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Antagonism of Rho and Rac signaling in the regulation of the actin cytoskeleton during cell migration
Antagonismus signalizace Rho a Rac v regulaci aktinového cytoskeletu během buněčné migrace

Bachelor´s thesis

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Prohlášení:

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 05.08.2024

Podpis

Abstrakt

Tato bakalářská práce se zabývá antagonismem signalizace Rho a Rac v regulaci aktinového cytoskeletu během buněčné migrace. Rho a Rac patří mezi malé GTPázy, což jsou signální proteiny, které se zabývají regulací a řízením mnoha důležitých procesů v buňce. Pro tuto práci je zejména důležitá jejich komplexní signální síť, která ovlivňuje dynamické změny v aktinovém cytoskeletu, jež jsou důležité během buněčné migrace. Signalizace Rho a Rac má nejen odlišné účinky na aktinový cytoskelet, ale také je často spojena s tím, že aktivace jednoho typu signalizace inhibuje druhý typ. Dále tato práce shrnuje, jak antagonistické interakce mezi Rho a Rac signalizací formují buněčné reakce na enviromentální podměty. Tento antagonistický mechanismu zajišťuje přesnou prostorovou a časovou koordinaci pohybu buněk, což je klíčové pro procesy, jako je hojení ran a imunitní odpověď. Pochopení mechanismu antagonismu těchto dvou proteinů je důležité pro identifikaci potenciální terapeutických cílů v rámci těchto drah, které by umožnily kontrolu abnormální migrace buněk pozorované u nemocí, jako je rakovina.

Klíčová slova: Rho, Rac, GAP, GEF, GDI, F-aktin, myosin

Abstract

This bachelor thesis addresses antagonism of Rho and Rac signaling in the regulation of the actin cytoskeleton during cell migration. Rho and Rac are small GTPases, which are signaling proteins that regulate and control many important processes in the cell. For this thesis, their complex signaling network is particularly important as it influences dynamic changes in the actin cytoskeleton, which are crucial during cell migration. Rho and Rac signaling not only have distinct effects on the actin cytoskeleton but are also often associated with the phenomenon where the activation of one type of signaling inhibits the other type. Furthermore, this thesis demonstrates how antagonistic interactions between Rho and Rac signaling shape cellular responses to environmental stimuli. This antagonistic mechanism ensures precise spatial and temporal coordination of cell movement, which is key for processes such as wound healing and immune response. Understanding the antagonism mechanism of these two proteins is important from identifying potential therapeutic target within these pathways to control abnormal cell migration observed in diseases such as cancer.

Key words: Rho, Rac, GAP, GEF, GDI, F-actin, myosin

Content

List of abbreviations

ABPs – actin-binding proteins

ADP – adenosine diphosphate

ADP-Pi – adenosine diphosphate and inorganic phosphate

Arp2/3 – actin related protein 2/3 complex

ATP – adenosine triphosphate

CAAX – C is cysteine, AA are aliphatic acids and X is any amino acid

Cdc42 – cell division cycle 42

DBL – diffuse B-cell lymphoma

DH – Dbl homology domain

DHR – Dock homology region

DNA – deoxyribonucleic acid

Dock – dedicator of cytokinesis

ECM – extracellular matrix

ERM – ezrin, radixin and moesin proteins

F-actin – filamentous actin

FAKs – focal adhesion kinases

G-actin – globular actin

GAP – GTPase-activating protein

GDI – guanine nucleotide dissociation inhibitor

GDP – guanosine diphosphate

GEF – guanine exchange factor

GPCRs – G protein coupled receptors

GTP – guanosine triphosphate

JNK – c-Jun- N-terminal kinase

kDa – kilodaltons

LIMK – LIM kinase

LPA – lysophosphatidic acid

MAPKs – mitogen-activated protein kinases

mDia – mammalian homolog of *Drosophila* Diaphanous

Mg^{2+} - magnesium divalent cation

MRCK - myotonic dystrophy kinase related Cdc42-binding kinase

MYPT - myosin light chain phosphatase

NF-kB - nuclear factor kappa-light-chain-enhancer of activated B cells

PAKs – p21-activated kinases

PDGF – platelet-derived growth factor

PH – Pleckstrin homology domain

PI3K – phosphoinositide 3-kinase

PKA – AMP-dependent protein kinase A

PTEN – phosphatase and tensin homolog

Rac – Ras-related C3 botulinum toxin substrate

Ras – Rat sarcoma

Rho – Ras homologous

ROCKs – Rho-associated protein kinases

Ser – serine

Thr – threonine

WASP – Wiskott-Aldrich syndrome protein family

WAVEs – WASP family Verprolin homologous proteins

WHD/SHD – WAVE homology/Scar homology

1 Introduction

1.1 Cell migration

Cell migration is vital biological process that is a part in various of physiological activities such as wound healing and immune cell response. The process of cell migration of polarized adherent cells includes several phases: extension of the protrusion at the front/leading edge; creating new focal adhesion complexes; focalized proteolysis; contraction of cell body by actomyosin complexes; realising of tail (Figure 1). These phases are tightly interlinked. Also, cell migration involves many specific actin filament-based structures, such as filopodia, invadopodia, lamellipodia, and blebs. Furthermore, it is associated with cell-extracellular matrix adhesions and cell-cell adhesion. Historically, PI3 kinase was considered as main intrinsic factor that promotes cell migration in response to external stimuli. However, members of Rho GTPases family represent main executive component of intracellular signaling in cell migration, by regulating the assembly and organizing the actin cytoskeleton (Guan et al., 2020).

4.Cell contraction by actomyosin: active myosin II binds to actin filaments (then termed actomyosin) and generates actomyosin contraction. Rho and ROCK involved.

1.Protrusion of the leading edge: actin polymerization at the leading edge of cell. Rac, Cdc42 and Arp2/3 involved.

3.Focalized proteolysis: cells move through matrix by using MMPs to degrade ECM.

Trailing edge

Leading edge

5.Detachment of the trailing edge: contractile force generated by actomyosin structures enables translational of movement.

Direction of cell movment

2.Cell-matrix interaction and formation of focal contacts: integrins come into contact with ECM ligands and cluster in the cell membrane. Rac involved.

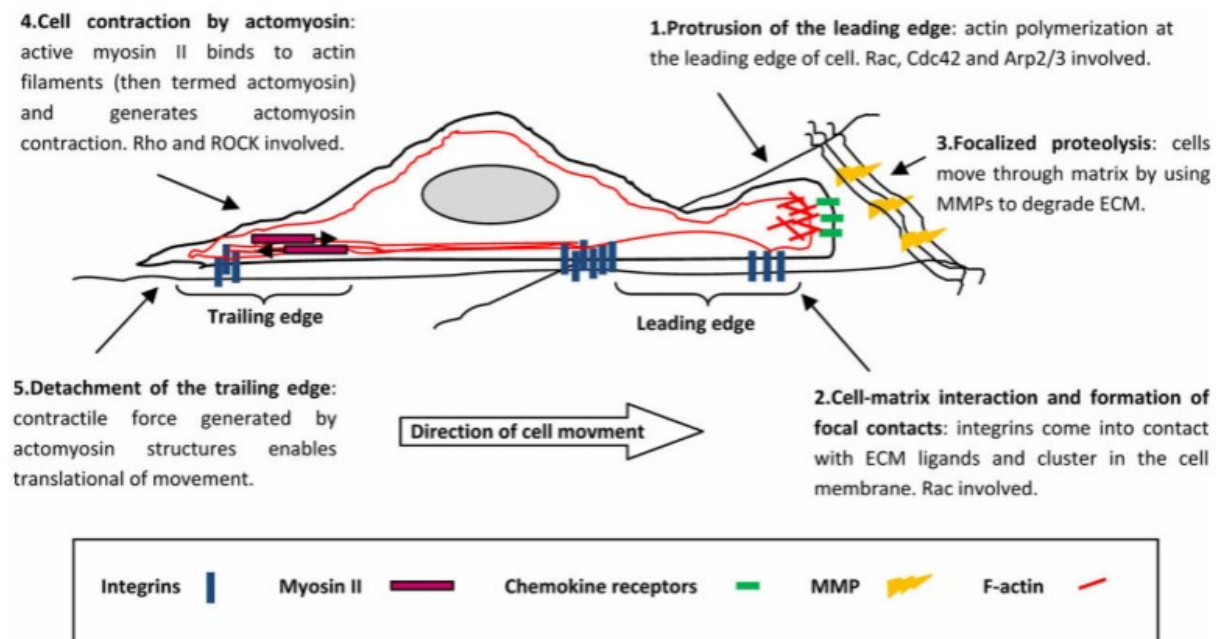| Integrins | Myosin II | Chemokine receptors | MMP | F-actin |

Figure 1 Phases of cell migration: 1. Protrusion of the leading edge, 2. Cell-matrix interaction and formation of focal contacts, 3. Focalized proteolysis (critical for movement in 3-D, mostly absent in 2-D), 4. Cell contraction by actomyosin and 5. Detachment of the trailing edge. Taken from: (Parri and Chiarugi, 2010).

