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MINI-REVIEW

Role of the small GTPase Rap1 in signal transduction, cell dynamics and bacterial infection

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ABSTRACT

Rap1 belongs to the Ras family of small GTPases, which are involved in a multitude of cellular signal transduction pathways and have extensively been linked to cancer biogenesis and metastasis. The small GTPase is activated in response to various extracellular and intracellular cues. Rap1 has conserved functions in *Dictyostelium discoideum* amoeba and mammalian cells, which are important for cell polarity, substrate and cell-cell adhesion and other processes that involve the regulation of cytoskeletal dynamics. Moreover, our recent study has shown that Rap1 is required for the formation of the replication-permissive vacuole of an intracellular bacterial pathogen. Here we review the function and regulation of Rap1 in these distinct processes, and we discuss the underlying signal transduction pathways.

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A conserved role for Rap1 in essential cell processes

Small GTPases represent molecular switches that control essential cellular processes such as signal transduction, cell adhesion, chemotaxis and motility, cell growth and division, membrane dynamics and vesicle trafficking, as well as interactions with pathogens. To this end, the small GTPases cycle between an inactive GDP-bound and an active GTP-bound state. The switch between these states is tightly controlled by cognate guanine nucleotide exchange factors (GEFs) and GTPase activating proteins (GAPs). GEFs facilitate the release of the bound nucleotide and allow the more abundant GTP to rebind, whereas GAPs stimulate the low intrinsic GTPase activity to stimulate the hydrolysis of the bound GTP to complete the cycle.[1]

Rap1 belongs to the Ras superfamily of small GTPases.[2] The switch between its GDP-bound and GTP-bound form is controlled by several specific GEFs and GAPs.[3] Rap1 is conserved in mammalian cells as well as in the haploid social soil amoeba *Dictyostelium discoideum*.[4,5] In mammalian cells Rap1 plays a pivotal role for cell growth, proliferation and survival.[5,6] In *Dictyostelium* antisense *rapA* RNA induction leads to a gradually decreased growth rate and cell viability, and in particular, Rap1-depleted amoeba show a reduced viability in response to osmotic stress.[7,8] Rap1 is likely essential for the amoeba, as attempts at generating the corresponding null mutant strain were unsuccessful. Despite extensive studies in various biologic systems, the Rap1 signaling pathways regulating these important processes are still not completely identified and characterized.[9] *Dictyostelium* is an excellent model for studying Rap1-dependent processes, because of its genetic tractability, as well as the evolutionary conservation of the Rap protein and the downstream signaling pathways that govern cytoskeletal rearrangements.[4,5] Here we highlight recent findings in *Dictyostelium* and mammalian cells that implicate Rap1 in the regulation of cytokinesis, cell adhesion, chemotaxis, and pathogen vacuole formation.

Rap1 governs cell adhesion and phagocytosis

Rap1 has been intricately linked to pathways regulating cell adhesion. In mammalian cells activation of Rap1 is triggered by adhesion molecules, cytokines, growth factors like tumor necrosis factor α (TNFα) and interferon γ (IFNγ), or second messengers that are coupled to GEFs.[5,10] During phagocytosis, Rap1 (and also the small GTPase Ras) is activated by diacylglycerol, which recruits the Rap1/Ras GEF RasGRP3.[11] Rap1 controls cell adhesion dynamics and phagocytosis, especially by mediating the functions of integrins and cadherins.[12,13] In this

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pathway Rap1 acts upstream of the integrin-associated factor talin, and controls the recruitment of the cytoskeletal protein to sites of particle binding and phagocytosis.[14] The activation of Rap1 indirectly activates talin, as active Rap at the cell membrane recruits the scaffold protein RIAM (Rap1-GTP-interacting adaptor molecule), which subsequently binds talin and stimulates integrin activation and formation of adhesion complexes.[15,16]

Rap1 is also essential for cell adhesion in *Dictyostelium* (Fig. 1).[8,17] The GEF GbpD is primarily responsible for activation of Rap1 during substrate attachment.[18,19] Active Rap1 mediates cell adhesion via the Ser/Thr kinase Phg2 and talin. Interestingly, in addition to indirectly regulating talin function, *Dictyostelium* Rap1 also directly binds and activates the cytoskeletal protein. Recent data from our laboratory revealed that the direct interaction of active

