ABSTRACT: The transient membrane engagement and reorientation of the soluble catalytic domain of Ras proteins has emerged as an important modulator of their functions. However, there has been limited information on whether this phenomenon is applicable to other members of the Ras superfamily. To address this issue, we conducted long-time-scale atomistic molecular dynamics simulations (55 μs aggregate simulation time) on representatives of the Ras, Rho, and Arf family proteins that differ in sequence, lipid modification, and the rigidity of the linker between the lipid anchor and the catalytic G-domain. The results show that the concept of membrane reorientation is generalizable to most but not all members of the Ras superfamily. Specifically, C-terminally prenylated small GTPases that are anchored to membranes via a single flexible linker adopt multiple orientations, whereas those that are N-terminally myristoylated and harbor a rigid linker experience limited orientational dynamics. Combined with published reports on Ras proteins, these observations provide insights into the common principles and determinants of the orientational dynamics of lipidated small GTPases on membrane surfaces and offer new ways of thinking about the regulation and druggability of the Ras superfamily proteins.

KEYWORDS: small GTPases, membrane orientation, protein−membrane interaction, lipid modification

INTRODUCTION

Membranes serve as a structural framework to organize proteins and lipids into signaling platforms. Many peripheral signaling proteins are targeted to membranes via lipid-based motifs generated by the co- or post-translational modification of glycine or cysteine residues by acyl and prenyl groups. The quintessential example of such lipid-anchored proteins is the guanine triphosphate (GTP) hydrolyzing Ras superfamily of small GTPases. Comprising the Ras, Rho, Rab, and Arf families, the predominantly lipid-modified Ras superfamily proteins are textbook examples of molecular switches with a conserved catalytic domain cycling between active GTP-bound and inactive guanine diphosphate (GDP)-bound states that differ in affinity for effectors and regulators. A guanine nucleotide exchange factor (GEF)/GTPase activating protein (GAP)-assisted switching between the two states enables this class of proteins to regulate a wide variety of cellular processes, including those controlling cell growth, motility, and trafficking.1−3 Dysregulation of the switching function can lead to intractable diseases.4−11 These include cancer and developmental disorders due to mutations in Ras proteins,6,10,11 or overexpression of Rho proteins6,7 and lysosomal and celiac diseases5,12 due to genetic defects of Rab and Arf proteins.8,12

The structure of lipid-modified Ras superfamily proteins consists of a conserved catalytic domain, a flexible linker, and a lipid anchor. The G-domain lacks a conventional lipid-binding domain, leading to the perception that this class of proteins passively attaches to membranes via the lipid anchor. Using molecular dynamics (MD) simulations and cell signaling assays, we have shown previously that the catalytic domain of H-Ras interacts with the membrane in a nucleotide-dependent manner.13,14 Subsequently, we and others have demonstrated that the catalytic domain of all three human Ras proteins interacts with membrane in multiple orientations.14−18 The functional significance of this process arises from the possibility that some orientations can be defective in signal transduction because of occlusion of the effector-binding region by the membrane.19 It has also been shown that the sequence and dynamics of the lipid-anchor encode lipid selectivity,20,21 whereas the conformational dynamics of the linker between the lipid anchor and the G-domain is a key determinant of Ras membrane reorientation.16−18

A few years ago, we reviewed the then available literature and proposed that the concept of membrane orientation may be broadly applicable to surface-bound lipidated proteins.22 To formally test this hypothesis, we have now conducted extended
all-atom MD simulations of full-length Rheb, RhoA, and Arf1 tethered to an anionic bilayer membrane composed of 20% POPS and 80% POPC lipids. Rheb, RhoA, and Arf1 were chosen as model systems because they represent the best-characterized members of the Ras, Rho, and Arf family, respectively (see, for example, ref 2). Although Rheb, RhoA, and Arf1 all utilize lipid-modification for membrane binding and share a high degree of structural homology at the catalytic domain, they differ in several important respects. Rheb is S-farnesylated (C-15 prenylated) at a C-terminal Cys residue (Figure 1), just like other members of the Ras family, including human N-Ras, H-Ras, and K-Ras. However, Rheb lacks the palmitoyl or polybasic motif near the site of farnesylation that is found in Ras proteins. RhoA is S-geranylgeranylated (C-20 prenylated) instead of being farnesylated; however, like K-Ras, it harbors a polybasic domain near its site of prenylation (Figure 1). Arf1 is not prenylated; it is targeted to membranes via a combination of a N-myristoyl lipid and a proximal amphipathic helix (Figure 1).

