-transformed epithelial cells, whereas inhibition of Rap signaling led to disruption of cadherin-based cell-cell contacts. This study suggests a broader role for this small GTPase to regulate the function of cell-surface adhesion receptors.

Chapter 3 presents a study on specific functions of two highly homologous RhoGTPases, Rac1 and Rac3. By utilizing short interference RNA technology, Rac1 and Rac3 specific contribution to the cell morphology and adhesion are dissected, with a surprising and intriguing outcome. Namely, we show here that Rac3 induces cell rounding and diminishes cell-matrix adhesions, fully opposing thereby the well-described stimulatory role of Rac1 in cell spreading and adhesion. This functional difference, as well as different subcellular localization of the two proteins, is dependent on the hypervariable domain just upstream to the carboxy terminus.

Chapter 4 impinges further on the mechanisms that underlay the remarkable functional difference between Rac1 and Rac3. It shows that both proteins interact with a downstream effector Git1, however, with an important difference that Rac3-Git1 interaction does not involve βPix, a GEF molecule that plays a positive role in cell adhesion stimulation through the regulation of paxillin distribution. As a consequence, Rac3-Git1 interaction is accompanied by loss of Git1-paxillin interaction, defective paxillin distribution and focal adhesion formation. Furthermore, Rac3-Git1 interaction stimulates Git1 GAP activity towards Arf6, a small GTPase involved in endosomal membrane trafficking.

Chapter 5 provides an overview of the results described in this thesis, followed up by discussion on different aspects of these findings, the correlation between them and possible future directions.