1.2 Actin cytoskeleton

The eukaryotic cell cytoskeleton consists of three main dynamic polymeric structures that differ in diameter of their constituent filaments. These structures are: microtubules, intermediate

filaments and microfilaments (Vázquez-Victorio et al., 2016). Microfilaments are polymers of actin and form actin cytoskeleton. In migrating cells actin cytoskeleton forms cell protrusions, which are extension of the extracellular plasma membrane. There are 4 types of cell protrusions: invadopodia, lamellipodia, filopodia and blebs. These protrusions are very important during cell migration (Guan et al., 2020).

Actin cytoskeleton is crucial component of cellular architecture. It is very important in many cellular processes and aspects of cell behaviour, that provides structural support, enables migration of cell and takes part in cytokinesis, signaling, polarity and transport (Ridley, 1995). Actin cytoskeleton is formed by actin filaments, which are polar linear polymers of actin folded into U-shaped double helix structure. Actin is abundant cytoplasmic protein that has a molecular weight of roughly 42 kilodaltons (kDa). In higher eucaryotes actin is known in 3 isoforms: α-actin, β-actin and γ-actin and has two molecular forms: globular and filamentous actin. Globular actin (G-actin) is monomeric form of actin. F-actin is polymeric form of actin made up of long chains of G-actin molecules connected together. This structure is a key component of the actin cytoskeleton (Vázquez-Victorio et al., 2016). Each actin monomer consists of four subdomains and contains a bound adenine nucleotide (ATP, ADP or ADP-Pi) and along with a bound divalent cation, especially Mg^{2+} (Bremer and Aebi, 1992) (Figure 2). ATP-actin monomers possess a higher affinity for the ends of filaments, which allows them to form easily into filaments. The assembly of filamentous actin activates ATP hydrolysis, thus this process forms two chemically distinct ends: plus- and minus-end. The plus-end or barbed end is enriched with ATP-actin, which allows it to grow faster than minus-end. On the other hand, the minus-end or pointed end is enriched with ADP subunits, which have opposite properties to ATP-actin, so the end grows more slowly and disassemble at a steady state. This process is essential for actin dynamics in the cells and is known as actin filament treadmilling (Hilpela K Vartiainen P Lappalainen, 2004). Actin cytoskeleton network continuously assembles and disassembles, which drives the development of filopodia, ruffles and lamellipodia. Actin cytoskeleton is regulated by a complex interplay of actin-biding proteins (ABPs), signaling molecules and mechanical forces, but the most important proteins regulators of actin cytoskeleton are members of the Rho GTPase family, mainly Rho, Rac and Cdc42 (Svitkina, 2018). Actin filaments are polymerized and organized into networks via actions of various nucleation factors, including Arp2/3 (actin-related 2 and 3) complex and other proteins such as formins and tandem-monomer-binding nucleators. The Arp2/3 complex takes part in developing cellular structures like lamellipodia (Guan et al., 2020).
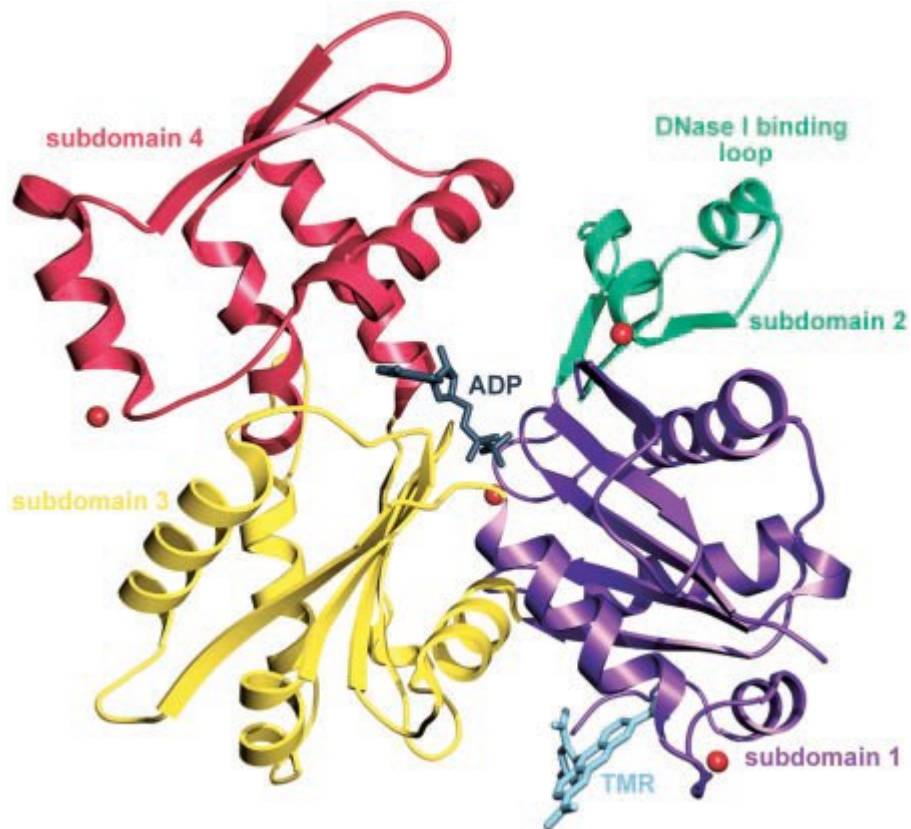
Figure 2 Ribbon representation of the structure of unbound actin in the ADP state, which is consist of 4 subdomains. Subdomain 1 is purple, subdomain 2 is green, subdomain 3 is yellow and subdomain 4 is red. Taken from: (Otterbein, Graceffa and Dominguez, 2001).

2 Small monomeric GTP-binding proteins

There are two types of regulatory GTP-binding proteins involved in cellular signaling: large heterotrimeric GTP-biding proteins and small monomeric GTP-binding proteins. Small monomeric GTPases are very important proteins, which can be found in eukaryotic cells. They are commonly known as small G proteins or small GTPases. These proteins have been discovered around 1980s and have been studied ever since. It has begun with a discover of Ras oncogene of sarcoma viruses (Madaule and Howard, 1985). Lately they were also found in humans, even mutations of these genes were recognised in humans carcinomas. In 1985 was found Rho gene as a homolog of Ras gene(Takai, Sasaki and Matozaki, 2001). Small monomeric GTPases are molecules that weight between 20 to 40 kDa (kilodalton). Families of small G proteins have many different functions that are important and affect nearly all processes in cell. Nowadays more than 170 monomeric GTPases have been found in eukaryotic cells. Ras superfamily of monomeric GTPases is usually divided into 5 families. These families are called Ras, Rho, Rab, Sar1/Arf and Ran family (Song et al., 2019). However, only the Ras and Rho families of Ras superfamily transmit signals from surface receptors of cell. Small monomeric GTP-biding proteins are molecular switches between two states: an "on" state and an "off" state. These states change structural conformation. As its name it says the "on" state happens when GTP is bound to proteins and the "off" state happens when GDP is bound to proteins. After binding GTP small monomeric GTPases bind and affect effector signaling proteins. Change between "on" and "off" states is activated by hydrolysis of GTP to GDP. These switching processes can be very slow without presence of regulatory proteins, so that is why in the cell are proteins which speed up first or second process, thus controlling the "on" state of GTP-binding proteins. There are GAPs (GTPase-activating proteins), these proteins control switch to the "off" state by helping it to hydrolyse its bound GTP to GDP. GDP stays bound to the deactivated monomeric GTPase. On the other hand, in cell are proteins that control process of the activation monomeric GTPases. These proteins are called GEFs (Guanine exchange factors). GEFs stimulate inactive monomeric GTPases by releasing bound between the GTPases and GDP (Alberts et al., 2022).

3 Ras superfamily

As it was already said Ras superfamily consists of five families of small monomeric GTPases with more than 150 members in mammals. Ras family regulates gene expression, apoptosis,

differentiation, cell cycle, cell growth etc. Main members of the Ras family are H-Ras, K-Ras, N-Ras, Rheb, RalA, RalB, Rap1 and many others (Cox and Der, 2010). Second is the Rho family regulating cytoskeletal dynamics, the most important members are Rho, Rac and Cdc42. Third family is the Rab family, they regulate endomembrane vesicular trafficking. The Rab family is the largest family and among members are Rab1-60. Fourth is the ARF family, members are for example: ARF1-6. This family regulates vesicular trafficking in the early secretory pathway (Takai et al., 2001; Hall, 2012). And last family is the Ran family which regulates directional transport between nucleus and cytoplasm and regulates mitotic spindle arrangement. This family has one member (Takai et al., 2001; Kalab and Heald, 2008).