On the basis of data from a 10−20 μs MD run per system, we show that all three proteins undergo large conformational changes upon membrane binding, which include multiple events of membrane engagement/disengagement and/or reorientation of the catalytic domain on the membrane plane, as well as in its membrane engagement/disengagement, are discussed in subsequent sections.

**Rheb, RhoA, and Arf1 Differ in Rotational Motions on a PC/PS Bilayer**

As noted above, a major finding from previous MD simulation studies of Ras proteins was the ability of the catalytic domain to adopt multiple distinct orientations on membrane surfaces. To check if this is applicable to Rheb, RhoA, and Arf1, we used Euler angles, defined in Figure 2, to quantify the tilt, rotation, domain and the HVR underwent major conformational reorganizations. Similarities and differences among the three proteins in terms of orientation and tilt of the catalytic domain on the membrane plane, as well as in its membrane engagement/disengagement, are discussed in subsequent sections.

**RESULTS AND DISCUSSION**

Following previous experiences on Ras proteins, the current simulations were started with the prenyl or myristoyl chain partially inserted into the hydrophobic core of the membrane. This ensured complete membrane insertion and stabilization of the lipid anchor during the equilibration period. Rheb and RhoA were then simulated for 20 μs each and Arf1 for 10 μs on Anton 2. In addition, Rheb and Arf1 were simulated for a 1 μs duration at Texas Advanced Computing Center (TACC) in single and three copies, respectively. During the entire duration of each of these simulations, the protein remained tethered to the membrane via the lipid anchor, whereas the catalytic

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**Figure 1.** Three-dimensional structure of Rheb, RhoA, and Arf1. Lobe1 is shown in green cartoon and lobe2 in orange. The myristoylated (Myr) N-terminus of Arf1 and the farnesylated (Far) and geranylgeranylated (Ger) C-terminus of Rheb and RhoA are shown in gray. Selected secondary structure elements as well as switch 1 (sw1) and switch 2 (sw2) are labeled using Ras numbering. The sequence of the membrane-targeting motif is shown at the bottom with positively charged residues in blue and lipid-modified cysteine or glycine residues in orange.

**Figure 2.** Rotational dynamics of bilayer-bound Rheb, RhoA, and Arf1. Distribution of Euler angles α, β, and γ representing tilt, rotation, and spin of the catalytic domain of Rheb (top), RhoA (middle), and Arf1 (bottom) with respect to the membrane normal. These data were derived from 20 μs-long MD simulations of farnesylated Rheb and geranylgeranylated RhoA bound to a POPC/POPS bilayer and a 10 μs long simulation of myristoylated Arf1 in the same bilayer.
and spin angles of the catalytic domain with respect to the membrane normal. The results show wide variations in the rotational dynamics of the three proteins. The catalytic domain of the N-myristoylated Arf1 experienced only modest rotational motion, as shown by the narrow distribution of its tilt and rotation/spin angles centered at \( \sim 30 \) and \( \sim 180^\circ \), respectively (Figure 2). In contrast, the catalytic domain of the C-terminally prenylated Rheb and RhoA proteins samples the entire \( 0^\circ - 90^\circ \) range of tilt and the \( 0^\circ - 360^\circ \) range of rotation/spin angles. Moreover, in each case, there are 1–2 broad peaks, suggesting that these two proteins not only undergo extensive fluctuations but also adopt a set of preferred orientations. These orientation preferences appear to be more clearly defined for Rheb than RhoA, as can be seen from the more diffuse distribution of the Euler angles in the latter (Figure 2). These results clearly show that the three proteins simulated here undergo distinct rotational motions, with the difference between the prenylated proteins being less pronounced than that between them and the myristoylated Arf1.