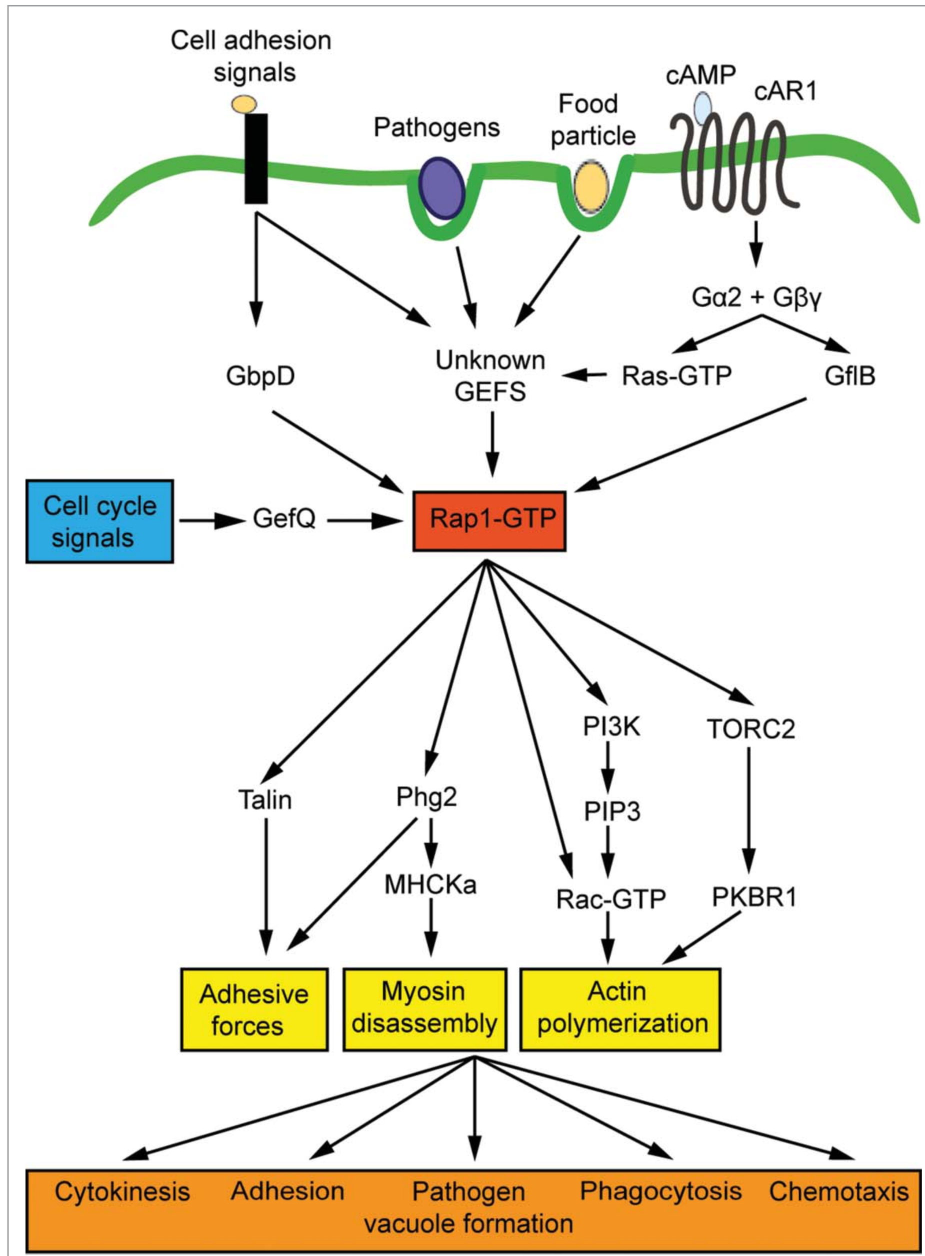

Figure 1. Role of the small GTPase Rap1 in signal transduction, cell dynamics and bacterial infection. Rap1 is activated in response to various extracellular and intracellular stimuli. During substrate attachment and cytokinesis, Rap1 is primarily activated by the GEFs GbpD and GefQ, respectively. In the course of chemotaxis, the Gα2-stimulated RapGEF GflB determines the balance between Rap1 and Ras activation at the leading edge of *Dictyostelium* cells. Other GEFs transducing the signals to Rap1 remain to be identified. Activated Rap1 is a major regulator of cytoskeletal dynamics and stimulates cellular adhesion, actin filament formation and myosin disassembly via the indicated pathways. Together, these cytoskeleton rearrangements are key for cytokinesis, adhesion, pathogen vacuole formation, phagocytosis and chemotaxis.