Proteins of Ras superfamily have universal globular structure that maintain GTPase core and two switch sequences. In their structure five common sequences called G boxes can be defined (Figure 3). The G1 box forms a conserved loop, binds beta phosphate of guanine nucleotide and thus G1 is called as P-loop (Di Magliano and Logsdon, 2013). The G2 box is situated in one of two sections that are rearranged based on whether the protein binds GTP or GDP. Thanks to its dynamic rearrangement it is called Switch I. Thr 35 (in Ras, Rac1, Cdc42) or Thr37 (in RhoA) is part of Switch I. Critical residue of G2 box Thr35/37 interacts with gamma phosphate and magnesium cation. The G3 box participates in binding of magnesium cation. Switch II and helix 2 are part of G3, which include another cancer mutation hotspot. This hotspot is Q61 and its sidechain takes major part during GTP-hydrolysis (Scheffzek et al., 2000). Mutation of Q61 often take place in NRas, HRas and Cdc42 (Wennerberg, Rossman and Der, 2005). G4 and G5 are loops that bind guanine bases and ribose and thus give specifity for guanine nucleotide binding. C-terminal of small GTPases often includes a common sequence CAAX (C is cysteine, AA are aliphatic acids and X is any amino acid), which is modified by prenylation (Vetter, 2014). Any changes in this terminal sequence are critical for subcellular localization. Because Switches I and II exhibit highest structural rearrangements between GTP or GDP biding, they decide which regulators (GEFs, GAPs or GDIs) members of the Ras superfamily will bind to and what their signaling response will be. Ras signaling pathways are affected by many factors, these factors can be regulated by processes such as growth factors and hormones (Roberts et al., 2008; Goitre et al., 2014; Yin et al., 2023).
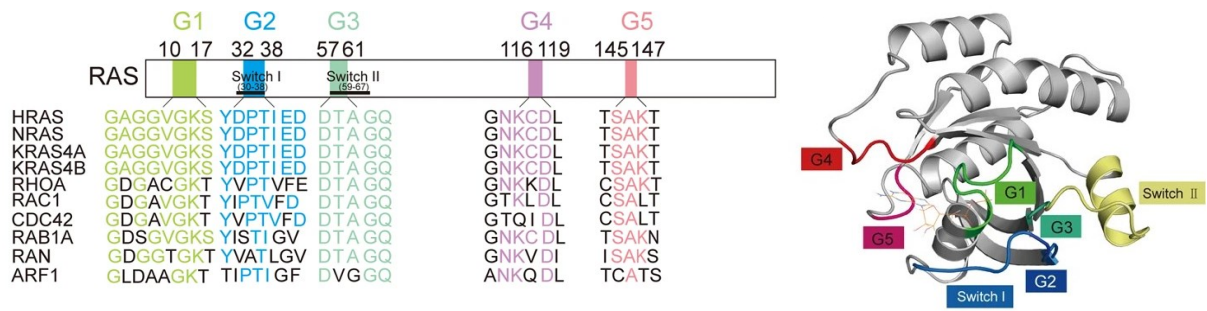
Figure 3 Five conserved G boxes and examples of G boxes of Ras superfamily members. These boxes represent 5 crucial loops for nucleotide binding and regulation of structures. Taken from: (Yin et al., 2023).

4 Rho family

Rho GTPases are commonly found in eukaryotic cells, their molecular weight varies between 21 to 25 kDa (Wang et al., 2022) A distinctive feature that sets Rho family apart from Ras proteins is special 13 amino acid helical insertion within their GTPase domain. This insertion creates a short, solvent-exposed 3_{10} helix, which is located in between α-helix number 4 and β-strand number 5 (Thapar, Karnoub and Campbell, 2002). Members of Rho family have many different important functions in cell as organisation of the actin and microtubule cytoskeleton, cell migration, regulation of gene expression, determinant of cell morphology and polarity (Hall and Nobes, 2000) Members of the Rho family are involved in cancerous growth, injuries and inflammatory processes too. (Haga and Ridley, 2016). Family of the Rho GTPases comprises 20 proteins in mammals and is divided into 8 subfamilies (Haga and Ridley, 2016). These subfamilies with members are: Rho (RhoA-C), Rac (Rac1-3 and RhoG), Cdc42 (Cdc42, RhoJ/TLC and RhoQ/TC10), RhoDF (RhoD, and RhoF/RIF), RhoH, Rnd (Rnd1, Rnd2 and Rnd3/RhoE), RhoBTB (RhoBTB1 and RhoBTB2) and RhoUV (RhoU/Wrch1 and RhoV/CHP) (Figure 4), these subfamilies have around 50 to 55 percent sequence identity. The best described subfamilies are Rho, Rac and Cdc42. Even though these subfamilies are divided on the basis of their various sequence homology, they can be divided into two groups based on if they behave as molecular switches, as typical, or not, as atypical. The typical subfamilies are considered as molecular switches, because they can be in the "on" state which binds GTP and after that switch to the "off" state which binds GDP. Those subfamilies that are considered as typical are Rac, Rho, Cdc42 and RhoF/RhoD (Haga and Ridley, 2016). For typical families activation of Rho GTPases occurs in the presence of regulatory proteins collectively called GEFs, and conversely inactivation of Rho GTPases occurs in the presence of regulatory proteins called GAPs (Alberts et al., 2022). To the atypical subfamilies group belong the rest of the subfamilies, they are called

atypical on the basis that there is no proof that they bind together with GAPs or GEFs. These subfamilies are predominantly GTP-bound, and no alternative regulatory mechanisms that influence them have yet been discovered (Haga and Ridley, 2016).
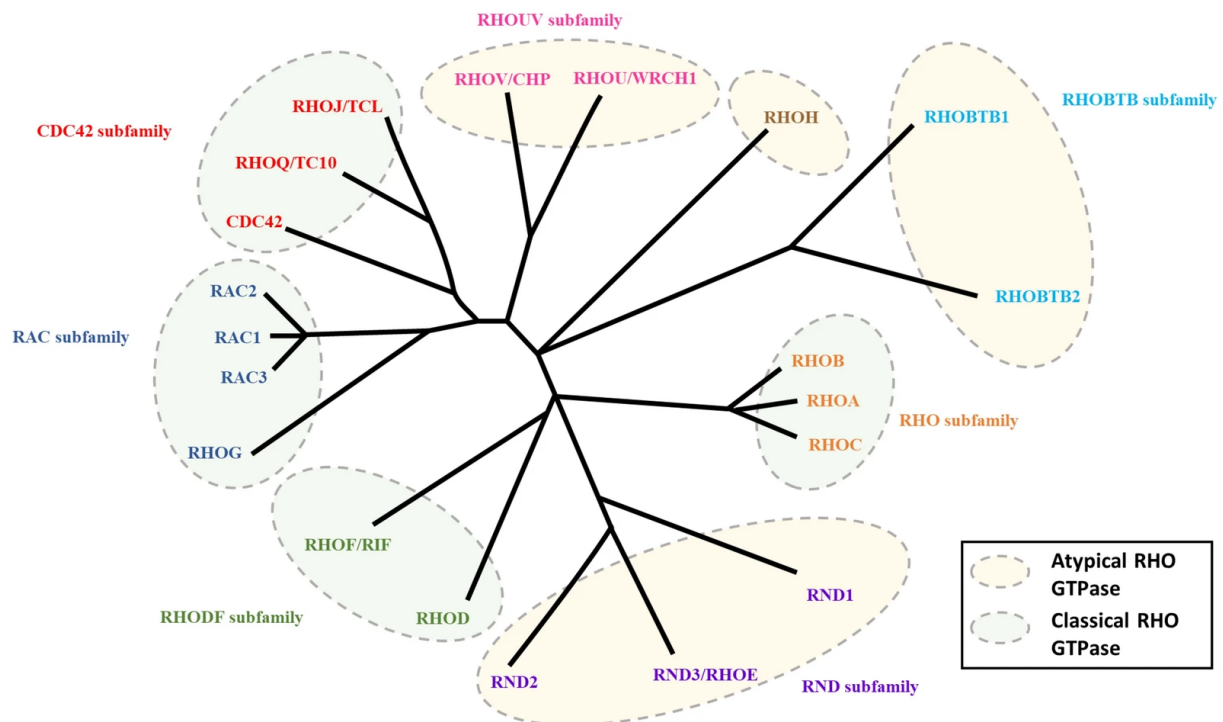

Figure 4 Rho subfamilies with members. Taken from: (Wang et al., 2022).

4.1 Regulation of Rho family GTPases

Signaling involving Rho GTPases can be initiated by activation of various receptors such as cytokine receptors, ion channel, GPCRs (G protein coupled receptors) or enzyme-linked receptor such as receptor tyrosine kinases (Hall and Nobes, 2000). Nowadays have been discovered more than one hundred effectros for members of the Rho family GTPases. Rho GTPases have 3 types of proteins that regulate their GTP/GDP state. These regulatory proteins are called GEFs, GAPs and GDIs (Hall and Nobes, 2000). In humans are known more than 80 Rho GEFs and more than 70 GAPs. Some subfamilies members can have their own specific GAPs and GEFs, but that is not common. In cytosol, switched off Rho GTPases are usually associated with GDIs (guanine nucleotide dissociation inhibitors). This connection happens to avoid connecting with GEFs and activating the Rho GTPases at the membrane of the cell (Alberts et al., 2022).