**Rheb Swings and Rolls on the Membrane Surface, Similar to Ras Proteins**

Rheb is a member of the Ras family and is farnesylated like all other Ras proteins. Therefore, one can expect its membrane dynamics to be more similar to those of Ras proteins than those of RhoA and Arf1. Along this line, an earlier solid-state nuclear magnetic resonance spectroscopy (NMR) study found that the three helices at the C-terminal lobe of Rheb (Figure 1) are membrane-proximal when the G-domain is GTP loaded, whereas the catalytic domain is distal from the membrane when it is GDP bound.\(^{12} \) These observations mirror those previously reported for H-Ras.\(^{13} \) With this in mind, we first conducted a 1 \( \mu s \) long simulation of Rheb with the GTP-loaded G-domain placed away from the membrane. We found that the G-domain quickly moved toward the membrane and engaged lipids via residues at the C-terminal helices 4 and 5 and the \( \beta2/\beta3 \)-turn, leading to the configuration shown in Figure 3A (left). This orientation is very similar to that observed in GTP-bound H-\(^{14} \) and in one of the states of GTP-bound K-Ras,\(^{15,16} \) and will be referred to here as OS1 following the terminology we introduced for K-Ras.\(^{16} \) We then conducted a 20 \( \mu s \) Anton 2 simulation starting from the configuration in Figure 3A (left). The goal was to test if, like K-Ras, GTP-bound Rheb samples additional orientation states. This longer simulation also allows us to determine if orientational dynamics is an intrinsic property of Rheb rather than one solely induced by the bound nucleotide. In other words, if Rheb samples multiple orientations in a single (GTP) nucleotide state, it would mean that its orientational dynamics is intrinsic and not just a consequence of nucleotide exchange.

It is clear from the snapshots in Figure 3A (and the plots in Figure 2) that Rheb undergoes extensive orientational dynamics. Specifically, lobe2 faces the bilayer at the start of the simulation (\( t = 0 \mu s \)) but away from the membrane at \( t = 3 \mu s \), where a portion of \( \beta \)-strands 2–3 in lobe1 interacts with lipids. The G-domain occasionally disengages the membrane, as shown by the snapshot at \( t = 15 \mu s \), and re-engages in an orientation similar to those at \( t = 18 \mu s \) and \( t = 20 \mu s \). To further examine this apparently fast membrane reorientation, we plotted the time evolution of the \( z \)-component of the distance between the center of mass of the membrane and lobe1 (\( Z_{\text{COM-B1}} \), Figure 3B), and the frequency of contact between each residue and bilayer lipids (Figure 3C). The plots show that the time scale of the reorientation is in the range of

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**Figure 3.** Membrane dynamics of GTP-bound Rheb. (A) Snapshots of Rheb embedded in an anionic membrane bilayer with lobe1 (residues 1–86) in green, lobe2 (residues 95–170) in orange, and the farnesyl and the flexible C-terminus in gray; a portion of the bilayer is shown in light blue lines. (B) Time evolution of \( Z_{\text{COM-B1}} \) (distance along the \( z \)-axis between the center of the mass of lobe1 and the membrane); the red line represents a 200 ns moving average. (C) Time evolution of Rheb-membrane contact per residue, with contact defined as any heavy atom of a residue within 5 Å of any phospholipid heavy atom. (D) Plot of the normalized 2D density distribution of \( Z_{\text{COM-B1}} \) and \( Z_{\text{COM-B2}} \), defined as the \( z \)-component of the distance of lobe1 and lobe2 from the bilayer center, respectively. Red color represents the most populated region.
tens to hundreds of nanoseconds. Moreover, a probability density plot of $Z_{\text{COM-lb1}}$ versus $Z_{\text{COM-lb2}}$ indicates that the simulated ensemble is dominated by two mutually exclusive membrane-proximal populations of conformers, with either lobe1 or lobe2 lying at $\sim 27$ Å from the bilayer center. The snapshots at $t=3$ μs and $t=18$ μs exemplify the former and are referred to as orientation state 2 or OS2, whereas the snapshots $t=0$ μs and $t=20$ μs represent OS1. In addition to these two dominant orientation states, Rheb also samples many other transient conformations, including those with the G-domain distal from the membrane, characterized by both $Z_{\text{COM-B1}}$ and $Z_{\text{COM-B2}} \gtrsim 30$ Å (example: see snapshot at $t=15$ μs). Each of these observations is remarkably similar to those in K-Ras and also H-Ras.