Dictyostelium Rap1 with the RA domain of talin provides additional strength, which is essential for processes demanding high adhesive forces, such as morphogenesis.[17] A direct interaction between Rap1 and talin has also been reported in mice;[20] however, the biologic significance of this interaction is not clear.

Rap1 coordinates cytoskeletal rearrangements during cytokinesis and chemotaxis

In *Dictyostelium* Rap1 functions as a general regulator of cytoskeletal dynamics (Fig. 1).[4,7] To regulate the cytoskeletal dynamics during cell division, Rap1 is uniformly activated in the cell cortex during the early stages of cytokinesis.[8] In contrast, at the final stages of the process, the small GTPase is restricted to the cell poles. GefQ appears to be important for regulating Rap1 activation during cytokinesis.[8] Furthermore, a recent study suggests that also RapGAP9 is crucial for Rap-mediated cytokinesis progression.[21] Decreased or increased Rap1 activation impairs the growth rate and cytoskeletal dynamics. Thus, *Dictyostelium* Rap1 drives cytokinesis progression, likely by coordinating the major cytoskeletal components, microtubules, actin and myosin II.[8] Similar to *Dictyostelium*, levels of Rap1 activation are tightly controlled during cell division in Hela cells.[22] Furthermore, hyper-activation of Rap1 in various human cell lines and *Drosophila* results in severe cytokinesis defects.[23,24] Together, this strongly suggests a conserved and essential role for Rap in the regulation of cytokinesis.

Rap1 and Ras also regulate the balance between F-actin and myosin dynamics during chemotaxis of *Dictyostelium* amoeba and mammalian leukocytes (Fig. 1).[25,26] In response to the chemoattractant cAMP, both Rap1 and Ras are rapidly activated at the leading edge of migrating *Dictyostelium*.[4,27,28] The Gα2-stimulated RapGEF, GflB, is an important regulator of the balance between Rap1 and Ras activation during chemotaxis.[29] In addition, Rap1 activity at the leading edge is regulated by an unknown GEF that acts downstream of active Ras.[30] Rap1 and Ras can activate the Rac, PI3K and TORC2 pathways, which subsequently results in actin polymerization and pseudopod extension from the front of the cell[28,31-40] (Fig. 1). Simultaneously, Rap1 inhibits myosin assembly at the leading edge through activation of its effector Phg2, while low levels of active Rap1 at the side and back of the cell allow myosin filament formation.[25,26] Taken together, the spatial and temporal Rap1- and Ras-mediated control of actin and myosin rearrangement is essential for proper chemotaxis.[41]

Rap1 localizes to the *Legionella* pathogen vacuole and controls infection

Pathogenic bacteria intimately interact with eukaryotic host cells to subvert immune functions and create a replication-permissive niche. *Legionella pneumophila* is an environmental Gram-negative bacterium, which can cause a severe pneumonia termed "Legionnaires' disease".[42] The facultative intracellular pathogen uses a seemingly conserved mechanism to replicate in environmental protozoa or immune system phagocytes within a unique membrane-bound compartment, the *Legionella*-containing-vacuole (LCV).[43] Host cells of *L. pneumophila* include free-living amoeba such as *Acanthamoeba* or *Dictyostelium* spp. as well as mammalian macrophages.