4.1.1 RhoGEFs

Guanine nucleotide exchange factors are proteins or protein domains that usually activate Rho small monomeric GTPases (Haga and Ridley, 2016). First mammalian Rho GEF was described

in 1985 as Beta-cell lymphoma, this discovery led to the finding of DBL oncogene and the realizing of the importance RhoGEFs (Eva and Aaronson, 1985). Binding GEFs to Rho protein lowers its affinity for the nucleotide biding and GDP is released. GTP is ten times more concentrated in cytosol than GDP, which increases its chance to bind nucleotide-free Rho. Binding of GTP further displaces GEF, which stabilizes GTP-bound Rho. Biding GTP by Rho GTPases is last step for them to interact with their particular effectors (Bos, Rehmann and Wittinghofer, 2007). More than 80 GEFs have been discovered (Hall, 2012). Rho GEFs are divided into two unconnected families Dbl-homology domain family (DH/PH) and Dock homology region domain family. In mammals have been discovered 70 members of Dbl family and 11 members of Dock family (Hall, 2012).

Dock means dedicator of cytokinesis. Dock proteins contain two region DHR1 and DHR2. DHR1 is composed of 200-250 amino acids. Function of DHR1 is to bind phospholipids. Dock Homology Region 2, also called CZH2, functions as catalytic domain and it is composed of 450 to 500 amino acids (Yang and Watsont, 1993; Côté and Vuori, 2002). Dock family is divided into four subfamilies (A-D). Dock proteins take a part during regulation of actin cytoskeleton, cell adhesion and cell migration (Côté and Vuori, 2002).

Dbl and related proteins have B200-amino-acid catalytic Dbl-homology (DH) domain and B100-amino-acid regulatory pleckstrin homology (PH) domain (Whitehead et al., 1997). Dbl exhibits no sequence or structural homology to DHR2 of Dock proteins. It has distinct sequences of N- and C- terminals that flank PH and DH domains. These sequences allow them to interact with varieties of motives or domains such as protein-protein or protein-lipid. This flexibility in sequences helps DH domain to catalyze GDP/GTP exchange reaction, forming protein complexes, structuring of actin cytoskeleton (Cook, Rossman and Der, 2014). Kinases often regulate Dbl domains by phosphorylation, in particular protein kinase A (PKA), protein kinase C (PKC) and some tyrosin kinases (Zheng, 2001). This phosphorylation happens near the PH/DH domains and phosphorylation sites include residues such as tyrosin, serin and threonine (Zheng, 2001; Case et al., 2011).

Core members of Dbl family are: Dbl, Tiam1, Ost, Vav, Tim, FGD1. Tiam1 is most active in cell migration (Guan et al., 2020).

4.1.2 RhoGAPs

GTPases-activating factors are proteins that swich off Rho GTPases. GAPs carry essential catalytic group (so called arginine finger) which increases GTP hydrolysis activity of Rho GTPases. That is why GAPs are considered as inhibitors and signal terminators. Without GAPs

GTP hydrolysis of small GTPases is very slow. GAPs contain around 150 amino acids GAP domain that greatly conserved arginine amino acid in a loop that is called as arginine finger (Figure 5) (Haga and Ridley, 2016). In 1988 first RhoGAP was discovered from human spleen and called p50RhoGAP (Garrett et al. 1989). Humans encode about 80 GAPs, but most of these GAPs have not been studied. RhoGAPs outnumbers Rho GTPases and many RhoGAPs are able to interact with a particular Rho GTPase (Scheffzek et al., 1997; Hodge and Ridley, 2016). For example p122RhoGAP and RA RhoGAP are specific for RhoA (Alberts et al., 1998). On the other hand, for Rac1 are specific α1-chimaerin and ArhGAP15 (Seoh et al., 2003). GAPs frequently carry other domains (2 or 3) to cooperate with various proteins to amplify or alter their signaling pathway. For example, α1-chimaerin C1 domain is able to bind phorbol esters to make very strong interaction between α1-chimaerin and NMDA receptor (Van De Ven, VanDongen & VanDongen, 2005; Huang et al., 2017). RhoGAPs are also able to take action as intermediator or scaffold proteins to transmit signal between Rho GTPases and nonRho GTPases signaling (Huang et al., 2017).
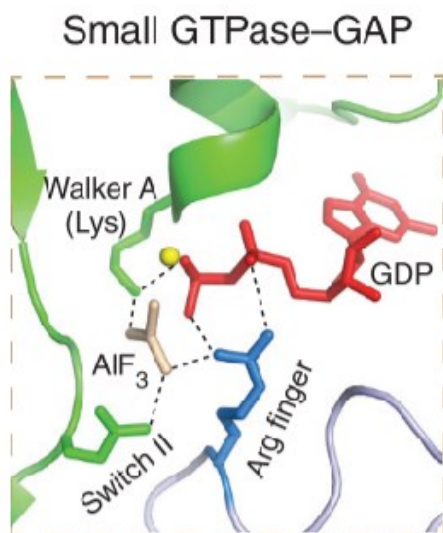

Figure 5 Arginine finger interactions in Rho GTPases. Taken from: (McCullough and Sundquist, 2014).

4.1.3 RhoGDIs

Guanine nucleotide dissociation inhibitors (GDIs) form a protein family of regulators that are pivotal for modulating activation and inactivation of Rho GTPases. They have two main domains C-and N- terminals. N-terminal domain associates with switch I and II of Rho GTPases. This association limits their plasticity essential for switching between GDP and GTP. And C-terminal domain has a pocket that allows the binding of geranylgeranyl, and consequently unbinds geranylgeranyled RhoGTPases from cell membrane (Haga and Ridley, 2016). On the other hand, they have another very important functions: facilitating the

solubilization and redistribution of Rho GTPases within the cell and controlling equilibrium of Rho GTPases between cytosol and membrane. Based on these functions Rho GDIs play a role in signaling pathways mediated by Rho GTPases. Conversely, they can serve as chaperons, transporting RhoGTPases trough membranes, which in certain cases, might facilitate their activation (Garcia-Mata et al., 2011). Rho GDIs family has three well-characterized members: Rho GDIα (or alpha or 1), Rho GDIβ (or beta or 2) and Rho GDIγ (or gamma or 3) (Garcia-Mata et al., 2011; Haga and Ridley, 2016).

Rho GDIα was first discovered and have been the most studied yet. It is found in every human tissues. RhoGDIα is important during cell migration, adhesion and supervising cell morphology (Garcia-Mata et al., 2011; Haga and Ridley, 2016).

Rho GDIβ known also as Ly-GDI, has more limited tissues expression, the highest hematopoietic tissues, Rho GDIβ participates in immune response. Rho GDIβ participates in many pathophysiological processes as cancer metastasis and immune cell migration (Garcia-Mata et al., 2011; Haga and Ridley, 2016).

RhoGDIγ has different structure than others, at the C-terminal it has a prenylation motif, which able to interact with membrane. It is present in many tissues, but it is specific for membrane association for example in Golgi apparatus. RhoGDIγ participates in vesicle trafficking, cell morphology and actin cytoskeleton organization (Garcia-Mata et al., 2011; Haga and Ridley, 2016).

5 Rho subfamily

Members of the Rho subfamily in mammals are RhoA, RhoB and RhoC share around 85% amino acid sequence identity (Parri and Chiarugi, 2010). Despite their similarities, GEFs, GAPs and downstream effectors preferentially interact with specific Rho isoforms, resulting in distinct roles for each of the 3 subfamilies of Rho proteins within cells. These proteins play a significant role in the cancer progression. RhoA, RhoB and RhoC have significant roles in inducing formation stress fibers and focal adhesion complexes, which influence cell shape, motility and adhesion. They are involved in many essential cellular processes such as adhesion, vesicular trafficking, proliferation, survival, morphology of cell and interactions between cell and matrix. Even though they all have similar functions, they exhibit distinct behaviours in cancer progression and chemoresistance (Ridley, 1995). RhoA and RhoC are frequently upregulated in various tumors in human, contributing to tumor progression and metastasis. On the other hand, RhoB tents to have opposite characteristic, it seems to act as tumor suppressor

that inhibits cancer cell proliferation, and it seems to exhibit pro-apoptotic functions (Mokady and Meiri, 2015). The difference between RhoB and RhoA/RhoC is also at the C-terminal CAAX box modification. RhoA and RhoC can be only modified by geranylgeranyl (Roberts et al., 2008). On the other hand, RhoB's prenylation includes both geranylgeranyl (RhoB-GG) and farnesyl (RhoB-F) isoprenoids. Additionally, RhoB can be palmitoylated at cysteines 189 and 192 (Wang and Sebti, 2005). All these various lipid modifications are essential for RhoB's localization and function, they allow RhoB to be localize at the plasma membrane, endosomes, MVB (multivesicular bodies) or nucleus. RhoA and RhoC are typically found in cytosol, where they interact with RhoGDI or on the plasma membrane (Wherlock et al., 2004; Gerald et al., 2013; Zaoui and Duhamel, 2023).