A closer look at the ensemble of conformers in the two well-populated peaks of Figure 3D shows surface residues from either lobe1 or lobe2 directly interacting with the membrane in OS2 and OS1, respectively. These interactions are mutually exclusive (i.e., when lobe1 contacts membrane, lobe2 is solvent exposed and vice versa). The exception is the β2/β3-turn (residues 48–52), where polar residues N50 and Q52 remained close to lipids in both orientations (Figure 4). As in K-Ras, membrane engagement of the G-domain is dominated by electrostatic interactions involving POPS and basic residues such as K5, R7, and K109 in OS2 and K135, R161, and R162 in OS1. Note that acidic and other polar residues proximal to these positively charged surface patches or the β2/β3 turn also approach the bilayer surface intermittently. These include D77 in OS2 and H124, Y131, E131, E132, and E139 in OS1. Figure 2 and the snapshots in Figure 3 further illustrate that, in addition to reorientation with respect to the membrane plane and translation along the membrane normal, Rheb also undergoes rotational motion similar to that documented for K-Ras.

**RhoA Rolls Like Rheb but Does Not Swing as Much**

A similar analysis of RhoA shows that the G-domain engages the bilayer within 1 μs (Figure 5), with residues at lobe1 making extensive contacts with lipids (Figure 5A; compare snapshots at $t=0$ μs and $t=2$ μs). After staying in this OS2 orientation for $\sim 4$ μs (Figure 5B), the protein adopted OS1,
Unlike Rheb (Figures 3 and 4) and K-Ras, where the N-terminus contacts the bilayer primarily in OS2, the RhoA N-terminus interacts with lipids even when the proximal β2/β3 turn (residues 46–51) disengages (Figure 5C). The N-terminus loses contact only when helix 4 (Ras numbering; residues 141–151) interacts with lipids in a parallel orientation (Figure 5A, t = 16 μs). This is probably because the first seven residues of RhoA consist of nonpolar (MAAI) and basic (RKK) amino acids that can form favorable interactions with the bilayer hydrophobic core and POPs head groups, respectively. Although K5, R7, and K8 of Rheb share similarity to R5, K6, and K7 of RhoA, Rheb (as well as Ras proteins) lacks the apolar/basic amino acid combination found in the RhoA N-terminus. If confirmed by further scrutiny, this observation has significant implications for RhoA function, as this interaction could stabilize selected orientations and thereby modulate the degree of occlusion of the effector-binding switch regions. Another unique feature of RhoA relative to Rheb and Ras is that its G-domain disengages the membrane more rarely. This can be seen from the nearly continuous contact of the G-domain residues with lipids (Figure 5C), or from the narrow distribution of the Z_{COM-lb1} and Z_{COM-lb2} distances (Figure 5D). As a result, RhoA mostly rolls, whereas Rheb and Ras both roll and swing (disengage/re-engage), to sample distinct membrane orientation states.

**Arf1 Does Not Roll or Swing**

Our current and previous studies on representatives of the Ras and Rho family consistently suggest that the flexible linker between the lipid anchor and the G-domain is critical for membrane reorientation. To test this further, we simulated Arf1, a member of the Arf family that is N-myristoylated at an amphipathic helix. This helix is connected to the G-domain by a short linker (~5 residues in the GTP-bound form). Thus, Arf1 lacks the long (>20 amino acids) intrinsically disordered domain preceding the lipid anchor of Ras and Rho proteins (Figure 1).

Figure 6A, B shows that Arf1 remained bound to the bilayer via the myristoylated N-terminus throughout the simulation while undergoing significant conformational changes. These conformational changes can be seen from the variations in the proximity of the G-domain to the bilayer surface and the insertion depth of the amphipathic helix into the bilayer core (Figure 6A), or the fluctuations in the protein–lipid contacts (Figure 6C). However, as already indicated by the data in Figure 2, the large-scale reorientation of the G-domain observed in RhoA and Rheb is absent in Arf1. This is further supported by (i) the lack of significant fluctuations in Z_{COM-lb1} (Figure 6B); (ii) only residues at lobe1 (residues 17–94) make direct if infrequent contact with the membrane (Figure 6C); and (iii) only a single peak is apparent in the Z_{COM-lb2}/Z_{COM-lb1} probability density distribution (Figure 6D). The same conclusion was obtained using other reaction coordinates (not shown).