LCV formation requires the bacterial Icm/Dot (intracellular multiplication/defective organelle trafficking) type IV secretion system (T4SS), which translocates approximately 300 (!) different "effector proteins" into host cells, where they modulate specific components of the machineries catalyzing transcription, translation, signal transduction or vesicle trafficking.[44-46] Some of these effectors target phosphoinositide lipids,[47] the retromer complex,[48] or small GTPases of the Arf,[49] Rab,[50,51] or Ran family.[52,53] LCVs avoid fusion with lysosomes, but extensively communicate with the host endosomal, secretory and retrograde trafficking path-ways, as well as with the endoplasmic reticulum (ER).[44,54,55]

Intact LCVs can be isolated and purified by a 2-step procedure, including an immuno-affinity purification step and density gradient separation.[56,57] To this end, the distinct and specific LCV localization of the Icm/Dot-secreted effector protein SidC is exploited.[58-60] Upon treatment with an anti-SidC antibody and a secondary antibody coupled to magnetic microbeads, LCVs are retained in a magnetic field, washed, eluted and further enriched by Histodenz density gradient centrifugation. Using this protocol, pathogen vacuoles harboring *L. pneumophila* have been isolated from *Dictyostelium*,[61] murine RAW 264.7 macrophages,[62] and bone marrow-derived macrophages (BMM) of infection-permissive A/J mice.[63] Proteomics analysis of these LCVs revealed more than 1150 host cell factors,[64] including 13 Rab GTPases, Ran and Rap1.[61,62] The localization to the LCV membrane and impact on intracellular growth of *L. pneumophila* of some of the Rab GTPases, as well as of Ran and Rap1, was validated by fluorescence microscopy and RNA interference, respectively.[52,62,65]

The presence of active Rap1 on LCVs was recently found to correlate with intracellular replication of *L. pneumophila*[65] (Fig. 1). In a comparative proteomics approach the proteome of isolated pathogen vacuoles

from *Dictyostelium* amoeba or RAW 264.7 macrophages infected with either the parental *L. pneumophila* strain Lp02 or the "pentuple" mutant ("Δpentuple") was determined. The Δpentuple strain, which lacks 5 gene clusters comprising ca. 13% of the genome and at least 31% of the effector proteins, is defective for intracellular replication in *Acanthamoeba* and *Dictyostelium*, but grows in BMM derived from the A/J mouse strain.[66] In the comparative proteomics study, Rap1 was identified on *Dictyostelium* LCVs containing the parental strain Lp02 but not the Δpentuple mutant and on macrophage LCVs containing either strain.[65] The localization pattern of active Rap1 was validated by fluorescence microscopy and quantitative imaging flow cytometry using *Dictyostelium* strains producing GFP-Rap1,[8,67] or the Rap1-GTP probe $RalGDS_{RBD}$-GFP.[26,67] In these experiments, GTP-bound Rap1 preferentially localized to LCVs harbouring strain Lp02 rather than to vacuoles containing Δpentuple mutant bacteria. Therefore, the accumulation of active Rap1 correlates with the formation of a replication-permissive pathogen vacuole. In agreement with this notion, the depletion of Rap1 by RNA interference reduced intracellular growth of *L. pneumophila*. In summary, Rap1 was found to represent a novel LCV host component that localizes preferentially to replication-permissive pathogen compartments and is implicated in intracellular bacterial replication (Fig. 1).[65]

Strikingly, the LCV localization pattern of a downstream target of Rap1, integrin-associated talin, mirrored that of the small GTPase. In *Dictyostelium* talin was exclusively identified in the Lp02 LCV proteome and not in the Δpentuple LCV proteome, whereas in macrophages talin was present in both proteomes. Taken together, the accumulation of Rap1 and talin on LCVs correlates with intracellular replication of *L. pneumophila*, and thus, the 2 host factors likely interact with each other not only during phagocytosis, but also in the context of bacterial infection and pathogen vacuole formation.

Concluding remarks

Rap1 is conserved in *Dictyostelium* amoeba as well as in mammalian cells, and its activation is regulated by various extracellular and intracellular stimuli. Rap1 functions mainly in controlling cytoskeleton rearrangements; it stimulates cellular adhesion, actin filament formation, and myosin disassembly. Rap1-mediated pathways are crucial for pleiotropic cellular processes, including cell adhesion, chemotaxis and motility, cell growth and division, membrane dynamics and vesicle trafficking. Moreover, our recent study has shown that the small GTPase is also required for pathogen vacuole formation and intracellular replication of a bacterial pathogen in *Dictyostelium* and macrophages.

Abbreviations

ER	endoplasmic reticulum
Icm/Dot	intracellular replication/defective organelle trafficking
LCV	*Legionella*-containing vacuole
T4SS	type IV secretion system

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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