5.1 RhoA

RhoA is the most studied member of the Rho subfamily, it has been studied for decades. It takes part in forming stress fibers and focal adhesions. These structures are essential for cell movement and adhesion to the extracellular matrix (Vega et al., 2011). RhoA is important in regulating the actomyosin contractility and cell proliferation. Moreover, RhoA is involved in cytokinesis, gene expression, and cellular reply to external signals. RhoA seems like a crucial nod in many different signalling pathways (Tkach, Bock and Berezin, 2005; Fan et al., 2024). RhoA has been considered as potential therapeutic target based on its highly expressed level in different types of cancers for example liver, skin, ovarian or gastric etc. (Ridley, 2013). Moreover, RhoA plays a critical role in cardiovascular diseases by controlling vascular tone and blood pressure, because RhoA influences contraction of smooth muscle (Mokady and Meiri, 2015).

5.2 RhoB

RhoB is mainly localized in the early endosomes, in multivesicular bodies, on plasma membrane and also found on nuclear membrane (Zhou et al., 2011). RhoB takes an important role as a regulator of endosomal trafficking within cell. This means that RhoB controls and regulates vesicle transport, effects receptor signaling and is involved in their recycling and degradation (Wheeler and Ridley, 2007). Thereby RhoB is involved in controlling cell growth, cell death, stress response, immune system and cell movement. All these functions are important for keeping cellular homeostasis and reacting to extracellular signals. Same as other members, RhoB provides cytoskeleton organization with a help of regulating actin filament dynamics and preserving the structural integrity of endosomes (Zaoui and Duhamel, 2023).

RhoB is also very important in stress responses such as DNA damage, oxidative stress and toxins (Ridley, 2013; Mokady and Meiri, 2015; Zaoui and Duhamel, 2023).

5.3 RhoC

RhoC and RhoA are quite similar, however RhoC has its own special functions and regulates various cellular processes. RhoC is very important protein in controlling/regulating actin cytoskeleton, cell movement, shape, adhesion etc. RhoC has been recognized as an important regulator of metastasis based on its regulation and organization of actin filaments. Overexpression of RhoC occurs in various types of cancer such as breast, pancreatic, gastric, melanoma etc (Islam et al., 2009; Vega et al., 2011). Additionally, RhoC is vital for formation and function of invadopodia, which are essential for cancer invasion (Guan et al., 2020).

6 Role of Rho during cell migration

RhoA is primally active at the rear of cell, but it is also able to be active at the front of the cell. To promote lamellipodium formation, mDia1 collaborates with Arp2/3 complex, which initiates polymerization of actin. This occurs at the front of cell. Furthermore, during membrane protrusion, RhoA is activated at the leading edge, whereas it is inactivated during membrane retraction (Haga and Ridley, 2016).

3-D environment induces different types of cell movement, which require different Rho GTPases activity. There are Rho-driven and Rac-driven types of movement. These types of movement are interchangeable, they inhibit each other signaling pathways, but they both promotes actomyosin contractility, driven by ROCK signaling. Rho activates ROCK, which results in phosphorylation of MYPT (myosin light chain phosphatase). Phosphorylation of MYPT leads into its inactivation. After this myosin II activity is enhanced. This whole process is vital in various cell contractions of smooth muscle and even in non-muscle cells (Kimura et al., 1996).

7 Downstream effectors of Rho

Downstream effectors of Rho are proteins that convert molecular signals from members of Rho family into targeted cellular responses and actions. These effectors encompass many diverse proteins and enzymes that can specifically interact with GTP-bound Rho proteins. The primary categories of downstream effectors include kinases like ROCKs (stands for Rho-associated

coiled-coil containing protein kinases). Main functions of ROCKs are to control actin cytoskeleton dynamics, cellular tension and to facilitate cellular contraction. Another important effectors are formins of mDia (mammalian homolog of diaphanous) subfamily, which mediate actin nucleation and polymerisation. (Amano, Nakayama and Kaibuchi, 2010; Guan et al., 2023) (Figure 7).

7.1 Rho-associated kinase/ROCK pathway and associated genes

The Rho-associated protein kinases are serine/threonine protein kinases recognized as critical downstream effectors of Rho. Human ROCKs include two isoforms: ROCK1 and ROCK2, which have more than 64% of amino acid identity (Amano et al., 2010). Both isoforms are located on different chromosomes, ROCK1 is on 18. chromosome and ROCK2 is on 2. chromosome (Guan et al., 2023). Both ROCKs are ubiquitously expressed across most tissues, but ROCK1 has higher expression in liver, testis and lung. On the other hand, ROCK2 has higher expression in brain and muscles (Amano et al., 2010). ROCKs bind to all isoforms of Rho in their GTP-bound state through theirs Rho-binding domains, targeting the switch region of activated isoforms of Rho GTPases. It has been shown that knock down of ROCK1 and ROCK2 have different effects. Silencing ROCK1, like RhoA but not like RhoC, changes cell shape, focal adhesions and stress fibers. In contrast, silencing ROCK2 changes phagocytosis, cellular protrusions and contraction of cell (Vega et al., 2011). ROCKs main functions are to regulate cell cytoskeleton by activatory phosphorylation of myosin light chain (MLC) and inhibitory phosphorylation of myosin light chain phosphatase (MLCP). This stimulates actin-myosin contractility, which is essential for formation of stress fibers and focal adhesions and overall cell migration (Mokady and Meiri, 2015). Smooth muscle contraction is through myosin II phosphorylation. This phosphorylation occurs at Ser-19 of myosin II (Field and Manser, 2012). Furthermore, ROCK activates phosphatase and tension homologue known as PTEN, downstream effector and tumor suppressor, which counteracts PI3K, inhibiting Akt signaling pathways. Akt signaling pathways regulate cell growth and proliferation (Guan et al., 2023). Additionally, LIMK (Lim kinase) is activated by ROCKs, which results in phosphorylation of cofilin, actin-depolymerizing protein. Phosphorylation of cofilin occurs on its Ser3 and it deactivates cofilin, which results in inhibiting its actin-depolymerizing activity (Pritchard et al., 2004). Also, ROCKs are able to activate adducin, cytoskeletal membrane protein, and ERM proteins, which allows both of them to immediately engage with F-actin and plasma membrane (Nalbant, Wagner and Dehmelt, 2023) (Figure 6).
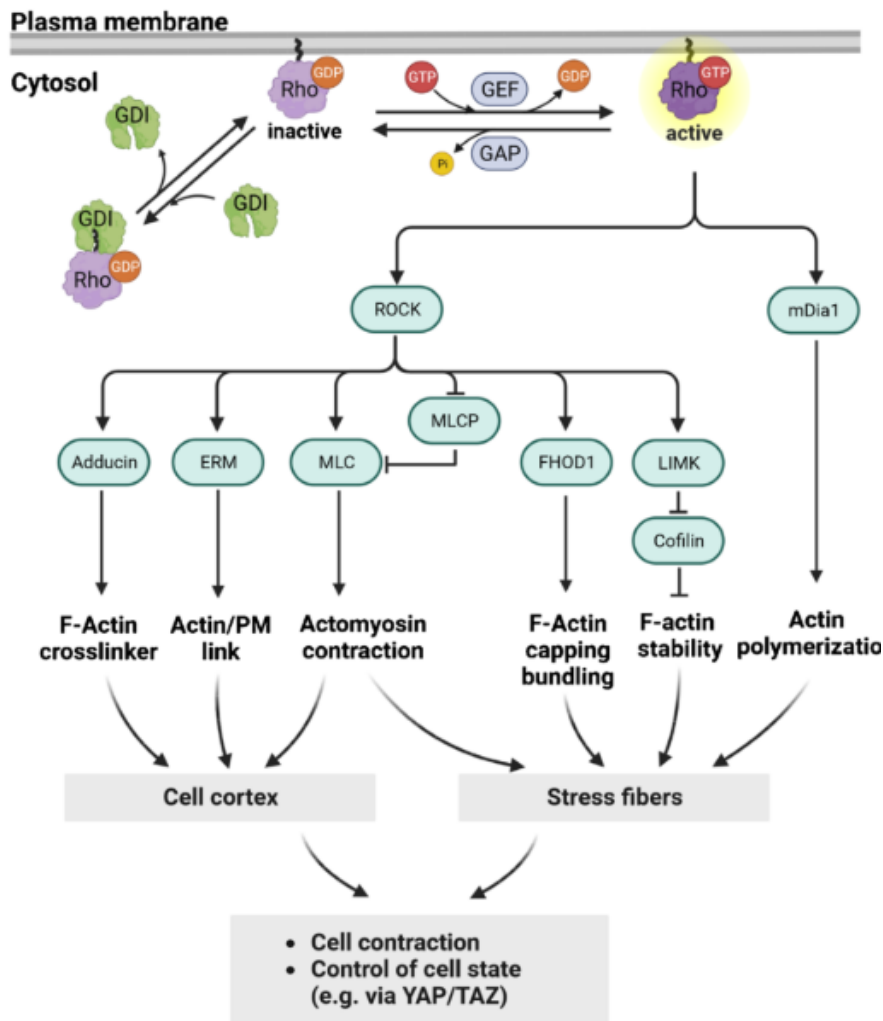
Figure 6 Control of actomyosin contractility by Rho subfamily. Taken from: (Nalbant et al., 2023).