To check if the single, roughly perpendicular (i.e., a small angle between the principal axis of the G-domain and the membrane normal) orientation of Arf1 is a consequence of the initial configuration, we conducted three 1 μs independent simulations started from different distance and orientation of the G-domain from the membrane surface. We found that all three runs converged to a very similar conformation/orientation. Thus, apart from few “pull-up” type motions that allow for the occasional disengagement of the G-domain from the membrane (Figure 6C), the short linker and the ordered helix in Arf1 do not allow for conformational fluctuations large enough to result in membrane reorientation. Other factors that may contribute to the restricted membrane dynamics of Arf1 include unique interactions involving the N-terminal amphipathic helix and a pair of C-terminal basic residues. Specifically, the N-terminal helix contains three phenylalanine residues at its hydrophobic face that insert into the hydrophobic core of the membrane, plus three lysine residues that ensure interfacial binding in a specific orientation. Although these interactions,
plus those of the myristoyl motif itself, provide the primary driving force for membrane binding of Arf1, the occasional penetration of the amphipathic helix deep into the bilayer core brings switch 2 residues N83 and Q85 and B2/B3-turn residue Y57 closer to the membrane (Figure 6C). In addition, residues R177 and K180 at the C-terminus make almost uninterrupted direct contact with lipids (Figure 6C). These interactions have the potential to impede orientational motion and reduce the frequency of complete membrane disengagement of the G-domain. Recall that we have made a similar observation in RhoA where, compared with Rheb (Figures 3 and 4), interactions of the N-terminus with lipids likely reduced its overall dynamics (Figure 5). Although it requires a systematic study to fully establish, these results suggest that a two-site anchorage of lipidated small GTPases may play a significant role in function by modulating dynamics at the membrane.

**Implications to Function**

When combined with previous observations on H-Ras,13–15 N-Ras,26,27 K-Ras,26,28,29 and some Rab proteins,30,31 the current results strongly suggest that most C-terminally prenylated proteins harboring a flexible linker undergo membrane reorientation. The modes of the conformational fluctuation underlying the orientation motion are similar among the prenylated proteins that have been studied thus far. However, there are also important differences. These include differences in the time scale and extent of conformational fluctuations and the role of basic residues at the N-terminus in stabilizing certain orientations. For example, using $Z_{\text{COM-b1}} < 26 \text{ Å}$ and $Z_{\text{COM-b1}} < 18 \text{ Å}$ as approximate cutoffs for Rheb and RhoA being in the OS2 orientation state, we estimated from the moving averages in Figures 3B and 4B that there are 10 events of each protein being in OS2 within the 20 μs duration of the simulation. This translates to a rate of 0.50/μs or $\sim 5 \times 10^{-2}/\text{s}$, suggesting a fluctuation time scale of $\sim 2 \mu\text{s}$. Whether or not membrane reorientation at this time scale could lead to measurable functional effects in the cell is yet to be determined and will likely depend on several factors including effects of scaffolding proteins or interaction with other proteins and lipids. Note, however, that K-Ras reorients at a similar time scale27 and yet K-Ras mutations and ligand binding have been shown to preferentially stabilize a given orientation state over others.19,34 In contrast to their OS2 sampling rate, Rheb and RhoA significantly differ in their rate of visiting OS1. Using the same cutoff values as above (i.e., $Z_{\text{COM-b2}} < 26 \text{ Å}$ for Rheb and $Z_{\text{COM-b2}} < 18 \text{ Å}$ for RhoA), we counted 12 events (0.6/μs) of Rheb visiting OS1 and just 5 (0.25/μs) for RhoA. This difference is also reflected in the equilibrium population distribution plots of Figures 3D and 4D. Longer simulations may be needed to further investigate this difference and its potential implications to function. It is clear, however, that sampling of different orientations depends on the sequence of the HVR (Figure 1), as well as the distribution of clusters of basic residues throughout the proteins (Figures 3–5).

Additional computational and experimental work on the proteins investigated here as well as other lipidated small GTPases will be required to clearly define the functional consequences of our observations. An issue that deserves attention on the MD simulations side is lipid composition. The current simulations utilized a simplified model of the plasma membrane inner leaflet, namely, a symmetric POPC:POPS bilayer at 4:1 ratio. This was important to facilitate direct comparison with previous studies where the anionic POPS lipid was specifically chosen as it preferentially interacts with the polybasic domain of the K-Ras lipid anchor,16–18 note in this context that RhoA also harbors a polybasic lipid anchor. Future simulations should be conducted in more complex asymmetric membrane models including bilayers containing cholesterol and phosphatidylethanolamine and, where relevant, phosphatidylinositol (PI) lipids. We could not find clear evidence that suggests differential lipid domain preference by the proteins studied in this work. Nonetheless, comparative analyses of myristoylated and prenylated proteins in domain-forming model membranes may be instructive. The results described in this work may also guide future experiments. One example would be to mutate key residues contributing to the dynamic engagement of the G-domain with the membrane and test their effect on function. Another would be to build chimeras of lipid anchors harboring a prenylated amphipathic helix as tools for investigating the biochemical and biological roles of orientational motion. We have previously employed similar approaches to gain insights into the functional relevance of Ras proteins membrane reorientation.13–15

**CONCLUSIONS**

In this report, we have shown that members of the Ras family of proteins, such as Rho, generally roll and swing on membrane surfaces with some variations in scope; Rho proteins, as exemplified by RhoA, appear to swing less but are able to roll over membrane surfaces; Arf1-like proteins do not swing or roll largely because of the myristoylated amphipathic helix; two-site anchorage dampens orientational dynamics. Taken together, these results strongly support the notion that the length and flexibility of the linker between the lipid anchor and the G-domain is the primary determinant of membrane reorientation; additional interactions of basic residues with anionic lipids fine-tune the mode and amplitude of the orientational motion.

**COMPUTATIONAL METHODS**

The simulations of GTP-bound full-length human RhoB and RhoA proteins were started from crystal structure 1XTS and 1A2B, respectively, downloaded from the protein data bank (PDB). The missing C-terminal residues were modeled in as an extended chain. Farnesyl was added to the C-terminal Cys residue of RhoB and geranylgeranyl to RhoA, with the new C-terminus capped by a caboxymethylated moiety using parameters and protocols previously described for Ras proteins.16–18 For human Arf1, we used PDB ID 4HMY for the catalytic domain, which was ligated to a myristoylated N-terminus derived from the yeast Arf protein PDB ID 2KSO, after mutating the yeast residues to human. Each protein was then placed on one side of a previously equilibrated bilayer made up of 320 POPC and 96 POPS (80:20%) lipids, with the lipid-modified moiety pointing toward the bilayer surface. Then, following previous experiences, the protein was pulled toward the bilayer until at least five carbon atoms of the prenyl or myristoyl tail were inserted into the hydrophobic core. The resulting constructs were solvated by the TIP3P water model, and counterions were added to neutralize the system and to achieve a physiological ionic strength of 150 mM, resulting in ~160,000 atom systems. Each system was then energy-minimized for 2000 steps with lipids and proteins fixed, and then equilibrated for multiple 200 ps steps using a time-step of 1 fs, with the lipid phosphate as well as protein and lipid heavy atoms harmonically restrained with a force constant initially set to 4 kcal mol$^{-1}$ Å$^{-2}$ and gradually scaled down by a factor of 0.75, 0.50, 0.25, and 0. Further equilibration, each system was simulated for 1 μs (in single copy except for Arf1, which was run in three copies) on
Stampede2 using the NAMD2.11 program.32 The simulation details are as described in the literature;6–8 the CHARMM36 force field and the CMAP dihedral correction were used.33 The final snapshot of these simulations (one per system) was used to start a 20 μs MD run on Anton 2 using Desmond, except for Arf1, where the simulation was stopped at 10 μs because there was no sign of membrane reorientation. The Desmond simulations used default parameters and cutoffs described in ref 18. Trajectory frames saved every 100 ps were analyzed as described previously,7,14 with the G-domain divided into two lobes based on superposition with Ras: residues 1–86, 5–86 and 17–94 represent lobe1, whereas residues 87–170, 87–180, and 95–180 constitute lobe2 of Rheb, RhoA, and Arf1, respectively. During analysis, the membrane was centered at the origin with the membrane normal aligned to the z-axis. Secondary structure numbering is based on Ras.

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**Author Contributions**

P.P. and A.A.G. conceived and designed the project; P.P. carried out the simulations; P.P. and A.A.G. analyzed the data and wrote the paper.

**Notes**

The authors declare no competing financial interest.

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**REFERENCES**

(1) Macara, I. G.; Lounsbury, K. M.; Richards, S. A.; McKiernan, C.; Bar-Sagi, D. The Ras superfamily of GTPases. *FASEB J.* 1996, 10 (5), 625–30.