7.2 mDia

Mammalian homolog of *Drosophila* Diaphanous or for short mDia promotes actin polymerization by stimulating nucleation and elongation of F-actin. mDia takes part in forming multiple actin-based structures in migrating cells (Rose et al., 2005). mDia contains Rho-binding domain and two formin homology domains (FH1 and FH2), which classifies it as a member of the formin family. mDia directly binds to plus-end of F-actin and promotes burst polymerization, which is essential for formation of stress fibers, cell polarity and migration. MDia cooperates with ROCKs to regulate formation of F-actin (Higashida et al., 2013). There are three isoforms of mDia that we know of, and they all interact with switch region of different Rho GTPases including RhoA, RhoB and RhoC. These isoforms are: mDia1, mDia2 and mDia3 (Rose et al., 2005). RhoA, RhoB and RhoC activate mDia1 to enhance actin stress fiber formation, and it is involved in filopodia formation, mechanotransduction, cell polarity and migration. Furthermore, mDia1 is involved in transcriptional activity of. In cell-cell adhension

has been noticed antagonistic relationship between mDia1 and ROCK (Ishizaki and Narumiya, 2014). MDia2 is able to be activated by RhoA (Staus, Taylor and Mack, 2011) and RhoB on endosome (Wallar et al., 2007), which is included in filopodia formation and cytokinesis. Finally, mDia3 is able to bind only RhoA, but has been shown to interact with other Rho GTPases such as Cdc42 and Rac1 (Schratt et al., 2002). Functions of mDia3 are less defined, but it is involved in spindle and chromosome alignment (Yasuda et al., 2004).

8 Rac subfamily

Members of the Rac subfamily in mammals are Rac1, Rac2 and Rac3, they share around 88-92% of protein identity. Their primary function is to regulate the formation of lamellipodia and membrane ruffles, which are crucial for cell migration, spreading and phagocytosis. The members of Rac subfamily are an important part of organization of actin cytoskeleton, affecting cell shape, movement, superoxide formation and adhesion (Etienne-Manneville and Hall, 2002). Individual members differ in their patterns of expression (Corbetta et al., 2005; Steffen et al., 2013). In general, Rac1 and Rac3 are found in many tissues, on the other hand Rac2 are only found in cells of hematopoietic origin. To completely understand mammalian Rac proteins, studies have been carried out focusing on the investigation of mouse proteins (Liu et al., 2019).

8.1 Rac1

Rac1 is the best characterize member of the Rac subfamily and is widely expressed in various cell types (Bustelo et al., 2012). Rac1 is composed of roughly 192 amino acids. It plays main role in regulating cell mobility (lamellipodia and filopodia), intercellular adhesion, and participates in numerous signaling cascades that influence gene activity, cell growth and cell cycle (Murali and Rajalingam, 2014). Rac1 influences cell cycle in transition from G1 to S phase and it takes part in cell survival by turning on anti-apoptotic pathways (Sugihara et al., 1998; Bustelo et al., 2012). Rac1 is important in controlling gene expression at the transcriptional level, mainly activating pathways like NF-kB, JNK (c-Jun- N-terminal kinase), PI3K and MAPKs (Mitogen-Activated Protein Kinases) (Bid et al., 2013; Steffen et al., 2013). Processes regulated by Rac1 are essential to processes linked to malignant transformation, such as tumor development, angiogenesis, invasion and metastasis. Therefore, it has been suggested that targeting Rac1 and its associated molecules could be a valuable strategy for developing drugs that would disrupt these malignancy-driving pathways. Rac1 shows signs of both

overexpression and altered activity in numerous tumor cell types (Bustelo et al., 2012; Steffen et al., 2013), and has been most implicated in testicular, gastric and breast cancers (Bid et al., 2013). There is an important difference in structure between Rac2 and Rac1, Rac3. The difference is that Rac1 and Rac3 contain domain analogous to the Ras hypervariable region located in primary sequence nearby C-terminal CAAL sequence. In Rac1 and K-Ras the hypervariable region exhibits a distinctly polybasic domain. In many different pathological states activation and pathways of Rac2 can be a potential therapeutic target for regulating immune responses, based on its crucial role in leukocytes and inflammation processes (Pai, Kim and Williams, 2010).

8.2 Rac2

Rac 2 is expressed in hematopoietic cells and its main function is to play distinct roles within the immune system. It controls the movement of leukocytes towards inflamed areas and the oxidative burst in neutrophils, crucial for combating pathogens (Zhang et al., 2022). These particular tasks highlight its crucial role in innate immunity and the body's inflammatory reactions. Its other function is regulator of actin cytoskeleton dynamics (lamellipodia and membrane ruffle formation), which is important for cell migration, phagocytosis and cell to cell interaction. Rac2 is comprised of roughly 192 amino acids, which correspond to a mass of 21 kDa (Liu et al., 2019).

8.3 Rac3

Rac3 was identified in 1996 in a chronic myeloid leukemia cell line (Haataja, Groffen and Heisterkamp, 1997). And ever since has been liked to human breast and ovarian cancer, its activity can be hyperactive and/or deregulated in various tumors. It is also implicated to cellular transformation and tumor invasion (Pai et al., 2010). Rac3 is expressed together with Rac1 protein in developing neurons and in many different cell types, but expression profile of Rac3 is more limited than Rac1. The primary sequence difference between Rac3 and Rac1 are in the carboxy-terminal hypervariable region (residues 180-192). This region is posttranslationally modified and is critical for determining the specific intracellular localization and interaction of GTPases with its target proteins. Rac3 is very closely related to the previously mentioned members of Rac subfamily (Corbetta et al., 2005). Recently Rac3 has been recognize in breast cancer for taking a crucial role in dynamics of invadopodia (Donnelly et al., 2017).

9 Role of Rac during cell migration

After that in 1995 was shown that Cdc42 influence actin dynamics by initiating filopodia formation and also it activates Rac (Nobes and Hall, 1995). Through interactions within the SCAR/WRC, Rac facilitates formation of lamellipodia. Engaging components such as Sra1 and WAVE1 within the SCAR/WRC, Rac triggers a conformational shift that shows the in WAVE1 the VCA motif. Exposure of the VCA motif is crucial for activation of the Arp2/3 complex. Activation of Arp2/3 complex facilitates the assembly of actin, crucial for development of lamellipodia. This process is vital for responses to environmental cues and for migration of cell (Chen et al., 2010). Research from Tang group has shown that dynamics of cell differently react to the inhibition of SCAR/WAVE-dependent activation of the Arp2/3 complex in 2-D versus 3-D environments. Achieved by reducing levels of Sra1 and Nap1, which are components of this regulatory complex. In 2-D environment inhibition of SCAR/WAVE restricts movement. On the other hand, in 3-D environment inhibition facilitates invasion. The inhibition of SCAR/WAVE in 3-D results in higher activity of FAK. Higher activity of FAK stimulates activation of N-WASP at the invasive front, which promotes invasion mediated by Arp2/3 (Tang et al., 2013). Moreover, research from Dang group highlighted other aspect of cellular regulation. Inhibition of SCAR/WAVE-dependent activation of Arp2/3 takes part in this regulation. This Rac-dependent signaling draws in and activates protein Arpin. Which binds to Arp2/3, however, is unable to activate Arp2/3 complex, because it does not have the VCA motif. Arpin function is to act as competitive inhibitor. Thus, Arpin is recruited by Rac to the edges of lamellipodia, where it suppresses Arp2/3 complex, which results in slower migration and possibility of directional shift in cell movement (Dang et al., 2013).

10 Downstream effectors of Rac

Downstream effectors of Rac are central regulator of actin cytoskeleton. Activation of Rac initiates downstream effectors that regulate and organize reorganization of actin filaments. These effectors, including WAVEs and PAKs, are very important during cell migration, wound healing, morphogenesis and many other cellular processes. (Parri and Chiarugi, 2010; El Masri and Delon, 2021) (Figure 7).

10.1 WAVEs

WAVE subgroup is part of Wiskott-Aldrich syndrome protein family (WASP), which have an important role in transmitting signals form the cellular environment to the actin cytoskeleton.