(2) Wennerberg, K.; Rossman, K. L.; Der, C. J. The Ras superfamily at a glance. *J. Cell Sci.* 2005, 118 (5), 843–846.

(3) Goitre, L.; Trapani, E.; Trabulzi, L.; Retta, S. F. The Ras superfamily of small GTPases: the unlocked secrets. *Methods Mol. Biol.* 2014, 1120, 1–18.

(4) Fitz, G.; Just, I.; Kaina, B. Rho GTPases are over-expressed in human tumors. *Int. J. Cancer* 1999, 81 (5), 682–7.

(5) Schubbert, S.; Shannon, K.; Bollag, G. Hyperactive Ras in developmental disorders and cancer. *Nat. Rev. Cancer* 2007, 7 (4), 295–308.

(6) Vega, F. M.; Ridley, A. J. Rho GTPases in cancer cell biology. *FEBS Lett.* 2008, 582 (14), 2093–101.

(7) Vasiliev, J. M.; Omelchenko, T.; Gelfand, I. M.; Feder, H. H.; Bonder, E. M. Rho overexpression leads to mitosis-associated detachment of cells from epithelial sheets: a link to the mechanism of cancer dissemination. *Proc. Natl. Acad. Sci. U. S. A.* 2004, 101 (34), 12526–30.

(8) Cox, A. D.; Der, C. J. Ras history: The saga continues. *Small GTPases* 2010, 1 (1), 2–27.

(9) Seixas, E.; Barros, M.; Seabra, M. C.; Barral, D. C. Rab and Arf proteins in genetic diseases. *Traffic* 2013, 14 (8), 871–85.

(10) Bos, J. L. Ras oncogenes in human cancer: a review. *Cancer Res.* 1989, 49 (17), 4682–4689.

(11) Karnoub, A. E.; Weinberg, R. A. Ras oncogenes: split personalities. *Nat. Rev. Mol. Cell Biol.* 2008, 9 (7), 517–31.

(12) Li, G.; Marlin, M. C. Rab family of GTPases. *Methods Mol. Biol.* 2015, 1298, 1–15.

(13) Abankwa, D.; Hanzal-Bayer, M.; Ariotti, N.; Plowman, S. J.; Gorfe, A. A.; Parton, R. G.; McCammon, J. A.; Hancock, J. F. A novel switch region regulates H-ras membrane orientation and signal output. *EMBO J.* 2008, 27 (5), 727–35.

(14) Gorfe, A. A.; Hanzal-Bayer, M.; Abankwa, D.; Hancock, J. F.; McCammon, J. A. Structure and dynamics of the full-length lipid-modified H-Ras protein in a 1,2-dimyristoylglycerol-3-phosphocholine bilayer. *J. Med. Chem.* 2007, 50 (4), 674–84.

(15) Abankwa, D.; Gorfe, A. A.; Inder, K.; Hancock, J. F. Ras membrane orientation and nanodomain localization generate isoform diversity. *Proc. Natl. Acad. Sci. U. S. A.* 2010, 107 (3), 1130–5.

(16) Prakash, P.; Zhou, Y.; Liang, H.; Hancock, J. F.; Gorfe, A. A. Oncogenic K-Ras binds to an anionic membrane in two distinct orientations: A molecular dynamics analysis. *Biophys. J.* 2016, 110 (5), 1125–38.

(17) Prakash, P.; Gorfe, A. A. Probing the conformational and energy landscapes of KRAS membrane orientation. *J. Phys. Chem. B* 2019, 123 (41), 8644–52.

(18) Prakash, P.; Litwin, D.; Liang, H.; Sarkar-Banerjee, S.; Dolino, D.; Zhou, Y.; Hancock, J. F.; Jayaraman, V.; Gorfe, A. A. Dynamics of membrane-bound G12V-KRAS from simulations and single-molecule FRET in native nanodiscs. *Biophys. J.* 2019, 116 (2), 179–83.

(19) Mazhab-Jafari, M. T.; Marshall, C. B.; Smith, M. J.; Gasmii-Seabrook, G. M. C.; Stathopoulos, P. B.; Iimagaki, F.; Kay, L. E.; Neel, B. J.; Ikura, M. Oncogenic and RASopathy-associated K-RAS mutations relieve membrane-dependent occlusion of the effector-binding site. *Proc. Natl. Acad. Sci. U. S. A.* 2015, 112 (21), 6625–30.