There are able to transmit the signals based on their VCA motif to bind and activate the Arp2/3 complex's actin nucleating activity (Ismail et al., 2009). The VCA motif is composed of verprolin homology, cofilin homolgy and acid regions at the C-terminus (Chen et al., 2010). WAVEs (WASP verprolin homologous), which have been also known as Scar protein (Derivery et al., 2009), are proteins that are associated with various of cellular processes such as cell migration, polarization, neuronal guidance, T cell activation (Ismail et al., 2009) and wound healing (Nakamura et al., 2023). Expect VCA motif WAVEs also have WHD (WAVE homology)/SHD (Scar homology) domain, basic region and prolin-rich region (Takenawa and Miki, 2001). In mammalian cell WAVE assembles into WAVE regulatory complex (WRC), which is a hetero-pentameric complex composed of Sra1/PIR121, Abi, Nap1 and HSPC300 proteins. The Scar/WAVE-WRC complex is trans-inhibited actin at the resting state. After binding WRC to Rac this trans-inhibited state is relieved, which enables WAVE to associate with Arp2/3 complex to promote nucleation of branched actin structures. Rac interacts with WRC through Sra1 (Nakamura et al., 2023).

The WAVEs consist of 3 key members (WAVE1, WAVE2 and WAVE3), each of them regulates actin cytoskeleton. WAVE1 and WAVE2 are extensively expressed across diverse types of cells and tissues, but WAVE3 is primarily expressed in neural tissues (Tang et al., 2020).

10.2 PAKs

P21-activated kinases are part of serine/threonine kinases family. They are important downstream effector of Rac, which take roles in many processes of cell such as cytoskeletal remodelling, focal adhesion assembly, cell migration, survival and gene expression (Knaus and Bokoch, 1998). PAKs bind with activated Rac, which changes their conformation and results in their activation by phosphorylation. Mammalian PAKs are divided into two large groups (I and II), that consist of 6 PAK isoforms. Kinases of group I are PAK1, PAK2 and PAK3 or PAKα, PAKβ and PAKγ, and kinases of group II are PAK4, PAK5 and PAK6. PAK1 has been the most studied isoform (Field and Manser, 2012). PAK1 and PAK2 have different functions in various experimental systems. PAK1 and PAK2 have been shown to differentially influence RhoA activity, adhesion, phosphorylation of MLC during migration of cell and tumor invasion. (Itakura et al., 2013). PAK1 acts as a positive regulator, it enhances degranulation via rearrangement of cytoskeletal. On the other hand, PAK2 is considered as negative regulator, based on its function to inhibit RhoA with a help of phosphorylation of GEF-H1 (Kosoff et al., 2013). PAK2 and PAK4 are ubiquitously expressed, whereas the rest of PAKs are

predominantly expressed in various tissues like brain and spleen, which is specific for PAK1 (Itakura et al., 2013). Overexpression of PAKs take place during cancer progression, for example PAK1 is connected to human breast cancer (Arias-Romero et al., 2010). Also, PAK1 activates LIM kinase by phosphorylation of threonine 508 within its activation loop. Activated LIM kinase catalyses phosphorylation of cofilin, which results in its inactivation. This inactivation enhances accumulation and stabilization of F-actin (Edwards et al., 1999).

11 Cdc42 subfamily

Cell division cycle 42 is member of Rho GTPases. Its function is to regulate cell division, cycle progression and migration (Du et al., 2016) via regulating actin cytoskeleton, especially formation of filopodia. Furthermore, Cdc42 is very important regulator of polarity of cell (Hall, 2012) and takes part in intracellular trafficking, influencing receptor endocytosis and transport through the Golgi apparatus, support tight junction integrity and regulate RNA processing (Erickson and Cerione, 2001; Farhan and Hsu, 2016). Also, Cdc42 has been shown to regulate metastasis of many human cancers such as gastric (Du et al., 2016), breast (Chander et al., 2013) and melanoma cancer (Woodham et al., 2017). Downstream effectors of Cdc42 are WASP, PAK, MRCK and PAR. (Chen, Wirth and Ponimaskin, 2012). Cdc42 regulates actin polymerization and formation of filopodia through its binding to WASPs, which recruits and activates Arp2/3 complex (Etienne-Manneville, 2004). It accomplishes this by engaging with and activating N-WASP. The binding of N-WASP to Cdc42, changes N-WASP's conformation, showing its binding site for Arp2/3 complex, which results in rapid actin polymerization (Rohatgi et al., 1999) (Figure 7).

MRCK (myotonic dystrophy kinase related Cdc42-binding kinase) also initiates phosphorylation of MYPT by downstream of Cdc42. Wilkinson research group found out that both Rho and Cdc42 together work to generate actomyosin contractility, which is required for elongated movement. Their research shows that any of MRCK or ROCK-dependent signaling pathways are able to phosphorylate MYPT, which results in its inhibition (Wilkinson, Paterson and Marshall, 2005).

Research study of Erickson and Cerione has shown complex interaction between RhoA and Cdc42 during cell division. Cdc42 is very important for exact localization of RhoA, which consequently influences assembly of actin for successful cytokinesis (Erickson and Cerione, 2001).
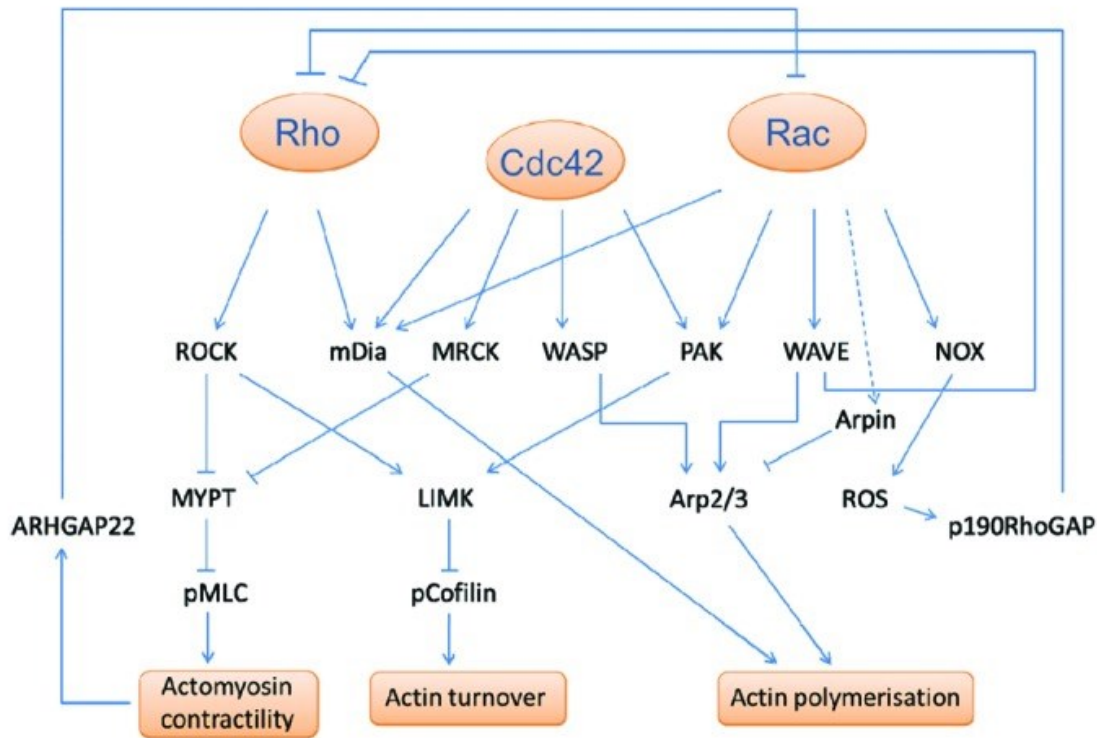
Figure 7 Crosstalk between Rho, Rac and Cdc42 regulates actin cytoskeleton during cell migration. Taken from: (Sadok and Marshall, 2014).

12 Antagonistic interactions between Rho and Rac during cell migration

The role of Rho GTPases in regulating cell cytoskeleton during migration in 2-D environment has been established by foundational research from Hall, Ridley and Nobes (Ridley and Hall, 1992; Nobes and Hall, 1995). Their research demonstrated that while Rac initiates formation of lamellipodia following stimulation of PDGF, RhoA is critical in promoting the assembly of contractile stress fibers or actomyosin fibers, which is activated by LPA signals (Ridley et al., 1992; Ridley and Hall, 1992). Rac1 and Cdc42 activates start to rise when activity of RhoA decreases. This shift marks a coordinated transfer in GTPases control, transitioning from RhoA's initiation of protrusion, lamellipodia, while Rac1 and Cdc42 stabilize and support forward movement (MacHacek et al., 2009). Rho and Rac signaling often exhibits opposite effect on cellular signaling and cytoskeleton organization. Activation of one protein, either Rho or Rac, typically counteracts or inhibits the function of other protein This antagonism is mediated by various of effectors, regulators and signaling pathways. Partially is mediated by activation and deactivation of their GAPs, GDIs and GEFs (Lawson and Burridge, 2014).