(20) Zhou, Y.; Prakash, P.; Liang, H.; Cho, K. J.; Gorfe, A. A.; Hancock, J. F. Lipid-sorting specificity encoded in K-Ras membrane anchors regulates signal output. *Cell* 2017, 168 (1–2), 239–51.

(21) Zhou, Y.; Prakash, P. S.; Liang, H.; Gorfe, A. A.; Hancock, J. F. The KRAS and other prenylated polybasic domain membrane anchors recognize phosphatidylserine acyl chain structure. *Proc. Natl. Acad. Sci. U. S. A.* 2021, 118 (6), e2014605118.

(22) Prakash, P.; Gorfe, A. A. Membrane orientation dynamics of lipid-modified small GTPases. *Small GTPases* 2017, 8 (3), 129–38.

(23) Gorfe, A. A.; Pellarin, B.; Caflisch, A. Membrane localization and flexibility of a lipidated ras peptide studied by molecular dynamics simulations. *J. Am. Chem. Soc.* 2004, 126 (46), 15277–86.

(24) Mazhab-Jafari, M. T.; Marshall, C. B.; Stathopoulos, P. B.; Kobashigawa, Y.; Stambolic, V.; Kay, L. E.; Iimagaki, F.; Ikura, M. Membrane-dependent modulation of the mTOR activator Rheb: NMR observations of a GTPase tethered to a lipid-bilayer nanodisc. *J. Am. Chem. Soc.* 2013, 135 (9), 3367–70.

(25) Neale, C.; Garcia, A. C. The plasma membrane as a competitive inhibitor and positive allosteric modulator of KRAS4B signaling. *Biophys. J.* 2020, 118 (5), 1129–1141.
(26) Kapoor, S.; Triola, G.; Vetter, I. R.; Erlkamp, M.; Waldmann, H.; Winter, R. Revealing conformational substates of lipidated N-Ras protein by pressure modulation. Proc. Natl. Acad. Sci. U. S. A. 2012, 109 (2), 460−5.
(27) Weise, K.; Kapoor, S.; Denter, C.; Nikolaus, J.; Opitz, N.; Koch, S.; Triola, G.; Herrmann, A.; Waldmann, H.; Winter, R. Membrane-mediated induction and sorting of K-Ras microdomain signaling platforms. J. Am. Chem. Soc. 2011, 133 (4), 880−7.
(28) Li, Z. L.; Buck, M. Computational modeling reveals that signaling lipids modulate the orientation of K-Ras4A at the membrane reflecting protein topology. Structure 2017, 25 (4), 679−89.
(29) McLean, M. A.; Stephen, A. G.; Slibar, S. G. PIP2 influences the conformational dynamics of membrane-bound KRAS4B. Biochemistry 2019, 58 (33), 3537−45.
(30) Edler, E.; Stein, M. Probing the druggability of membrane-bound Rab5 by molecular dynamics simulations. J. Enzyme Inhib. Med. Chem. 2017, 32 (1), 434−43.
(31) Edler, E.; Schulze, E.; Stein, M. Membrane localization and dynamics of geranylgeranylated Rab5 hypervariable region. Biochim. Biophys. Acta, Biomembr. 2017, 1859 (8), 1335−49.
(32) Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kalé, L.; Schulten, K. Scalable molecular dynamics with NAMD. J. Comput. Chem. 2005, 26 (16), 1781−802.
(33) Klauda, J. B.; Venable, R. M.; Freites, J. A.; O’Connor, J. W.; Tobias, D. T.; Mondragon-Ramirez, C.; Vorobyov, I.; MacKerell, A. D., Jr.; Pastor, R. W. Update of the CHARMM all-atom additive force field for lipids: validation on six lipid types. J. Phys. Chem. B 2010, 114 (23), 7830−43.
(34) Fang, Z.; Marshall, C. B.; Nishikawa, T.; Gossert, A. D.; Jansen, J. M.; Jahnke, W.; Ikura, M. Inhibition of K-RAS4B by a unique mechanism of action: Stabilizing membrane-dependent occlusion of the effector-binding site. Cell Chem. Biol. 2018, 25 (11), 1327−1336.

**NOTE ADDED AFTER ASAP PUBLICATION**

This paper was published ASAP on December 3, 2021, with errors in Figure 2 and the related text. The corrected version was reposted on December 23, 2021.