12.1 RhoA mediated suppression of Rac

The molecular mechanisms of Rho antagonism against Rac include various levels of regulation of Rac GAPs and GEFs depending on signaling associated with Rho. Examples of these signaling relationships are described below (Kuo et al., 2011).

One of the known mechanisms of antagonism is the effect of ROCK signaling on Rac's GEF and GAP. ROCK-mediated myosin II contractility has been shown to negatively regulate Rac GEFs β-Pix and DOCK180 (Kuo et al., 2011). Both β-Pix and DOCK180 are part of activation of Rac at the front of cell. They are primarily located at focal adhesions. Both of these Rac GEFs are sensitive to mechanical stress therefore ROCK-activated contractility results in downregulation of β-Pix and DOCK180 (Kuo et al., 2011; Vicente-Manzanares et al., 2011) (Figure 8).

ROCKs are also able to stimulate activity of Rac's FilGAP by its direct phosphorylation. As a result, FilGAP activated in this way is a mediator for Rho's antagonistic effect on Rac. This antagonistic effect suppresses protrusion of cell and promotes cell contraction in 2-D environment (Saito et al., 2012). Cancerous cells apply mesenchymal or amoeboid movement to progress migration. RhoA and ROCK can further influence migration by inhibiting activity of Rac by ROCK-ArhGAP22 pathway. Activation of ROCK increases the GAP activity of ArhGAP22, which is closely related to FulGAP. When ArhGAP22 is activates, it accelerates the conversion of activated Rac to its inactivate state, which results in suppression of Rac (Sanz-Moreno et al., 2008; Lawson and Burridge, 2014) (Figure 8).

12.2 Rac1 mediated suppression of RhoA

Most examples of Rac1 mediated suppression of RhoA include effect of Rac-activated PAK kinases on specific Rho GAPs and GEFs (Kiyokawa et al., 1998). This includes inhibitory phosphorylation of GEF-H1 (Zenke et al., 2004) and activatory phosphorylation of p190RhoGAP (Nimnual, Taylor and Bar-Sagi, 2003; Bustos et al., 2008). Regulation of phosphorylation p190RhoGAP further inhibition of its phosphatase. This is part of a redox pathways activated by signals that induce EMT (epithelial-mesenchymal transition). It begins with extracellular activation of Rac1, which leads into increasing production of ROS (reactive oxygen species) by activating NADPH oxidase. Increasing level of ROS inhibits LMW-PTP (low molecular weight protein tyrosine phosphatase) enzyme, which enhances the phosphorylation of p190RhoGAP. Moreover p120-catenin interacts with phosphorylated p190RhoGAP, simplifying its localization to adherens junctions and after that anchoring it to

cadherin complex. This association is very important for regulation of cell-cell adhesion and localized downregulation of RhoA (Wildenberg et al., 2006). Interestingly, it was also known that Rac can directly activate p190RhoGAP (Bustos et al., 2008) (Figure 8).

An interesting example of Rac1 mediated suppression of RhoA involves the inhibition of Smurf1, which is E3 ubiquitin ligase. Smurf1 is activated and recruited to the leading edge of migrating cells by PAR6-aPKC complex. This activation results into targeted proteasomal degradation of RhoA, which suppresses RhoA activity (Sahai et al., 2007).
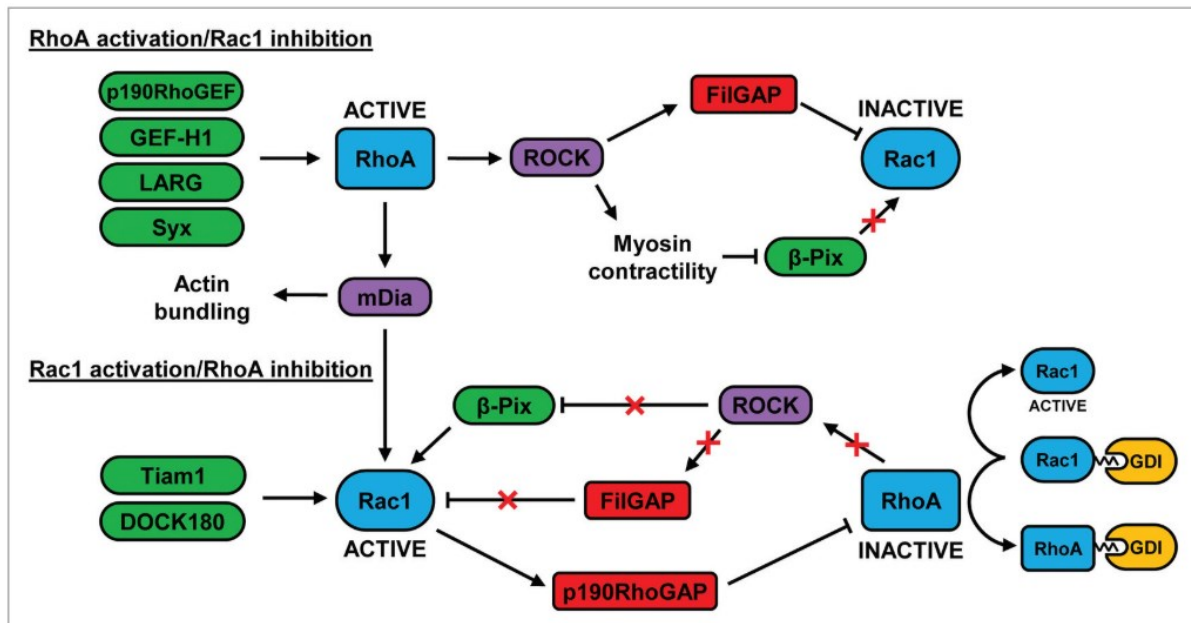

Figure 8 Crosstalk between RhoA and Rac1 in migrating cells. Taken from: (Lawson and Burridge, 2014).

12.3 GDIs influences Rho/Rac antagonism

GDIs are shared by all members of the Rho family, and the binding of individual inactive Rho proteins to GDIs results in competition. Disruption of this competition, for example by post-translational modification to Rho or Rac, can modulate the balance of binding of Rho-GDI, favouring a specific Rho protein, resulting in its inhibition. GDIs directly modulate balance between Rho and Rac opposite signaling pathways to impact cell adhesion and migration. Cyclin AMP-dependent protein kinase A (PKA) is activated at the leading edge of some cell types, and it regulates cellular processes such as migration. In smooth muscle cell PKA is important in formation of membrane ruffles and regulation of protrusions of cell. PKA phosphorylates RhoA, specifically its 188 serine. This phosphorylation enhances RhoA's affinity to bind RhoGDI, which results in its removal from plasma membrane and therefore inactivation of RhoA. Because of the competitive binding of RhoA and Rac1 to GDI, this allows

Rac1 release from Rac1/GDI complex and increases activity of Rac1 (Ellerbroek, Wennerberg and Burridge, 2003; Tkachenko et al., 2011) (Figure 8).

12.4 Synergy between Rho and Rac

Besides antagonism between Rho and Rac, it has been also observed synergy between these two proteins. For example, effector mDia1 is linked to RhoA activity, it stabilizes microtubules, and it is also connected to activation of Rac. Additionally, mDia1 has impact on Rac's activity by influencing Src localization at focal adhesions, which in turn facilitates phosphorylation of p130Cas (Yamana et al., 2006). Furthermore, RhoA-dependent pathways activating Rac also includes Tiam1 and DOCK180, which are activated as a result of signals relayed downstream from p130Cas and Src (Nishimura et al., 2005).

13 Conclusion

This bachelor's thesis has examined the antagonistic relationship between Rho and Rac GTPases in the regulation of actin cytoskeleton during cell migration. Rho and Rac, members of small GTPases family, take crucial roles in controlling cell shape, motility and various cellular processes. This thesis highlights the complex signaling network of Rho and Rac, which exhibit distinct yet interrelated effects on the actin cytoskeleton. These networks ensure precise spatial and temporal coordination of cell movements, which are vital for cellular processes such as wound healing and responses of immune system.

The antagonistic interactions between Rho and Rac are mediated by many diverse downstream effectors, including ROCKs, PAKs and partially is mediated by the regulatory proteins GEFs (β-Pix, GEF-H1, p190RhoGEF), GAPs (p190RhoGAP, ArhGAP22, FilGAP) and GDIs. GEFs activate Rac1 to promote formation of lamellipodia and counteracts the assembly of stress fibers, which are mediated by RhoA. On the other hand, GAPs suppress RhoA to promote Rac1-driven migration of cell. Additionally, GDIs regulate balance between Rho/Rac antagonism via their competitive binding. This antagonistic relationship is necessary for dynamic regulation of actin structures vital for successful migration.

Understanding this molecular mechanism of Rho and Rac antagonism has significant implications for therapeutic strategies. Abnormal cell migration takes place in diseases including cancer. Targeting their signaling pathways and regulatory proteins has potential for therapeutic strategies to control cell migration. Inhibiting Rho or Rac specific effectors and regulatory proteins could modulate migration of cells, which could potentially find new treatment for cancer through limiting metastasis and tumor progression.

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